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DELAYED PENICILLIN EXCRETION BY A DIET INCREASING HIPPURIC ACID SYNTHESIS.

BY LÁSZLÓ MOSONYI, ERZSÉBET OBLATT AND MARGIT GÖTTINGER SURJÁN.

FROM THE II. MEDICAL CLINIC OF THE PÉTER PÁZMÁNY UNIVERSITY OF BUDAPEST (DIRECTOR PROF. IMRE HAYNAL).

One way of augmenting the degree of effectiveness of penicillin is to slow up its rapid excretion. *Fleming* and his co-workers, in the course of investigating the causes of penicillin-retention in kidney patients, thought to employ experimentally the substances excreted through the tubuli, as it could be established by clearance investigations that a good part of the penicillin left the organism by that route. *Rammelkamp* and *Bradley* first experimented with the contrast-substance diodrast, but their results were far surpassed by the investigations of *Beyer* and his associates, carried out with para-aminohippuric acid. In their experiments on dogs the animals were given 10,000 units of penicillin with simultaneous drop-infusions of hippuric acid (HA), by which they obtained a 25 mg % concentration in the dogs' blood. In this way they succeeded, as opposed to serum which otherwise was penicillin-free $1^1/_2$ hours after the injection, in attaining one which after 2 hours still contained 0,2 units, that is, 4 times the strength of the concentration *Fleming* found effective (0,05). At the same time, only about 30% of the penicillin was present in the urine in the time given, as opposed to the 60—62% usual in the absence of hippuric acid. In the case of penicillin and HA drop-infusion employed simultaneously, if the penicillin was taken into the organism at a rate of 15 u/min., a constant concentration of 0,08—0,10 units could be maintained in the blood; while if the same quantity was given without HA, the blood level fell to 0,02. The hippuric acid itself was found to be completely harmless to mice, guinea pigs and dogs, though in mice the dose of 5300 mg per kg b. w. was mortal — but this had to be injected in such high concentrations (20—40%) that one could rightly suppose the solution to be primarily the physically detrimental cause.

The composition and excretion of hippuric acid (benzoyl glycine) belong to chapters of biochemistry which have so far not been entirely cleared up. *Neuweiler* has reviewed the most recent research in the subject,

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according to which *Bunge* and *Schmiedeberg's* experiments on surviving kidney, where hippuric acid could be shown in the urine after perfusion of benzoic acid and glycine, showed the decisive importance of the kidneys. But later *Kingsbury* and *Bell* also found HA synthesis in nephrectomized rabbits and dogs. Glycine and benzoic acid combining is brought about by the ferment hippurase (histozym) which, according to *Waelsch* and *Busztin* is demonstrable equally in the kidneys, liver and blood of horses. The combining of benzoic acid and glycine is in reality homologous to the production of conjugated glucuronic acids, that is, to the organism's detoxicating processes, which fact draws attention to the role of the liver. In respect to just this question *Quick* et al. worked out their hippuric acid tests of liver function, which many later investigators have found useful. The liver's primary role is also demonstrated by *Tulane* and *Lewis*, according to whom hydrazin-poisoned guinea pigs do not excrete HA after a diet of carrots and oats, but die, while the urine contains free benzoic acid. But recently *Quick* has also found HA in dogs after ligature of the ureter but not after nephrectomy. As indicative of the kidneys'very essential role, besides this communication of *Quick's Leuthardt* too has observed that with kidney slices HA could be synthetised in vitro from benzoic acid and different amino acids (glycocoll, glutaminic acid, glycilglycin, asparagin, serin and glutamin).

The important constituent common in all the varieties of penicillin thus far known is a dipeptide of two amino acids (cystein and serin); but these amino acids, according to the above, could be the basic materials of HA synthesis. After the chemical laws of mass action asserting themselves through common radicals, the radical in greater concentration tries to expell from the solution — in our case from the kidneys' tubular celle — the radical acting on the same side with it in the same chemical reaction.

As hippuric acid does not combine chemically with penicillin, and thus prevention of a sudden drop in its serum-level is exclusively effectuated in the kidneys, this can really be attained by venous HA. But *Fleming* himself emphasises that the penicillin injections are necessary even so every 3—4 hours, so that the additional use of drip infusions mean an almost insupportable burden for the patient. This clumsy and disagreeable method, however, can be avoided by giving the HA perorally. In the *Quick* liver function test one gives 6 g N. benzoicum to the fasting individual; in the case of good liver function a good part of it is excreted within 4 hours. On the other hand, it is known too that in the urine of herbivorous animals HA reaches a high concentration. For these reasons we made experiments, on the one hand by giving, perorally, Na-benzoicum and anaesthesin, chemically closely related to it and which is reduced

in the organism to benzoic acid; and on the other hand by suitable changes in diet.

Anaesthesin (which as an aromatic compound with an unpaired number of carbon atoms — ethyl ester of aminobenzoic acid — according to *Lehnartz* is also excreted from the organism in the form of hippuric acid) is a quite harmless but bad-tasting powder which, on being swallowed, causes an insensibility in the lips and a feeling of pressure in the region of the stomach, and for that reason cannot be given in large doses. Na. benzoicum has similarly a disagreeable taste. *Hara* carried out animal experiments on HA excretion with different diets. He found that fasting, copious animal protein and fat-content of the meals diminished the HA synthesis to such an extent that nearly 20% of the Na. benzoicum taken in failed to be demonstrable.

In composing our diets we took these facts into consideration. Most of the caloric requirement was covered by materials of vegetable origin, while limiting the animal proteins and fats. The use of oat flakes seemed to be of the greatest importance. The great hippuric acid content of horse-urine is well known, and that it increases still more on a pure oat diet. Oats stand out among the grains by reason of their high protein content (oat-flake has one of 13,5%). Cinchona-cortex and different fruits, such as prunes, also increase hippuric acid excretion. The diet we composed (milk, oat-flake, potatoes, clear soup made of bones, vegetables, prunes, some sugar) contains about 40 g protein, 30 g fat, and 200 g carbohydrate and, in an appetising and easily digestible form, offers those factors which bring about the necessary increase in HA excretion. The diet was completed by as much bread and dry biscuits made of oat-flake as desired. In reducing penicillin excretion it is not the serum's HA content which is decisive, but that it reaches the necessary concentration in the tubuli of the kidneys. We convinced ourselves of the extent of HA concentration attained through this diet by determinations of HA-content in the urine. The determinations were carried out after *Krauss* and *Dulkin's* method.

50 ml urine were acidified with acetic acid, boiled and if it vas not clear filtered. To the filtrate 30% NaCl was added, and heated, until the salt melted. After cooling a 50% sulphuric acid was added drop-wise until the entire HA precipitated. Then the fine white powdery precipitate was filtered off after standing for 15 minutes and washed with a 30% cold NaCl solution. After drying, the filtrate is dissolved in 100 ml distilled water and titrated with 0,5 normal NaCH phenolphthalein serving as indicator. 1 ml lye corresponds to 0,072 g hippuric acid.

At first we examined the HA content of the urine of the experimental individuals for several days. 10 persons free from kidney diseases were

found with endogenous HA excretion between 0,6 and 1,62, agreeing with the bibliographic data. For two days thereafter they were given the diet above and the HA determined in 24 hours' urine. The urines were also examined for two days after stopping this diet. In general we found that on the second day without the diet the HA had returned to its beginning value.

Hippuric acid excretion. (g)

Name	Before diet	During the diet		After the diet	
		1st day	2nd day	1st day	2nd day
J. F.	0,62	1,53	12,40	28,08	2,8
F. E.	0,68	1,62	8,8	4,6	1,7
R. J.	1,35	45,2	44,6	13,2	3,5
H. R.	0,8	5,79	6,35	32,0	2,5
A. J.	1,1	33,0	—	17,40	4,0
K. J.	1,62	5,6	42,0	22,40	5,3
P. E.	1,05	12,0	56,0	62,6	5,2
K. T.	0,70	9,0	7,0	28,0	2,1
S. J.	1,14	4,3	34,0	—	—
R. I.	1,17	2,7	27,0	—	—

The table shows that on the first day of the diet the HA excretion did not reach in general a high value. The maximum was usually reached on the second day of the diet, in some cases on the first day after stopping the diet. This circumstance, which obviously is explained by the slow digestion of the vegetable protein, is well suited to our purpose, for thus the HA level can be kept uniformly high through diet. If the diet was continued for more than two days the amount of HA excreted no longer increased after the third day, but with slight fluctuations remained at the same level.

We tried to determine how the vegetable protein given in one day influenced the HA content of the urine by a diet differing from the above in that morning, noon and evening equal but greater amounts of oat-flake were given, leaving the other restrictions of the diet unchanged (poor in animal protein and fat). The urine was collected accordingly, in theree samles.

Hippuric acid excretion. (g)

Name	I.	II.	III.
R. I.	1,5	6,9	20,8
A. J.	5,0	7,2	21,6
K. J.	5,67	8,29	42,1
K. E. I.	5,24	7,86	10,68
II.	12,50	28,0	50,0
P. E. I.	10,0	7,5	13,45
II.	25,57	19,0	40,0

20,000 units penicillin (4 cases).
Serum penicillin concentration:

Time	Before diet	After diet
1 hr	0,2 U	0,25 U
3 „	0,04 „	0,11 „
6 „	No penicillin was found	
60,000 Units (9 cases).		
1 hr	0,2 U	0,3 U
3 „	0,03 „	0,12 „
5 „	—	0,08 „
6 „	—	0,04 „
100,000 units (4 cases).		
1 hr	0,48 U	0,58 U
3 „	0,11 „	0,35 „
6 „	—	0,15 „
120,000 units (3 cases).		
1 hr	0,56 U	0,65 U
3 „	0,15 „	0,20 „
5 „	—	0,12 „

The second experiment on K. E. and P. E. was carried out the day immediately following the previous experiment, so that the repetition of the phenomenon to be seen in Table I. can here be remarked, namely that on the second day of the diet the excretion increases markedly. For determining the serum-level of the penicillin we chose *Heatley's* method, with the modification that the substance to be examined was not put into porcellain cups placed on an agar plate, but into holes made in the agar itself. We poured 13 ml 2% agar into a Petri dish of 9 cm diameter and after a 24 hour sterilization test spread 0,2 ml of a 16 hrl. bouillon culture of staphylococcus pyog. aureus Oxford strain on the discs with a glass rod. Then we prepared holes 5 mm in diameter and into them put an average amount of 0,05 ml serum with a sterile Hagedorn pipette.

We first investigated the bactericide action with „normal" sterile serum containing known but differing amounts of penicillin, and drew the curve of the values thus obtained. After this the method described

by *Heatley*, of examining the different dilutions of the substance investigated was unnecessary, as by reading the inhibition zone-values on the curve we could get the height of the serum-level in one determination. It is essential for the substance in the holes prepared in the agar plates to be exactly measured.

The penicillin was always given i. m. in different concentrations. We investigated the serum-level in the same patient without diet, then after a 2-day diet of oat-flake, and found that the urine attains on the second day adequately high HA value.*

After abandoning the diet the serum levels returned to their original values. Since our tables show that the high HA content of the urine had no marked effect on the rise in serum-level, this means a drawback as compared with a continuous infusion. On the contrary, comparison of the 3, 5 and 6 hour figures proves conclusively that a significant decrease in penicillin excretion can be atteined through the diet. If we consider the standpoints of confortability and economy, the 4-times dose of 60,000 units seems the most suitable for, since though with a 100,000 dose a consideraby higher serum-level can be reached, it speaks against it that a 66% increase in the dose is necessary for this purpose. There is no significant difference between the effects of doses of 100,000 and 120,000 units. Although with advances in the technic of penicillin manufacture the appearance can be counted on of a preparation, like zinc protamin insulin, which assures a higher level in a single dose, until that time we recommend the diet and method of dosing mentioned in the treatment of patients requiring large amounts of penicillin.

Experiments made with anaesthesin show that 10 g taken once in the morning or at intervals three times a day are eliminated the same day.

Name	Excretion within 24 hours
J. E.	6,47
F. E.	4,57
V. M.	7,68
J. A.	8,0
K. J.	8,0
R. J.	4,48
Sz. A.	12,04
K. J.	7,4

As this proved that a protracted effect could not be attained with anaesthesin and as its disagreable incidental effects make its continued use difficult, further experiments with it were abandoned.

* See : Table on p. 5.

We got the same results on giving Na. benzoicum. We found, agreeing with *Quick's* data, that most of it left the organism in 4 hours, and, no matter what the circumstances, always the day taken. In respect to the calculations it must be remarked that, according to the greater molecular weight, the HA value obtained by titration must be multiplied by a factor of 0,68 to get the value of sodium benzoate.

It appears from our Tables that in many cases the excreted HA surpasses the amount to be expected from the protein content of the diet. This also occurred in one single case in the anaesthesin experiments, but this exception might have been due to some unknown factor in the diet. In the diet experiments, however, this phenomenon occurred in large numbers. We imagine the explanation to be that the synthetic process set in action by the hippuric acid enzyme exceeds the vegetable protein existing and the always more abundant glycocoll, and even the break-down products of penicillin, cystein and serin, form HA with the benzoic acid deriving from the destruction of animal proteins. *Kohlstaedt* and *Helmer's* findings show that this can occur in other circumstances too; in their opinion "endogenous" HA excretion depends to a great extent on the prevailing carbamide level.

It consequently seemed necessary to observe whether the great degree of penicillin-retention in kidney diseases described by *Fleming* was not parallel with the level of the non-protein nitrogen, and whether it did not depend upon the possible increase in amount of endogenous HA.

After 20,000 units penicillin i, m,

Name	Diagnosis	Non-protein Nitrogen mg %	Hippuric acid g	Serum-level after 3 hrs.
P. J.	Nephritis chr.	155	4,76	0,06
B. J.	,, ,,	90	trace	—
T. S.	,, ,,	66	11,0	0,4
K. J.	Nephrosis	28	5,81	—

As we see, the urine of two nephritics and of a patient with nephrosis, abundantly nourished on different proteins, contains a great amount of HA. In spite of this, in three hours penicillin was not to be found in the serum of the patient with pure nephrosis. It disappeared likewise from the serum of the third nephritic, in whose urine HA was scarcely demonstrable. It follows that the nephritic kidney retains penicillin only if the hippuric acid synthesis increases at the same time. Penicillin retention does not occur in nephrosis. Naturally, the number of cases we had is too small for drawing any final conclusions.

SUMMARY.

On a diet containing vegetable proteins, principally oat-flake, prepared in an easily digestible, appetising fom, we succeeded in raising the urine HA level uniformly and to a great extent. Through the use of this diet the decrease of the penicillin-level of the serum could be inhibited so far that we could show an active bac bacteriostatic concentration 6 hours after the injection.

In delaying penicillin-excretion in kidney patients it seems that the extent of endogenous hippuric acid synthesis also plays a part.

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THE FACTOR IN PERNICIOUS SERUM WHICH INHIBITS THE MATURATION OF RED BLOOD CELLS.

BY ISTVÁN RUSZNYÁK, SIMON LÖWINGER AND LÁSZLÓ LAJTHA.

FROM THE I. MEDICAL CLINIC OF THE PÉTER PÁZMÁNY UNIVERSITY IN BUDAPEST (DIRECTOR, PROF. ISTVÁN RUSZNYÁK).

In our last work, two of us demonstrated (1) that on peroral administration of synthetic folic acid the megaloblasts of bone marrow in a very short time became macroblasts, and these latter normoblasts. In the course of our investigations the possibility arose that folic acid was identical with the hypothetical extrinsic factor. Apparently supporting this was the idea that folic acid — in respect to its structural form too — could fill the role of co-enzyme which got into the organism with the food, like cocarboxylase, lactoflavin, nicotinic acid amide, etc. It binds itself to the protein in the organism and forms an effective holoenzyme. Also supporting our hypothesis is the fact that in folic acid molecules produced from yeast and liver there are varying amounts of glutaminic acid. The glutaminic acid would be that part of the molecule by which the co-enzyme bound itself to the protein and the amount of glutaminic acid contained would depend on the greater or less perfection of the hydrolysis. It seemed probable that natural folic acid occurs bound to protein and just needs the gastric juice in order to separate the active prosthetic group from the foreign proteins, and thus enable it to bind itself to the organism's own proteins and create efficacious holoenzymes. The anti-anaemic factor would be identical with this holoenzyme.

Speaking against this hypothesis were the significant physical and chemical differences between crystallized folic acid and the anti-anaemic factor, which made it unlikely that the protein molecule would be the cause of the difference between the two substances. There are other serious biological differences between the two materials, the most significant being that folic acid is active in chickens, while the liver factor is inactive.

As opposed to our first hypothesis, which for the reasons above stated it would have been difficult to maintain, the possibility of another explanation offered itself. It is known (1) that folic acid is not merely a growth factor of streptococcus lactis and lactobacillus casei, but that

almost every microorganism has need of it. They either synthetise it themselves or must acquire it from outside. Folic is acid therefore an important biotic factor, which can also affect the immature cells of the bone marrow.

The question can be determined only by in vitro experiment, and we therefore decided to administer crystallized folic acid to cultures of megaloblastic bone marrow and investigate the development of the cells.

We wanted to carry out our experiments on bone marrow cultivated in a liquid medium, as on a solid medium a great many of the pluripotent cells of bone marrow change into fibroblasts, histiocytes and connective tissue cells, making a qualitative evaluation impossible: neither can the quantitative conditions (amount of folic acid to be given, number of cells) be exactly established. The culture medium recommended by *Osgood* (2) seemed the most suitable for our purposes. *Osgood* demonstrated that the *Gey* solution (3) which is exceptionally suitable for the culture of normal human and of tumour cells is also more advantageous for bone marrow cultivation than Tyrode solution. We used two sorts of solutions, one citrated, the other without citrate.

As *Osgood's* method had never been controlled, we carried out preliminary experiments, the results of which confirmed the author's statement that a medium which contains 35% serum from human placenta blood and 65% Gey solution is particularly suitable for bone marrow culture.

Somewhat modifying *Osgood's* method to suit our purposes and possibilities, our experiments were carried out in the following way:

1. We made a regular sternum puncture in a patient with pernicious anemia. With a sterilised 20 ml syringe, 4 ml bone marrow was rapidly withdrawn.

2. This substance was very quickly injected into two previously prepared sterile bottles (we used empty, carefully cleaned and sterilized "neutraglas" penicillin bottles), in each of which was 18 ml sterile citrated Gey solution, so that there was 2 ml bone marrow in each. The suspension was immediately thoroughly shaken. With the bone marrow remaining in the syringe we prepared the so-called "beginning smears".

3. We centrifuged the citrated bone marrow suspension for 15 minutes at 1500 revs/min.

4. In the meantime we prepared the vessels necessary for the experiment: sterile bottles (see above), into which we put 3,5 ml sterile serum from placenta veins, and 6,5 ml sterile uncitrated Gey solution. At the beginning of the experiment we prepared 3 experimental bottles, in one folic acid, in one campolon mixed with the above culture medium, while

one of the bottles served as control. We put into the bottles 0,1 ml of a 3 mg% fresh sterile solution of folic acid, which corresponds to about a 200 mg dose for a 70 kg man, calculated to 1 g bone marrow; of the campolon 0,2 ml of a 1 : 1000 diluted sterile solution, which in the conditions mentioned above corresponds to 10 ml campolon in man.

5. Carefully taking from the centrifuge the bottles containing the centrifuged citrated bone marrow suspension, the citrated Gey solution was drawn off to the last possible drop in sterile conditions, while with 10 ml uncitrated sterile Gey solution from the centrifuge we prepared a new suspension. 1 ml of the suspension was then injected into the experimental bottles. The bone marrow was then in 11 ml of fluid. The small amount and the very dilute folic acid (or campolon) changed neither the dilution nor the pH conditions essentially. In our first experiments we prepared two parallels from each bottle. Later, as the results coincided perfectly, this proved superfluous.

6. Our bottles were put into a thermostat at 37⁰ and after 48 hours, shaking the bottles well, we took up sterilely 1 ml suspension, centrifuged it and prepared a smear from the precipitate. The bottles containing the media were again centrifuged (1500 revs, 15 minutes) and the medium changed under very carefully sterile conditions.

7. The smears were stained according to *May—Grünwald—Giemsa*, thoroughly examined, after which we calculated the percentual relation of the different corpuscular elements. At first we counted all the nucleated elements together, that is, we worked out a complete qualitative picture from 500 cells (Cases 1—4); later it seemed indicated to take the nucleated red blood cells separately, as the mature white blood cells died very rapidly in the culture. (This phenomenon has also been observed by other investigators.) We thus calculated 200 nucleated red blood cells.

Osgood maintains that with his method he could keep cells of human explantates alive for 50 days. We did not need so long a time, as we had previously demonstrated (1) that the effect of folic acid comes into force in bone marrow after 24—48 hours. With the method described, in our first experiment we found living cultures after 72 hours (Case 1.).

DETAILS OF THE EXPERIMENTS.

In Case 2. we transplanted the bone marrow of a syphilitic anaemic patient. At the outset there were no megaloblasts in the bone marrow, the red blood cell formation shifted to the left. Results of the 48 hour culture: in the control bottle, 0,5% macroblasts, 23% young normoblasts, and 15% mature normoblasts. In the bottle containing campolon were irrecognisable degenerated cells. Hence the phenol used for conservation is poisonous

to the cells, even in such a very dilute state. The folic acid bottle: 3% macroblasts, 12% young normoblasts and 32,5% mature normoblasts. By doubling the number of mature normoblasts the folic acid undoubtedly increased the maturation of the red blood cells (Table I.).

TABLE I.

Type of cells	No. 2.			No. 3			No. 4		
	Begin.	48 hr		Begin.	48 hr		Begin.	48 hr	
		Contr.	Fol. acid		Contr.	Folic		Contr.	Folic
Megaloblast	not	—	—	50,2	15,5	4,0	23,5	12,5	4,5
Macroblast......	pern	0,5	3,0	3,6	5,5	8,0	5,5	3,0	7,0
Young normo. ..		23,0	12,0	11,4	4,5	16,0	0,5	10,0	12,0
Mature normo. .		15,0	32,5	1,4	59,0	51,0	0,5	30,5	29,0

Type of cells	No. 5.			No. 6.			No. 7.		
	Begin.	48 hr		Begin.	48 hr		Begin.	48 hour	
		Contr.	Folic		Contr.	Folic		Contr.	Folic
Megaloblast	41,5	9,5	5,0	79,0	12,0	1,0	37,5	16,5	8,5
Macroblast......	20,5	23,5	16,0	5,5	15,0	4,0	30,5	17,5	19,5
Young normo. ..	21,5	34,5	30,0	9,0	44,0	17,0	21,0	29,0	15,5
Mature normo. .	16,5	32,5	49,0	6,5	29,0	78,0	11,0	37,0	57,0

In Case 3. we used pernicious bone marrow. Starting value: 50,2% megaloblasts, 3,6% macroblasts, 11,4% young normoblasts, and only 1,4% mature normoblasts. In a 48 hour culture the results were modified as follows: in the control bottle 15,5% megaloblasts, 5,5% macroblasts, 4,5% young and 59% mature normoblasts. In the bottle containing campolon, again degenerated cells. In the folic acid bottle only 4% megaloblasts, 8% macroblasts, 16% young and 51% mature normoblasts. Here the effect of the folic acid in maturing the megaloblasts is already avident.

In our 4th case (pernicious bone marrow) there were at the start 23,5% megaloblasts, 5,5% macroblasts, 0,5% young and likewise 0,5% mature normoblasts. After 48 hours there were, in the control bottle, 12,5% megaloblasts, 3% macroblasts, 10% young and 30,5% mature normoblasts. In the folic acid bottle 4,5% megaloblasts, 7% macroblasts, 12% young and 29% mature normoblasts. The effect of the folic acid in maturing megaloblasts is evident here also.

In Case 5. (pernicious bone marrow) the proportion of nucleated red blood cells in the beginning was: 41,5% megaloblasts, 20,5% macroblasts, 21,5% young normoblasts and 16,5% mature normoblasts. In the 48 hour culture there were, in the control 9,5% megaloblasts, 23,5% macroblasts, 34,5% young and 32,5% mature normoblasts. In the folic acid bottle were 5,0% megaloblasts, 16% macroblasts, 30% young and

49% mature normoblasts. The cell-maturing effect of folic acid is again apparent.

In Case 6. (pernicious bone marrow) the proportion of nucleated red blood cells at the start was: 79% megaloblasts, 5,5% macroblasts, 9% young and 6,5% mature normoblasts. In the 48 hour culture, in the control were 12% megaloblasts, 15% macroblasts, 44% young and 29% mature normoblasts. In the folic acid bottle the maturing effect was striking: 1% megaloblasts, 4% macroblasts, 17% young and 78% mature normoblasts.

In Case 7. (pernicious bone marrow) the proportion of nucleated red blood cells at the outset was: 37,5% megaloblasts, 30,5% macroblasts, 21% young and 11% mature normoblasts. In the control bottle after 48 hours: 16,5% megaloblasts, 17,5% macroblasts, 29% young and 37% mature normoblasts. The cell-maturing action was again evident in the folic acid bottle: 8,5% megaloblasts, 19% macroblasts, 15,5% young and 57% mature normoblasts.

We thus succeeded in demonstrating that crystallized folic acid acts on megaloblastic bone marrow in a direct, cell-maturing way. In the course of our experiments it appeared, however, that the maturation of the megaloblasts was brought about not only by the folic acid culture, but also — though not to the same extent — on the control culture medium. There was *placenta serum* in the culture medium according to the prescription, and it might therefore have been thought that the unknown maturing substances in it influenced the experiment. Thus in Case 3. at the beginning there were 50,2% megaloblasts in the bone marrow: as effect of the folic acid their number diminished to 4%, but in the medium without folic acid it also fell to 15,5%.

Beginning with Case 4., therefore, we used ***healthy adult serum***, to eliminiate the disturbing effect of embryonic materials in placental serum stimulating hypothetical maturation. But the result was similar (Table II.); in the control bottles the megaloblasts' "value" was unchanged.

TABLE II.

Case No.	Beginning value	48 hour culture Control	Remarks
3	50,2	15,5	placenta s.
4	23,5	12,5	normal s.
5	41,5	9,0	" "
6	79,0	12,0	" "
7	37,5	16,5	" "
20	60,0	0,0	diabet. s.

Megaloblast %.

This significant decrease in the control bottles was so remarkable that at first we attributed it to a mistake in the method. We thought that a part of the megaloblasts died in our cultures. We tried to check this in two ways. First, we prepared a "Gey control": 11 ml Gey solution was put into the bottles without serum. After 48 hours we prepared the usual smear. But this picture differed essentially from that which we saw in bone marrow preparations cultivated in a serum medium. In the greater part of the nuclearted elements signs of cell dissolution were apparent —the nuclear structure was washed out, in some cells the boundaries of the nucleus — plasma had become indistinct, the nuclei had taken a star shape, we observed many more caryorrhexes than normally, and mitosis could not be found. Thus this picture differed entirely from that seen in the serum culture medium, *this* indicating a decaying bone marrow culture. The other method of control was by following the maturation of the megaloblasts and the other young nuclear red blood cells. We took the increase in number of the reticulocytes as indicators of maturation (in serum culture medium). We determined the number of reticulocytes in the beginning smear and in a 48 hour culture. We found that the number of reticulocytes increased simultaneously with a decrease in the number of megaloblasts. These two proofs we took as sufficient, in view of the fact that the megaloblasts in our cultures did not die off, but matured.

From this we could suppose that the explanted megaloblasts matured in normal serum because the anti-anaemic factor was *not* lacking in it, as, according to the hypothesis, it is lacking in pernicious serum. So, therefore, in our succeeding experiments we further modified *Osgood's* method and prepared a further control. In this bottle *pernicious serum* was present in the 35 vol % prescribed and also used elsewhere. This control was made to eliminate the effect of the anti-anaemic factor.

The results were excessively surprising (Table III a.). Contrary to our expectations, in the bottles containing the pernicious serum too — though to a smaller extent — the number of megaloblasts diminished. If before we said that in normal serum (as effect of an anti-anaemic substance) the megaloblasts matured, we must here also say: the megaloblasts matured significantly, it is true, in comparison with the beginning value, but decidedly less than in normal serum.

The above results raised the question of why the megaloblasts also mature in pernicious serum not containing the anti-anaemic factor. At first we wondered whether it were a local in hibition in the bone marrow of pernicious patients, which disappeared when the bone marrow was taken out of the organism and put into another milieu. In this case, however, the difference between normal and pernicious sera is incom-

prehensible; in both of them the bone marrow ought to mature equally, since, removed from the organism — in the case of a local inhibition — the inhibiting circumstance ceases. On the other hand, on the culture medium containing pernicious sera, the maturation was at least 100%less than that observed on the normal ones. We were therefore obliged to think of something else. We formed the slightly bold hypothesis that the substance which inhibited maturation of the cells must be in the pernicious serum — which we in our experiments had diluted, indeed (according to prescription) we used only a 35 vol % serum. In this way the phenomenon that megaloblast maturation was also to be seen in the bottles ontaining the pernicious serum could be explained (Table III a.).

TABLE III.

Case No.	Begin. value	Normal serum		Pernicious serum		Remarks	Stage of exp.
		Dilute	Conc.	Dilute	Conc.		
4	23,5	3,5		6,5		72 hr cult.	a
5	41,5	9,0		16,5		48 „ „	
6a	79,0	12,0		36,0		48 „ „	
6b	79,0	0,0		16,0		72 „ „	
19	29,0	0,5		2,5		48 „ „	
7a	37,5		16,5		36,5	Beside the same normal serum two separate pern. sera 48 hr cult.	b
7b	37,5		16,5		27,0		
11	14,0	1,0	0,0	0,0	2,0		c
13	32,5	4,5	2,0	9,5	16,5		
14	4,0	1,0			6,0	Remission bone marrow + pern s.	
15	85,0	17,0	10,0				
16	35,0	7,0	5,0	7,5	13,0		
22	76,0			8,0	27,0		
23	2‘5			0,0	3,0	Not pern.	
24a	90,0			3,5	21,0		
27	23,5	1,0	0,5	1,5	4,0		

Maturation of magaloblasts in different sera.
Stage *a:* pernicious serum matures, but badly.
„ *b:* concentrated pernicious serum inhibits maturation.
„ *c:* normal concentrated serum *matures* better than normal dilute.
Pernicious concentrated serum *inhibits* more than pernicious dilute.

Therefore in the next stage of our experiments, to eliminate the consequences of diluting the serum, we prepared the medium so as to contain 65 vol % pernicious serum ("concentrated serum"). We also prepared a "concentrated" parallel from normal serum. These experiments showed that while in concentrated normal serum the megaloblasts decreased from 37% to 16,5%, the number in concentrated pernicious serum

scarcely changed: 36,5% and 27% (Table III b). These experiments seemed to prove our hypothesis.

To confirm it we now prepared the following culture medium: *normal dilute* serum (35 vol % serum + 65 vol % Gey solution), *normal concentrated serum* (65 vol % serum + 35 vol % Gey solution), *pernicious dilute serum* (35 vol % serum + 65 vol % Gey solution), and *pernicious concentrated serum* (65 vol % serum + 35 vol % Gey solution). The results are shown in Column c of Table III. It appears clearly therefrom that the concentrated normal serum *matures* better that the dilute normal serum, while the concentrated pernicious serum *inhibits* more than the dilute. The differences are significant and far beyond the limits of error, because the normal serum matures in rough approximation about 100% better than the dilute normal serum, while the concentrated pernicious serum inhibits in at least the same proportion more vigorously than the dilute. The fact that the normal concentrated serum matures while the pernicious concentrated serum inhibits is important. because it shows that the inhibiting effect is not merely the consequence of the serum's concentration. This part of our experiments at the same time contradicts *Osgood's* assertion that 25—40% serum gives the best results as medium.

The presence of the inhibiting factor is further proved by Cases 22, 23 and 24, where we prepared a "medium" culture, between the dilute and the concentrated, in a combination of 50% pernicious and 50% Gey solution. The results, to be seen in Table IV, show very clearly that as we

TABLE IV.

Serum concentration	Percentual ration of Megaloblasts		
	No. 22.	No. 23.	No. 24b.
Dilute (35 : 65)............	8,0	0,0	7,5
Medium (50 : 50)	15,0	2,5	12,5
Concen. (65 : 35)	27,0	3,0	15,5

48 hr cultures.

increase the concentration of pernicious serum the inhibiting effect increases, i. e., more and more megaloblasts remain in the cultures. We must mention Case 23, which was not pernicious. In this bone marrow the shift to the left did not extend to the megaloblasts, but only to very young macroblasts. It is worthy of note that the inhibiting effect acted on these cells too.

Case 14 must also be mentioned separately, a pernicious case in spontaneous remission, with a beginning value of 4% megaloblasts, which in a 48 hour culture of concentrated pernicious serum *increased* to 6%.

Hence in vitro the maturation of the megaloblasts stops as consequence of the inhibiting factor (Table III c).

In qualitative investigation of cultures of pernicious bone marrow it could be established beyond question that *only a small part of the megaloblasts mature directly into megalocytes.* The maturation takes place principally in the form of pyknosis and karyorrhexis. The shrunken or disintegrated nucleus is ejected from the plasma. In most of them, however, this process cannot be observed, but these cells develop further into macroblasts, resp. normoblasts. *Hence most of the megaloblasts mature gradually into red blood cells.* With this our experiments prove one (4) of our earlier suppositions, that the original Ehrlich-Naegeli theory, according to which only megalocytes are formed from megaloblasts, must be revised.

We felt it necessary to complete our experiments by investigating the cellmaturing effect of folic acid in pernicious serum too. The results are shown in Table V. From it will be seen that folic acid has a cell-

TABLE V.

Effect of folic acid in pernicious serum.

	No. 5.		No. 6.		No. 7a.		No. 7b.		No. 27.	
	pern. serum	pern. s. + fol. a.	pern. serum	pern. s. + fol. a.	pern. serum	pern. s. + fol a.	pern. serum	pern. s. + fol. a.	pern. serum	pern. s. + fol. a.
Megaloblast........	16,5	12,0	36,0	13,0	36,5	30,5	27,0	27,0	4,0	0‘0
Macroblast	21,0	24,0	19,0	22,0	7,0	13,0	19,0	9,0	20,0	8,5
Young normob. ...	35,0	26,0	15,0	22,0	21,0	7,0	42,0	21,5	48,5	28,5
Mature normob. ...	26,5	37,5	30,0	43,0	35,5	49,5	18,0	40,5	27,5	60,5

Nos. 5 and 6 dilute sera. Nos. 7a, 7b and 27 concentrated sera.
48 hour cultures.

maturing effect even in concentrated pernicious serum. On the dilute pernicious medium (less inhibiting factor) the number of megaloblasts diminishes more, actually, than on the concentrated pernicious medium (greater inhibiting factor), but the shift to the right is everywhere pronounced.

We have mentioned that we could not investigate the effect of liver in vitro with Campolon. We therefore attempted it with Pernaemon forte in which there is a less toxic conserving material (butyl alcohol). This preparation proved suitable, 0,1 ml of a 1 : 1000 dilution being in no way harmful to our cultures. Our results contradicted *Overbeck* and *Gaillard's* statement that liver has no effect on bone marrow explantates. In our experiments the effect of the liver was to diminish the number of megaloblasts, and in the nuclear red blood cells in every case a shift to the right took place (Table VI.).

TABLE VI.

Effect of liver in pernicious serum.

	No. 7a.		No. 7b.		No. 8.	
	Pernicious serum	pern. s. + liver	pern. serum	pern. s. + liver	pern. serum	pern. s. + liver
Megaloblast	36,5	15,5	27,0	15,5	7,0	1,0
Macroblast	7,0	8,5	13,0	15,5	18,0	27,0
Young normob....	21,0	21,5	42,0	28,5	25,0	20,0
Mature normob. .	35,5	44,5	18,0	40,5	50,0	52,0

48 hour cultures.
Concentrated sera.

We consider Case 15 very significant (Table VII.). Here as pernicious serum we used the serum of Patient No. 14, who — as mentioned above — was in spontaneous remission. Strangely enough, this serum *in its concentrated form* behaved differently from other concentrated pernicious sera, for compared with its diluted form — exactly like normal sera — *it had a maturing effect.*

TABLE VII.

Remission serum.

	Beginning smear	Normal serum		„Pernicious serum"	
		Dilute	Concen.	Dilute	Concen.
Megaloblast...............	85,0	17,0	10,0	31,0	26,0
Macroblast	9,0	17,0	14,0	23,5	22,5
Young normob.	5,0	10,0	10,0	14,0	17,0
Mature normob.	1,0	56,0	66,0	29,0	35,0

DISCUSSION.

From our experimental results it follows that there is a factor in pernicious serum which inhibits the maturation of red blood cells. On this basis the pathogenesis of pernicious anaemia can be imagined in two ways:

1. We might suppose that the increase of a *substance* (toxin?) *inhibiting* the maturation of red blood cells plays a role in creating pernicious anaemia. This is also proved by the circumstance that in pernicious serum cell-maturation is impeded. (Not only that fewer megaloblasts mature than in normal serum, but also that with concentration of the serum the inhibiting effect increases.)

2. We can, however, also suppose that the cause of the pernicious anaemia is not the increase in some inhibiting material, but exclusively the lack, or decrease, of the anti-anaemic substance — as has generally

been accepted. In this case, according to our experimental results, we must suppose that *every serum* contains substances (physiologically too) which in vitro inhibit red blood serum maturation. In non-pernicious serum this inhibiting substance is counter-balanced by the anti-anaemic substance undoubtedly present in the serum. As, according to general opinion, the anti-anaemic factor is lacking in pernicious serum, because of the preponderance of the inhibiting materials, the prevention of red blood cell maturation gets the upper hand. There must generally be substances in the serum injurious to cell-function, as the serum conveys to the kidneys those poisonous substances which it excretes, and the increase of which cause well-known intoxications in cases of deranged kidney function.

Our remission case (No. 15) can accordingly also be explained in two ways:

We believe either that the amount of the hypothetical pernicious "inhibiting substance" diminishes from some cause or other, permitting the action of the anti-anaemic factor in the serum (it, accordingly, therefore is present in pernicious serum as a physiological red blood cell maturing factor).

Or we suppose that the patient again produced enough of the anti-anaemic factor to counter-balance the physiologically poisonous metabolic products.

In the final analysis the present experiments do not entitle us to take a stand beside any of the possibilites. Further experiments will be required for solution of the question.

SUMMARY.

1. In cultures of bone marrow folic acid has a direct cell-maturing effect, acting as a biotic.

2. On a medium containing placenta serum pernicious bone marrow explantate shows spontaneous maturation of red blood cells.

3. Serum of healthy adult humans has the same effect.

4. The serum of pernicious patients contains a factor inhibiting the maturation of red blood cells, the effect of which depends on the concentration.

5. Most of the megaloblasts do not form megalocytes, but develop further into macro- or normoblasts respectively.

6. The direct effect of folic acid is also observable in pernicious serum.

7. Direct action of liver is also found in vitro in pernicious serum.

8. The serum of a patient in remission behaves nearly like normal serum.

9. We suggest two possibilities as to the pathogenesis of pernicious anaemia:

a) We suppose either the presence of a substance (toxin?) which prevents the maturation of red blood cells, with an insufficient amount of anti-anaemic factor to counter-balance it,

b) or, in consequence of a decrease in the anti-anaemic factor present in *every* serum, the inhibiting effect of metabolic products poisonous to cell activity gets predominating control.

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DIE PHOSPHORYLIERUNG UND DER BLUTHISTAMIN-SPIEGEL BEI FLECKFIEBER.

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Nach dem heutigen Stande der medizinischen Wissenschaft wird das Fleckfieber durch die Rikettsia Prowazeki verursacht. Mehr darüber, namentlich über die durch den Krankheitserreger verursachten Grundveränderungen steht uns kaum zur Kenntis. Es stehen uns aber die allgemein bekannten klinischen Symptome und die pathologischen anatomischen Befunde zur Verfügung und von diesen ausgehend versuchten wir nach gewissen Grundveränderungen zu forschen.

Die für das Fleckfieber charakteristische, leicht verhängnissvoll werdende Blutdrucksenkung wird in allen Lehrbüchern hervorgehoben, die dieselbe auf Grund der übrigen dürftigen pathologischen anatomischen Befunde zu erklären versuchen. Histologisch ist nämlich im ganzen Gefässsystem eine Thrombovasculitis zu beobachten, die sich auch in der grauen Substanz des Hirnstammes auffinden lässt. Ein grosser Teil der Verfasser ist geneigt, in Analogie mit anderen, in dem klinischen Bild auftretenden und auf die Läsion des zentralen Nervensystems hinweisenden Symptome, auch die Blutdrucksenkung darauf zurückzuführen. Während aber die Erscheinungen des zentralen Nervensystems völlig fehlen, oder den schwersten Grad erreichen und in ein und demselben Falle während der Krankheit sehr abwechslungsreich sein können, zeigt sich die Blutdrucksenkung in jedem Falle sozusagen als ständiges Symptom, das während der ganzen Krankheit, vom Frühstadium an bis zu Ende zu bestehen pflegt und einen regelmässigen Ablauf zeigt. Den peripherischen Charakter der Blutdrucksenkung berücksichtigend, versuchten wir auf Grund theoretischer Überlegungen nach der Anwesenheit eines solchen Stoffes zu forschen, der imstande ist eine Blutdrucksenkung hervorzurufen. In erster Linie kam als solcher das Histamin in

Frage, da die Erscheinung am meisten der Histaminwirkung entspricht und dessen Entstehung in den Geweben unter pathologischen Verhältnissen sich am meisten erklären lässt.

Die Versuche haben wir möglichst im Frühstadium der Krankheit begonnen, abhängig davon, wie früh die Kranken in die Klinik eingeliefert wurden. Im allgemeinen lässt sich sagen, dass die Patienten kurz vor dem Erscheinen oder eher noch zur Zeit des bereits bestehenden Exanthems in unsere Beobachtung kamen.

Die Untersuchungen haben wir bei 15 Patienten und zwar bei fast sämtlichen auch wiederholt durchgeführt. Die Ergebnisse waren im Wesentlichen völlig übereinstimmend. Von unseren Fällen haben wir einige ausführlich beschrieben, die endgültigen Feststellungen entnahmen wir aus den Gesamtresultaten.

Zur Bestimmung des Histamingehaltes des Blutes wurde das Blut aus der Armvene entnommen, wobei die Zeitdauer der minimalen Kompression aufs Notwendigste herabgesetzt wurde, um dadurch der Wahrheit entsprechende Resultate erzielen zu können. Von 10 ccm Blut haben wir nach dem von *Barsum u. Gaddum* beschriebenen und von *Code* modifizierten Verfahren mit Hilfe eines selbstkonstruierten automatischen Extraktionsapparates zunächst eine salzsäurige, dann eine alkoholische Extraktion gemacht, und das von den blutdruckaktiven Mitteln allein Histamin enthaltende Extrakt wurde in verschlossenen Ampullen im Eisschrank solange aufbewahrt, bis die Menge der Praeparate genügte, um ihren Histamingehalt mit der biologischen Methode zu prüfen. Die biologische Titrierung wurde entweder an Katzen oder am überlebenden Jejunumteil von Meerschweinchen durchgeführt. In die Einzelheiten der Methodik wollen wir uns bei dieser Gelegenheit nicht einlassen.

1. *Fall* (Abb. 1.). *H. Gy.* 36 j. Mann wurde am 5. VI. 1944 mit Fleckfieber in die Klinik aufgenommen. Die Krankheit nahm ihren Anfang etwa 8—10 Tage vor der Aufnahme. Bei der Untersuchung lassen sich lediglich die allgemeinen, bekannten Fleckfiebersymptome beobachten. Zwecks Charakterisierung des vorgerückten Stadiums und der Schwere der Krankheit sei folgendes hervorgehoben: ausserordentlich verfallener Zustand, gut entwickelte Exantheme, gestörtes Sensorium. Am 5. Tag tritt eine mässige Nackenstarrheit auf, es zeigt sich eine allgemeine Hypersensibilität und Hyperaesthesie, die Störung des Sensoriums erreicht immer höheren Grad. Harn- und Stuhlinkontinenz. Vom 10. Tag an stellt sich eine auffallende Besserung ein, vom 14. Tag an klärt sich auch das Sensorium auf.

Körpertemperatur am Tag der Aufnahme 39,8° C, die Temperaturkurve weist auf den übergang zwischen Continua und Lysis, die

einzelnen Phasen lassen sich aus der Kurve gut erblicken. Puls 100—120 pro Min., im allgemeinen der Temperaturkurve entsprechend.

Die serologischen Untersuchungsresultate am Tage der Aufnahme: *Weil—Felix*'sche und *Bien—Sonntag*'sche Reaktion bis 1 : 1600 positiv, *Gruber—Widal*'sche Reaktion gegenüber Typhus abd., Paratyphus A, B, C, sowie *Bang*—Antigen negativ. Harnbefund: auf Sulfosalicylsäure Opaleszenz, Urobilinogen ++; diese Erscheinungen verschwinden in den ersten zehn Tagen. Haematologischer Befund: rote Blutkörperchen 4,400.000, Haemoglobin 87%, Färbeindex: 1. Leukozytenzahl 13200, qualitatives Blutbild: Jugendf. 3%, Stabkernige: 11%, Segmentierte 65%, Eosinophile 0%, Basophile 0%, Monozyten 1%, Lymphozyten 20%. Restnitrogen 90 mg%, Serumchlor 336 mg%, Serum-Bilirubin 3,43 mg% (direkt), Serumgesamtcholesterin 145 mg%, Estercholesterin 58 mg%, *Takata—Ara*'sche Reaktion negativ, Refraktion 1,35037 und der entsprechende Eiweissgehalt: 8%. Der Blutdruck zeigt bei der Aufnahme einen niedrigen Wert (85/60 mm Hg RR.). Kennzeichnend ist, dass in erster Linie der systolische Druck bedeutend abnahm, wodurch das Blutdruckamplitudo auffallend eingeengt wurde. Während der ersten Behandlungswoche weist der Blutdruck nur eine sehr mässige Steigerung auf, das Amplitudo bleibt im Wesentlichen unverändert. In der zweiten Woche kommt es dann infolge eines steilen Anstieges des systolischen Druckes und einer gleichzeitigen geringen Abnahme des diastolischen Druckes zu einer plötzlichen, sogar über das Normale hinaus reichenden Erweiterung des Amplitudo. Während der folgenden Tage und Wochen erreicht dann diese zusammengesetzte Blutdruck reaction nach kleineren oder grösseren Schwankungen wieder die physiologischen Werte.

Gleichzeitig mit diesen Beobachtungen wurde auf die bereits geschilderte Weise der Histaminspiegel des Blutes bestimmt. Der auf biologischem Wege bestimmte Histamingehalt wurde in Gamma % ausgedrückt. Davon ausgehend, dass der normale Histamingehalt unter physiologischen Verhältnissen 3—5, im Mittelwert 4 Gamma % beträgt, haben wir die Differenz in Prozenten ausgedrückt und diese Werte neben den anderen graphisch dargestellt.

Für die erste Bestimmung wurde das Blut am 4. Tage nach der Aufnahme des Patienten entnommen, das einen Histamingehalt von 155 Gamma % aufwies, was in Vergleich zur Norm eine 3775%-ige Zunahme bedeutet. Am 8. Tage beträgt der Histamingehalt des Blutes 35 Gamma %, d. h. + 775%. Zu dieser Zeit zeigt der Blutdruck bereits die erwähnte steile systolische Steigerung und Amplitudoerweiterung. Am 10. Tage ist der Histaminwert auf 35 Gamma %, d. h. + 520% herabgesunken, am 13. Tag zeigt er 15 Gamma %, d. h. + 275%, am 21. Tag sinkt dieser

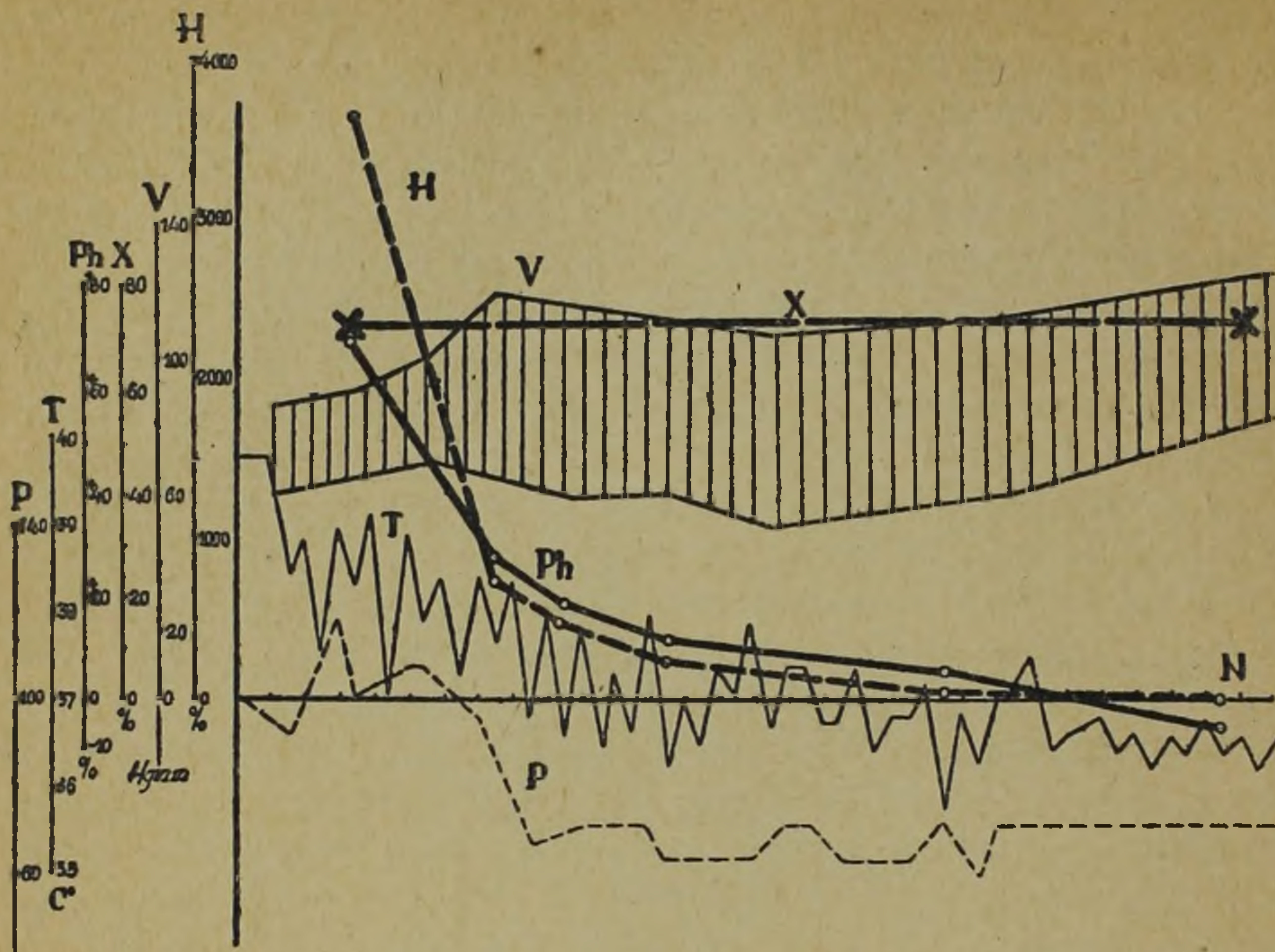

Abb. 1. P = Puls, T = Körpertemperatur, Ph = Phosphorylisierungsausfall (Glucoseretention), X = Xyloseretention, V = Blutdruck, H = Histamingehalt des Blutes, N = Tage.

Wert auf 6,65 Gamma % (+ 66%) und am 29, Tag auf 4,8 Gamma %, also auf die Norm zurück. Bei den biologischen Wertbestimmungen sind die Fehlerquellen naturgemäss grösser als bei den exakten naturwissenschaftlichen Messverfahren, aber mit entsprechender Übung und besonders durch sorgfältige und wiederholte Durchführung der Bestimmungen lassen sich sehr gute Resultate erzielen. In unserem Falle würde dieser Umstand keine wesentliche Rolle spielen, da die Differenzen derart gross sind, dass eine Abweichung von 1—2 Gamma % an den Resultaten nichts ändern würde. Wir haben darum in allen Fällen, wo sich eine Streuung zeigte, auf Kosten der Abweichung die niedrigeren Werte in Betracht gezogen. Bei den niedrigen Werten wurden die Bestimmungen mit erhöhter Sorgfalt durchgeführt.

Sowohl in diesem Falle, als auch in den übrigen, wo wir die Bestimmungen durchgeführt haben, fällt das spiegelbildartige Verhalten der Histamin- und der Blutdruckkurve auf. Der Zusammenhang zwischen den beiden deutet darauf hin, dass in dem Zustandekommen der bei Fleckfieber auftretenden und oft leicht verhängnissvoll werdenden Blutdrucksenkung auch das Histamin eine Rolle spielen dürfte.

In dem intermediären Grundumsatz des Organismus kommt dem Phosphatase-Fermentsystem eine wichtige Rolle zu. Auf Grund theoretischer Überlegungen nahmen wir an, dass im Laufe der Krankheit auch eine Herabsetzung der Phosphorylierung stattfinden kann. Wir haben daher zugleich mit den Histaminbestimmungen unsere Untersuchungen auch auf die Phosphataseaktivitat ausgebreitet. Da uns die Rolle der Phosphorylierung in dem Kohlehydratstoffwechsel wohl bekannt ist und ebenso der Umstand, dass sich eine der sog. selektiven Resorption ähnliche Erscheinung auch bei der Elimination des Zuckers aus der Blutbahn nachweisen lässt (*Minibeck u. Verzár* (1), *Thaddea u. Sarkady* (2)), haben wir nach intravenöser Glykosebelastung (40 mg pro Kg/Körpergew.) nach *Hagedorn—Jensen* den Blutzuckergehalt bestimmt um daraus auf die Aktivität des Phosphatase-Fermentsystems zu folgern. Wegen Platzmangel sind wir gezwungen bei dieser Gelenheit von der Beschreibung der Methodik Abstand zu nehmen.

Bei dem vorhin bereits ausführlich beschriebenen Patienten haben wir die Untersuchung zum erstenmal am 4. Tag nach der Aufnahme vorgenommen. Das Verhältnis zwischen dem Blutzuckerwert in der 2. der Glykosebelastung folgenden Stunde und dem Nüchternwert haben wir in Prozenten ausgedrückt graphisch dargestellt. Die am 4. Tage durchgeführte Bestimmung zeigt eine Zunahme von 68%, also einen beträchtlichen Ausfall der Phosphorylierung. Unter normalen Verhältnissen kehrt nämlich der Blutzuckerwert zwei Stunden nach der Belastung innerhalb einer Grenze von ± 5% auf den Nüchternwert zurück.

Die am 8. Tage wiederholte Untersuchung ergab + 27%, am 10. Tag + 18%, am 13. Tag + 12%, am 21. Tag + 5% und am 29. Tag — 4%. Die ursprünglich bedeutende Verminderung der Phosphatasewirkung hörte also am Ende der 3. Woche völlig auf. Auffallend ist der streng parallele Ablauf der Bluthistaminkurve und der Phosphataseaktivitätskurve, ferner der enge Zusammenhang zwischen ihrer Rückkehr zur Norm und der Normalisierung des Blutdruckes und der Besserung der klinischen Erscheinungen.

Trotz aller theoretischer Beweisführungen und des Vertrauens in die Methodik könnte sich die Frage stellen, ob die beobachtete schlechte Glykoseaufnahmefähigkeit der Gewebe tatsächlich in ihrer herabgesetzten Phosphorylierungstätigkeit ihren Ursprung hat? Zur Klärung der Frage haben wir an dem der ersten und der letzten Glykosebelastungsprobe folgenden Tag die Untersuchung auf ähnliche Weise auch mit einer nicht phosphorylierenden Zuckerart und zwar mit Xylose durchgeführt. Nach Xylosebelastung zeigt am Beginn der Krankheit, am 5. Tage, der 2 Stunden-Wert in Vergleich zu dem Nüchternwert eine Zunahme von

72%. Der am vorigen Tage mit Glykose gewonnene Wert von + 68% dürfte beweisen, dass die Glykose auch bei teilweise stattfindender Hemmung des ihre Aufnahme fördernden Fermentprozesses, wenn auch in geringem Masse, aber noch immer rascher aus dem Kreislauf in die Gewebe übergeht.

Bei der am 30. Tag durchgeführten Xylosebelastung überstieg der Blutzuckerwert nach 2 Stunden den Nüchternwert um 71%, wobei der Wert nach der Glykosebelastung am vorigen Tage — 4% betrug. Durch diesen Versuch liess es sich demnach voll bestätigen, dass die im Laufe der Krankheit gewonnenen Werte die Hemmung der Phosphorylierung treu widerspiegeln.

Da auf Grund der klinischen Symptome dieser Fall zu den schweren Fällen gehörte, haben wir die Ergebnisse unserer experimentellen Untersuchungen zugunsten des Patienten verwertet, in dem wir täglich Rindenhormon (Cortenil—Bayer) und grosse Mengen Vitamin C verabreichten.

Zur Ergänzung sei noch erwähnt, dass in der 2. Behandlungswoche als Komplikation Parotitis, vom 14. Tag an eine linksseitige Mittelohrentzündung mit Schwerhörigkeit und später Hyperthyreose (Grundumzatzwert + 30%) auftraten.

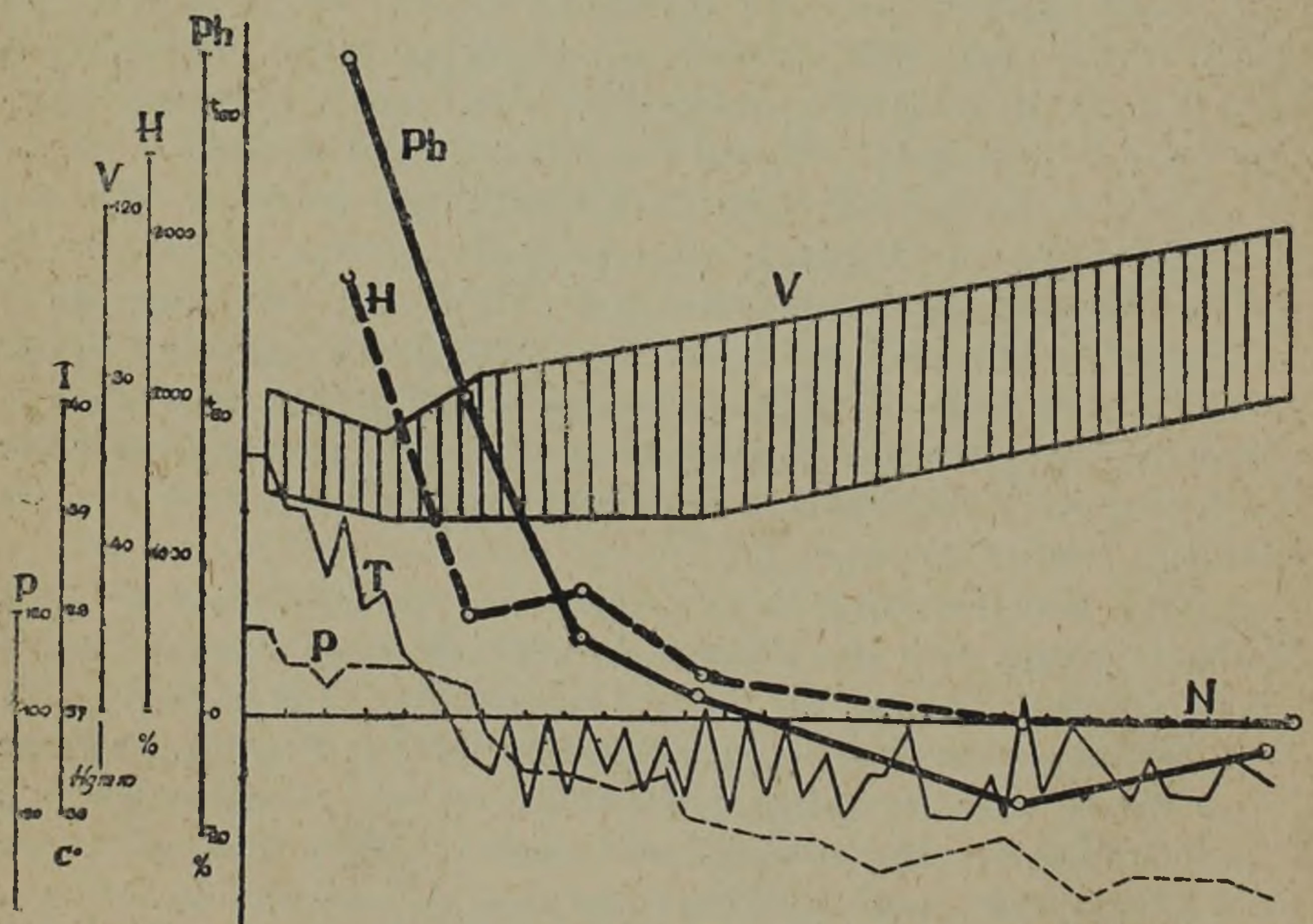

Abb. 2. *P* = Puls, *T* = Körpertemperatur, *V* = Blutdruck, *H* = Histamingehalt des Blutes, *Ph* = Phosphorylisierungsausfall (Glucoseretention), *N* = Tage.

2. *Fall.* (Abb. 2.) *K. A.* 45 j. Mann, ist seit 6 Tagen krank, hat hohes Fieber, Kopf-, Lumbal- und Extremitätschmerzen. Bei der Aufnahme (2. VII. 1945) stark verfallener Zustand, Schwäche, Schwerhörigkeit, gestörtes Sensorium. Die Ausschläge sind gut entwickelt und bleiben noch eine Woche nach der Aufnahme bestehen. Körpertemperatur 39,5° C, die Fieberkurve deutet den Beginn der lytischen Phase an. Pulszahl pro Min. 116, Pulskurve der Temperatur entsprechend. *Weil—Felix*'sche Reaktion in Verdünnung von 1 : 1600 positiv, *Gruber—Vidal*'sche Reaktion auf Typhus abd. und Paratyphus negativ. Blutdruck 78/54 mm Hg RR., am 4. Tage 68/48 mm Hg RR.; von nun an nimmt der systolische Druck stufenweise zu und zugleich damit erweitert sich das Amplitudo.

Der Histamingehalt des Blutes beträgt am 3. Tage 100 Gamma % d. h. in Vergleich zu dem physiologischen Wert + 2400%. Am 6. Tag ergibt die Bestimmung 25 Gamma % (+ 525%), am 9. Tag 20 Gamma % (+ 650%), am 12. Tag 13 Gamma % (+ 222%) und schliesslich vom 20. Tag an 4 Gamma %, d. h. den Normalwert.

Von den Ergebnissen nach der Glykosebelastung sind ebenfalls die 2 Stunden-Werte angeführt: am 3. Tag + 108%, am 6. Tag + 50,7%, am 9. Tag + 12,6%, am 12. Tag + 3,8%, am 20. Tag — 12% und am 26. Tag — 4%, also der anfangs mächtige Ausfall der Phosphorylierung ist in kaum zwei Wochen verschwunden.

3. *Fall.* (Abb. 3.) Frau *J. I.* 43 j. wurde mit mittelschwerem Fleckfieber am 3. VII. 1945 eingeliefert. Die Krankheit besteht seit etwa 7 Tagen. Die Körpertemperatur schwankt zwischen 38,1 und 38,8° C, die Fieber-

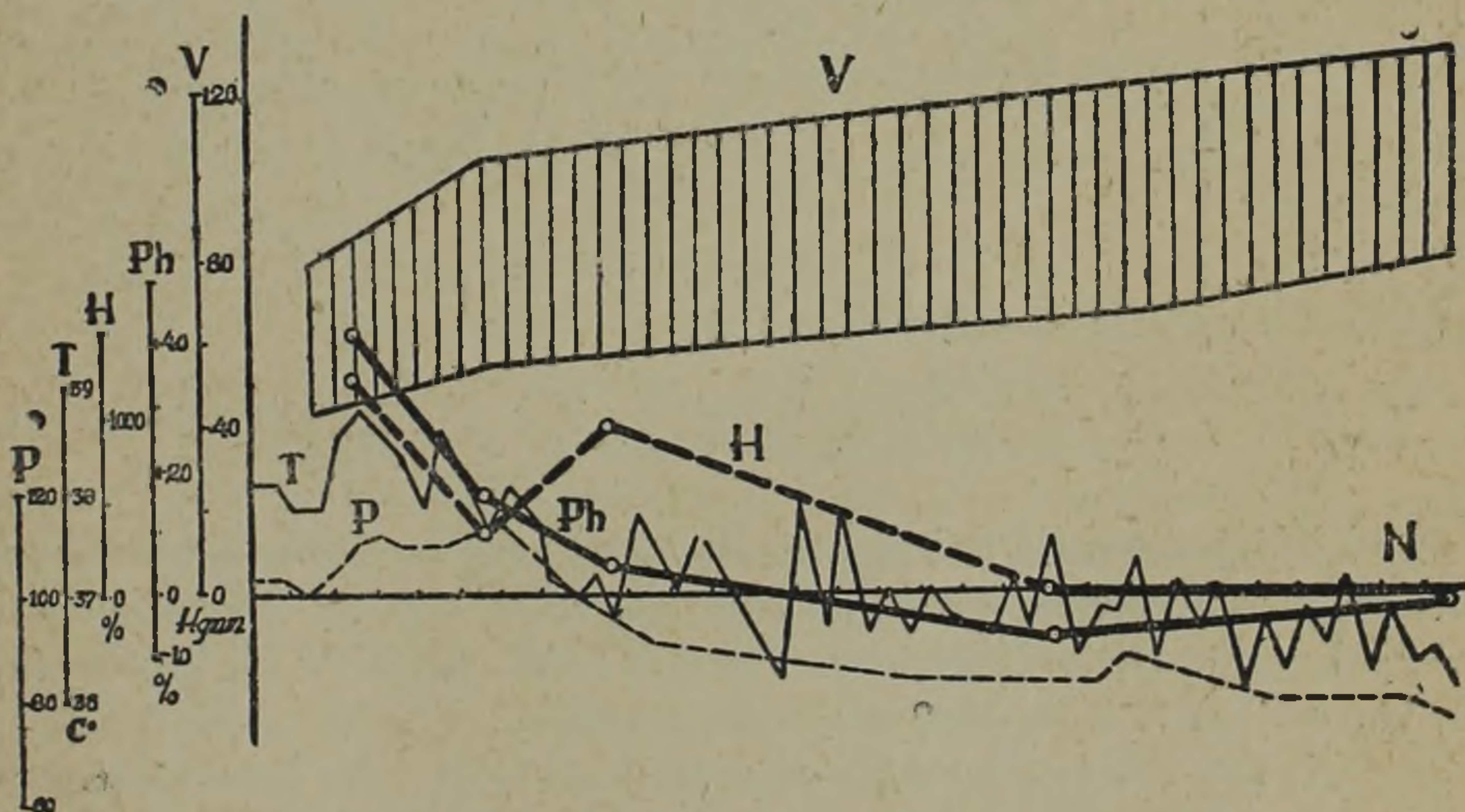

Abb. 3. *P* = Puls, *T* = Körpertemperatur, *H* = Histamingehalt des Blutes, *Ph* = Phosphorylisierungsausfall (Glucoseretention), *V* = Blutdruck, *N* = Tage.

kurve weist auf den Übergang zwischen Continua und Lysis hin. *Weil—Felix*'sche Reaktion in Verdünnung von 1 : 100, nach einer Woche in 1 : 1600 positiv. Die *Gruber—Widal*'sche Reaktion war in beiden Fällen negativ. Blutdruck 80/40 mm Hg RR., der in den nächsten Tagen zugleich mit dem Blutdruckamplitudo zunimmt.

Der Bluthistamingehalt betrug: am 3. Tage nach der Aufnahme 50 Gamma % (+ 1150%), am 6. Tag 18 Gamma % (+ 350%), am 9. Tag 40 Gamma % (+ 900%), am 19. Tag 3,8 Gamma %, erreichte also bereits die physiologischen Grenzen.

Die Resultate nach der Glykosebelastung zeigten folgendes: am 3. Tag + 41,2%, am 6. Tag + 15,4%, am 9. Tag + 5,1%, am 19. Tag — 5,1%, und am 29. Tag — 2,1%.

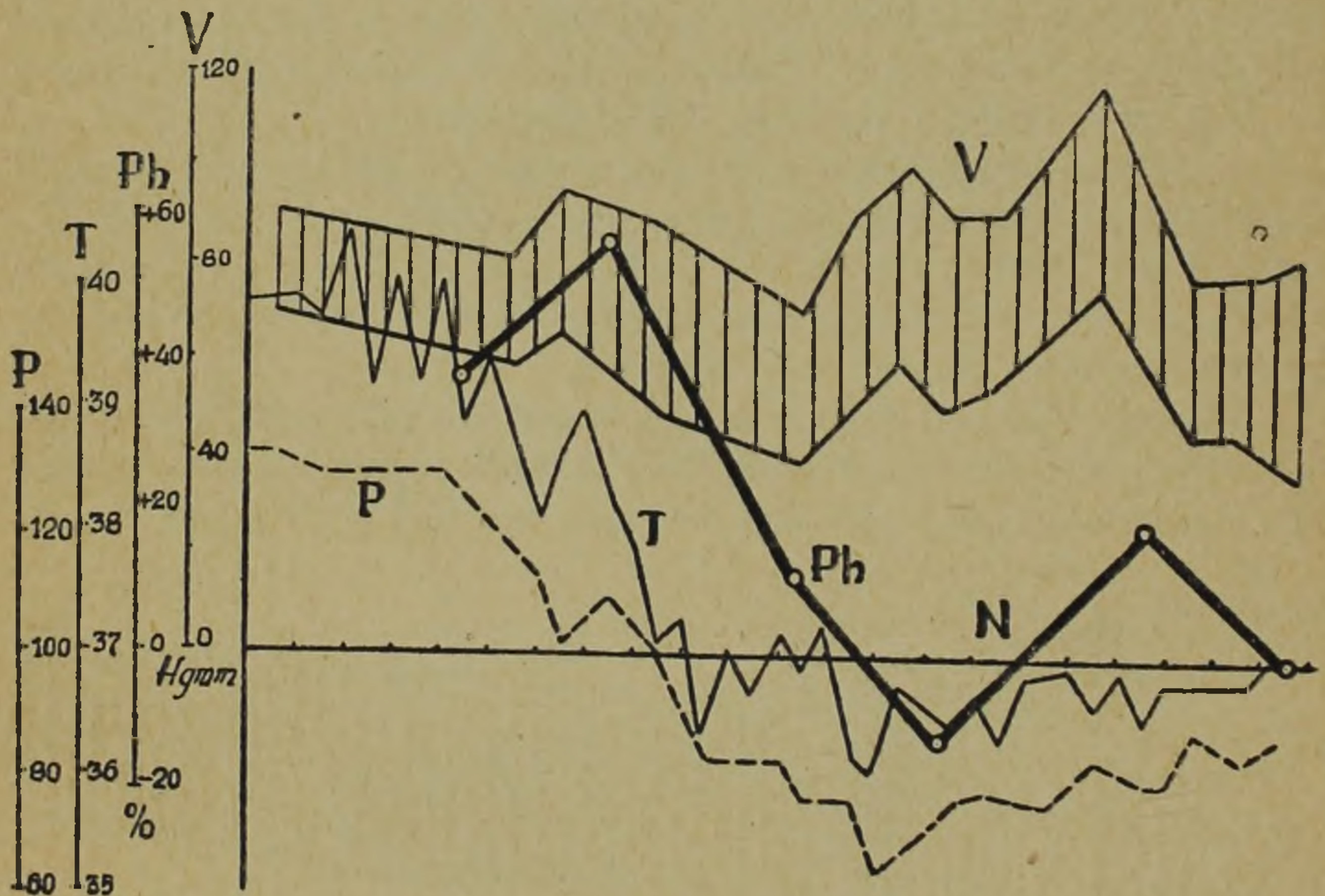

Abb. 4. *P* = Puls, *T* = Körpertemperatur, *Ph* = Phosphorylisierungsausfall (Glucoseretention), *V* = Blutdruck, *N* = Tage.

Ein interessantes Beispiel für die der Phosphorylierungsschädigung folgenden Überkompensation und für die Rückkehr der Werte auf das physiologische Niveau bietet unser 4. *Fall* (Abb. 4.), die 33 j. Zigeunerin *S. G.*, die am 8. XII. 1945 eingeliefert wurde.

Die Einzelheiten der Abbildung sind auf Grund der bereits Erörterten leicht verständlich, wir wollen lediglich auf die Wellenlinie der Phosphorylierungskurve aufmerksam machen. Die der Abweichung der Phos-

phataseaktivität von den physiologischen Werten folgende elastische Schwankung derselben um das normale Niveau ist kein unbekannter Begriff, und beweist die Reaktionsfähigkeit des Regelungssystems. In diesem Falle lässt dieses Verhalten der Kurven darauf schliessen, dass das phosphorylierungsregelnde System — aller Wahrscheinlichkeit nach die Nebennierenrinde — vielleicht über eine bessere Reaktionsfähigkeit verfügt, als in den vorigen Fällen. Die Reaktionsfähigkeit zeigt natürlich individuelle Unterschiede, die in demselben Krankheitsbild bei verschiedenen Personen in Form individueller Variationen in Erscheinung treten. Da wir diese Erscheinung öfters bei Zigeunern beobachtet haben, besteht auch die Möglichkeit, dass sie auf der bei dunkelhäutigen Individuen beobachteten gesteigerten Reaktionsbereitschaft der Nebennieren beruht. So ist u. a. die Feststellung von *Cramer* (3) bekannt, dass bei in den Tropen lebenden Europäern — im Gegensatz zu den Dunkelhäutigen — infolge der starken Inanspruchnahme der Wärmeregulation eine Nebenniereninsuffizienz auftreten kann. Ausser den funktionellen Erscheinungen hat diese Möglichkeit auch eine organische Grundlage, indem die Untersuchungen von *Kokas* (4) zeigten, dass die dunkelhaarigen Tiere eine grössere Nebenniere besitzen als die hellhaarigen Individuen derselben Tierart.

Es sei noch erwähnt, dass bei einzelnen neu aufgenommenen Kranken die Resultate der ersten Untersuchungen mit den oben gemachten Feststellungen nicht übereinstimmten, d. h. das Ergebnis der Phosphorylierungsuntersuchung entsprach nicht dem klinischen Bild. Jedoch bereits bei den ersten solchen Kranken vermochten die weiteren Untersuchungen diese scheinbaren Abweichungen soweit erklären, dass wir aus dem Resultat der ersten Untersuchung der neuen Patienten auf das Stadium der Krankheit und die eventuell auftretenden Komplikationen schliessen konnten.

Als Beispiel diene der 5. *Fall* (Abb. 5.).

F. P. 35 j. Mann. Nach Angaben des Kranken besteht die Krankheit seit 2 Wochen. Bei der Aufnahme (9. XII. 1945) Febris continua, ausgeprägte Exantheme, die in einigen Tagen verschwinden. Der Patient befindet sich in stark verfallenem Zustand, das Sensorium ist etwas gestört, das klinische Bild entspricht im allgemeinen dem des Fleckfiebers. Der am 4. Tage zwei Stunden nach der Belastung bestimmte Blutzuckerniveau war in Vergleich zu dem Nüchternwert negativ, was auf eine erhöhte Reaktionsfähigkeit hinweist, wogegen wir in den vorigen typischen Fällen bei ähnlichen klinischen Erscheinungen noch eine verminderte Funktion beobachten konnten. Aehnliche Fälle liessen uns vermuten, dass der Patient im letzten Krankheitsstadium war als er in die Klinik

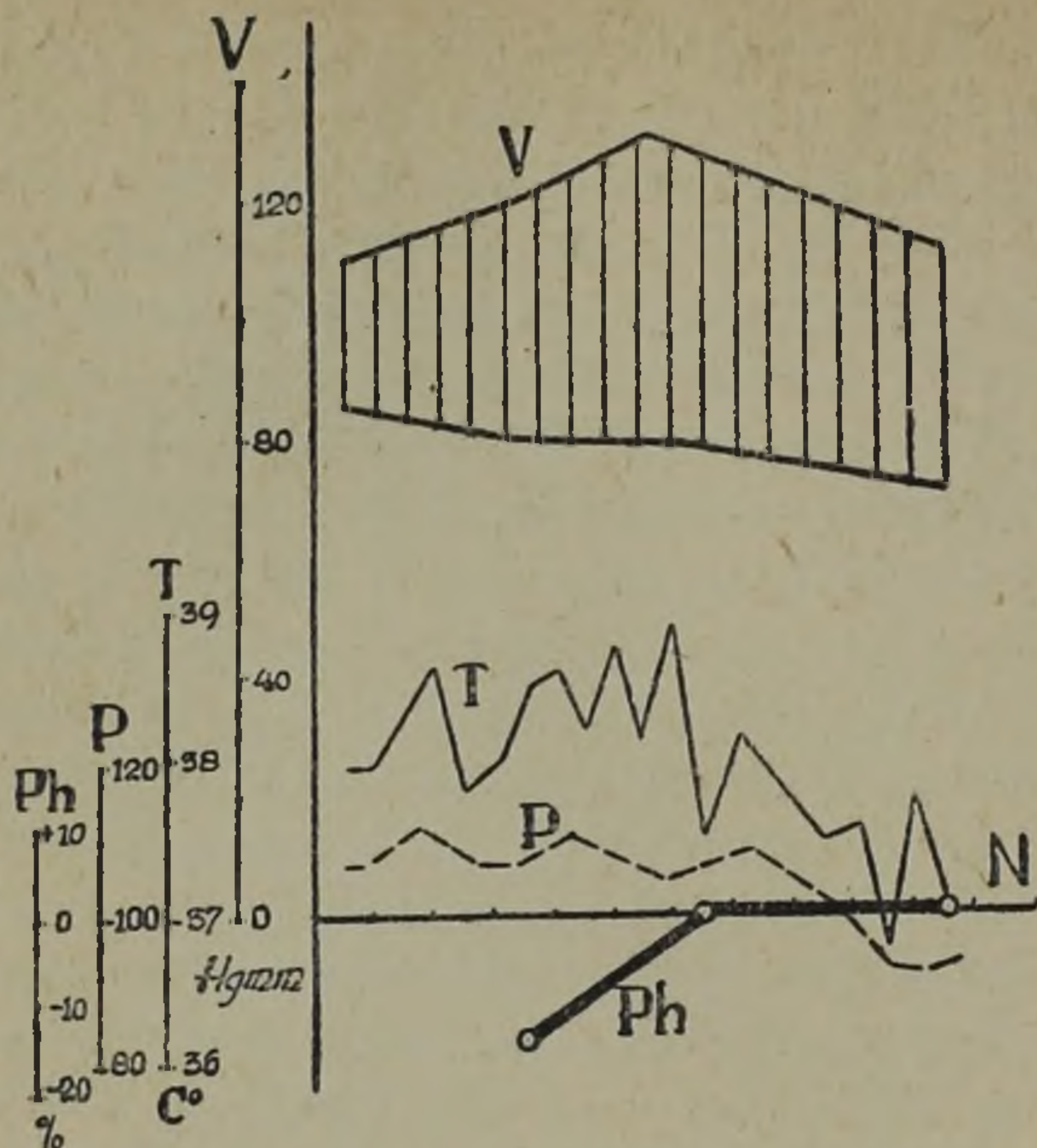

Abb. 5. Ph = Phosphorylisierungsausfall (Glucoseretention), *P* = Plus, *T* = Körpertemperatur, *V* = Blutdruck, *N* = Tage.

eingebracht wurde und dass das hohe Fieber, der verfallene klinische Zustand nicht durch das Fleckfieber selbst, sondern durch irgendeine Komplikation aufrechterhalten wurde. Darauf wies auch das Verhalten des Elutdruckes hin, da bei der Aufnahme des Patienten bereits eine entschieden steigende Tendenz zu beobachten war und das Amlitudo die physiologische Erweiterung zeigte. Die eingehende Untersuchung des Patienten, die wegen der bestehenden Schwierigkeiten erst nach einigen Tagen auch durch röntgenologische Untersuchung ergänzt werden konnte, hatte bewiesen, dass der Patient nach dem eigentlichen klinischen Abklingen des Fleckfiebers mit anfänglicher Komplikation eingebracht wurde. In den emphysematischen Lungen war eine in Entwicklung begriffene Bronchopneumonie zu beobachten, die am 7. Tag der Behandlung bei lytischem Fieber, Husten und Expektoration sich zu lösen anfing, wonach noch ein rechtsseitiges Empyem auftrat.

Wir hatten Gelegenheit mehrere lehrreiche Fälle zu beobachten, in welchen wir aus dem Verhalten der Phosphorylierung und des Histaminspiegels auf das Krankheitsstadium und die Entwicklung der verschiedenen Komplikationen bereits dann schliessen konnten als diese aus dem klinischen Bild eventuell noch nicht hervorging.

Diese Fälle deuten darauf hin, dass die Hemmung der Phosphorylierung bei Fleckfieber ihre Wurzeln tiefer in den während der Krankheit

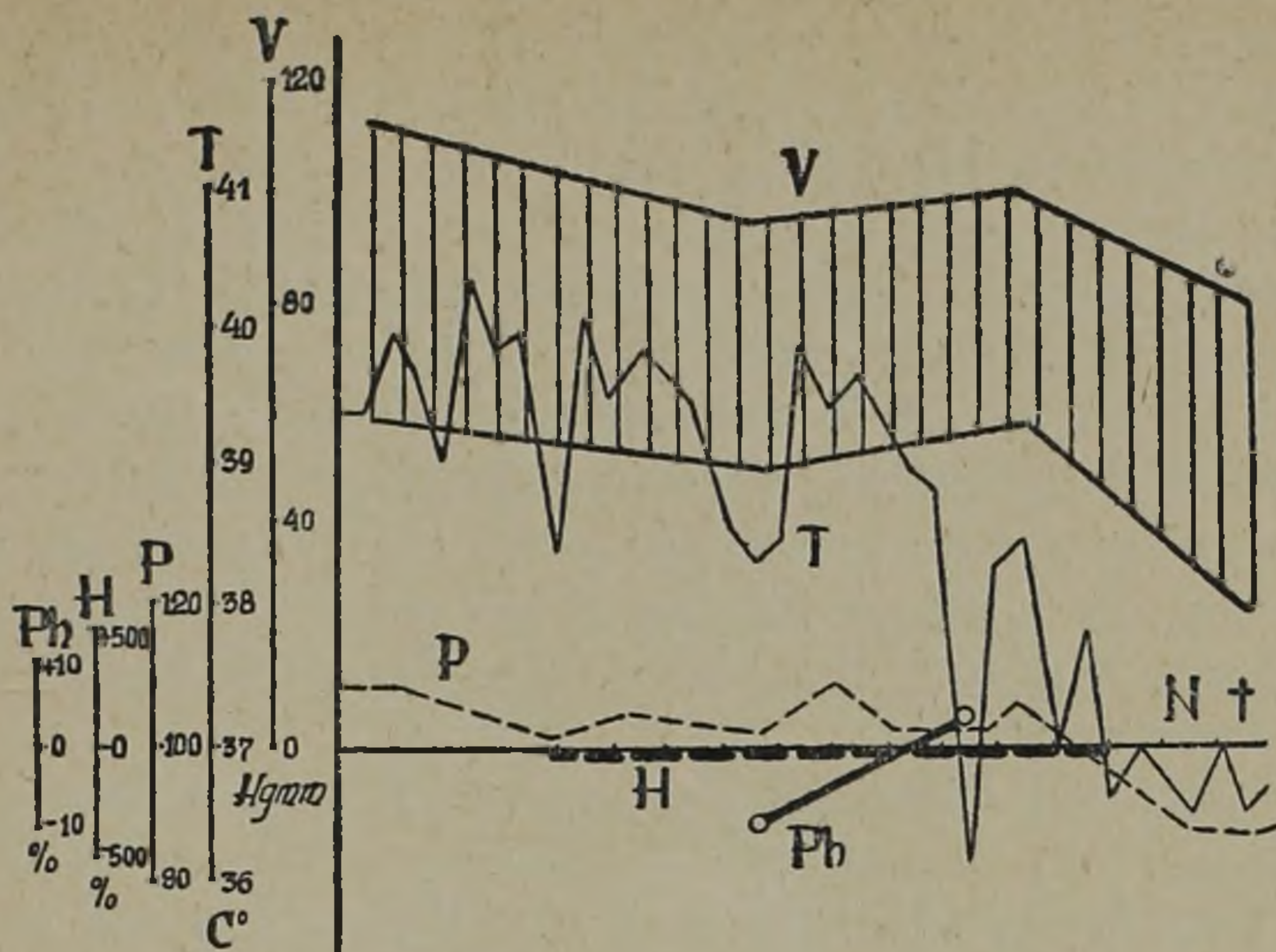

Abb. 6. Ph = Phosphorylisierungsausfall (Glucoseretention), *H* = Histamingehalt des Blutes, *P* = Plus, *T* = Körpertemperatur, *V* = Blutdruck, *N* = Tage.

sich abspielenden pathologischen Prozessen hat und nicht mit dem allgemeinen fieberhaften Krankheitszustand in Zusammenhang steht.

Noch besser lässt sich dies aus der Abb. 6. erblicken, in welcher die Untersuchungsresultate eines mit dem Tode der Patientin (*V. E.* 16 j. Mädchen) endenden Typhus abdominalis dargestellt sind.

Die schweren klinischen Erscheinungen boten — trotz der relativen Bradykardie, die neben dem Verhalten des Blutdruckes als einziges positives Symptom für Typhus abd. sprach, eher das Krankheitsbild des Fleckfiebers. Wegen der damals bestehenden schweren Arbeitsmöglichkeiten bekamen wir die serologischen Befunde frühestens in einer Woche zur Hand. In diesem Falle wurde ausserdem die Klärung der Diagnose noch dadurch erschwert, dass die *Gruber—Widal*'sche und die *Weil—Felix*'sche Reaktion bei der Aufnahme (24. VI. 1945) negativ ausfielen, dann nach vier Tagen beide in Verdünnung 1 : 200 positiv waren und erst die am 8. Tage entnommene Blutprobe, deren Untersuchungsresulate erst nach einer guten Woche einliefen, sich in der *Gruber—Widal*'schen Reaktion in 1 : 400 und in der *Weil—Felix*'schen Reaktion in 1 : 200 als positiv erwies. Der Agglutinationstiter nahm spezifisch für Typhus abd. später noch weiter zu (am 11. Tag Typhus abd.: Antigen H 1 : 800, Antigen O 1 : 1600, Typhus exanth. negativ), während die Fleckfieberagglutination negativ wurde. Dieses Resultat lief leider erst nach dem Tode der Patientin ein, nachdem die Sektion die Richtigkeit der Diagnose Typhus abdominalis bewiesen hatte. Von unserem Gesichtspunkt haben

wir lediglich den Umstand hervorzuheben, dass der bei der hohen Continua und schwerem klinischem Zustand zweimal geprüfte Histaminspiegel und die Phosphataseaktivität sich als normal erwiesen, und dass, da die Phosphorylierungsuntersuchungen nicht laufend durchgeführt worden sind, die für den Typhus abd. in dem grösseren Teil der Fälle charakteristische Regelmässigkeit ebenfalls *(Obál, Ivády, Kelemen* u. *Nagy* (5)) nicht zu beobachten war. In Besitz der Untersuchungsergebnisse bezüglich der Phosphataseaktivität und des Histamingehaltes, ergänzt durch das Verhalten des Blutdruckes und des Pulses, hielten wir auf Grund unserer Beobachtungen eher die Diagnose Typhus abdominalis für richtig, was dann später durch den spät eingetroffenen serologischen Befund und die Sektion bestätigt wurde.

In typischen Fällen der *Addison*schen Krankheit lässt sich Blutdrucksenkung mit verengertem Amplitudo, sowie verringerte Phosphorylierung ebenfalls beobachten. Während diese Kranken eine herabgesetzte Adrenalinempfindlichkeit zeigen, konnten wir bei Fleckfieber auch in dem hypotonischen Stadium normale Adrenalinempfindlichkeit feststellen.

Die Angaben einiger unserer an Fleckfieberkranken durchgeführten Versuche haben wir tabellarisch zusammengestellt. Die Resultate sind überall übeinstimmend.

Wir wollen uns nicht in weitläufige Erörterungen einlassen und weisen mit einigen Worten lediglich darauf hin, wie sich die beobachteten Erscheinungen in das Krankheitsbild fügen. Es erübrigt sich näher zu besprechen, was für eine Bedeutung es für den von Infektionskrankheiten schwer betroffenen Organismus hat, wenn ein so vielseitiges und in einer jeden Zelle des Organismus wirkendes Fermentsystem, wie die der Phosphorylierung, eine Störung erleidet. Es ist wahrscheinlich, dass dabei auch sonstige, dem intermediären Umsatz dienende Funktionen geschädigt werden, wodurch dann die Widerstandsfähigkeit der Gewebe und des ganzen Organismus beträchtlich herabgesetzt wird. Nach Nebennierenexstirpation oder nach Behandlung mit phosphataselähmenden Giften treten zahlreiche pathologische Erscheinungen auf. Aehnliche pathologische Symptome lassen sich auch bei Fleckfieber beobachten, wie z. B. in unserem L. Falle unter den laboratorischen Untersuchungen der Reststickstoffwert von 90 mg %, ferner der Serumchlorgehalt von 336 mg %, was den Werten von 554 mg % NaCl und 220 mg % Serumnatrium entspricht. Diese Befunde weisen ebenfalls auf eine gestörte Phosphorylierung hin *(Verzár)* (6).

Zur Aufklärung des Ursprungs der erwähnten Erscheinung sind unsere Untersuchungen im Gange und wir wollen den Resultaten an

TABELLE I.

Kranke	Tag d. Exp.	Blutzuckerwert vor Glykosebelastung mg %	Blutzuckerwert 2 Stund nach Glykosebelastung mg %	Differenz mg %	Differenz %
H. Gy.	8. 6. 1944.	70	118	+ 48	+ 68,5
	12. 6.	81	103	+ 22	+ 27,1
	14. 6.	82	97	+ 15	+ 18,2
	17. 6.	88	99	+ 11	+ 12,5
	25. 6.	100	104	+ 4	+ 4,0
	3. 7.	100	96	— 4	— 4,0
K. A.	4. 7. 1945.	82	171	+ 89	+ 108,0
	7. 7.	134	166	+ 68	+ 50,7
	10. 7.	119	134	+ 15	+ 12,6
	13. 7.	105	109	+ 4	+ 3,8
	21. 7.	94	82	— 12	— 12,7
	27. 7.	98	94	— 4	— 4,0
J. I.	5. 7. 1945.	80	113	+ 33	+ 41,2
	8. 7.	104	118	+ 14	+ 15,4
	11. 7.	98	103	+ 5	+ 5,1
	21. 7.	96	91	— 5	— 5,1
	31. 7.	92	90	— 2	— 2,1
H. R.	1. 7. 1945.	123	154	+ 31	+ 25,2
	7. 7.	102	102	± 0	± 0,0
	13. 7.	106	72	— 34	— 31,0
	19. 7.	102	96	— 6	— 5,8
S. G.	12. 12. 1945.	94	130	+ 36	+ 38,3
	15. 12.	60	94	+ 34	+ 36,6
	19. 12.	102	114	+ 12	+ 10,7
	22. 12.	116	102	— 14	— 12,0
	26. 12.	91	107	+ 16	+ 17,5
	29. 12.	92	93	+ 1	+ 1,0
K. S.	27. 12. 1945.	48	59	+ 11	+ 22,9
	29. 12.	122	119	— 3	— 2,4
F. P.	27. 12. 1945.	66	74	+ 8	+ 12,1
	30. 12.	79	75	— 4	— 5,0
F. Á.	27. 12.	82	65	+ 13	+ 25,0
	30. 12.	75	67	— 8	— 10,6
R. E.	28. 12.	95	108	+ 13	+ 13,6
G. G.	8. 1. 1946.	124	124	± 0	± 0,0
	11. 1.	83	122	+ 39	+ 47,0
	13. 1.	102	93	— 9	— 8,8
	16. 1.	70	72	+ 2	+ 2,8

dieser Stelle nicht vorgreifen. Es sollen jedoch kurz die Möglichkeiten erwähnt werden, die zu dem Zustandekommen der gefundenen Ergebnisse verholfen haben durften. Nach der Beobachtung von *Feldberg, Kellaway* (7), *Keogh* (8) und *Trethewie* (9) wird auf Wirkung verschiedener Schlangengifte, Bienengift und sogar durch Staphylokokkentoxin in den Organen durch Lysozytin Histamin frei. Die Annahme liegt auf der Hand, dass infolge des toxischen Zustandes bei der durch die Rikettsia Prowazeki verursachten Erkrankung aus den Zellen Histamin frei werden dürfte. Die Insuffizienz der Nebennierenrinde selbst kann ebenfalls zum Freiwerden von Histamin führen *(Riml* (10), *Wilson* (11), *Verzár* (6).) Das Erscheinen von grösseren Mengen Histamin im Kreislauf findet seine Erklärung zum Teil auch in der geringgradigen Leukozytose, da nach *Code* und *McDonald* (12) ein bedeutender Teil des im Blute nachweisbaren Histamins in den Granulozyten zu finden ist. In unserem Falle jedoch kann diese Menge bloss einen Bruchteil des von uns gefundenen Histamins darstellen.

Die Bedeutung des beobachteten hohen Histaminniveaus lässt sich in bezug auf den normalen menschlichen Organismus garnicht erwägen, da die Tatsache bekannt ist, dass der seiner Nebenniere beraubte Organismus gegenüber Histamin viel empfindlicher ist als der normale, wie dies die Untersuchungen von *Marmorston—Gottesmann* und *Perla* (13), *Wyman* und *tum Suden* (14), *Rose* (15), *Wilson,* sowie *Noble* und *Collip* (16) zeigen. Nach denselben beträgt z. B. die minimale tödliche Dosis bei normalen Ratten 100 mg pro Kg/Körpergew., während bei nebennierenexstirpierten Tieren 10 mg genügen.

Das Histamin spielt in der Entstehung des Kreislaufshockes eine allgemein bekannte Rolle. *Parkins, Swingle, Taylor* und *Hays* (17) haben durch zahlreiche Beispiele darauf hingewiesen, dass die ihrer Nebenniere beraubten Tiere einen chirurgischen, oder traumatischen Shock verschiedenen Ursprungs viel weniger ertragen als die normalen Tiere. Die Einwirkung von einer Menge, die das normale Tier noch gut verträgt, führt bei einem Organismus mit verringerter Phosphorylierung bereits zum Tode. *Perla, Freiman, Sandberg u. Greenberg* (18) berichten, dass ihre operierten Kranken die Magendarmoperation, Thorakoplastik usw. bei Vor- und Nachbehandlung mit NaCl und Rindenhormon viel besser vertragen haben. Hierher gehört noch die auffallende Aehnlichkeit, die zwischen den Symptomen der Rindenhormoninsuffizienz und des Histaminkollapses besteht. Beide kennzeichnen sich durch Eindickung des Blutes, Verringerung der zirkulierenden Blutmenge, Zunahme des Milchsäuregehaltes im Blute, Acidose, Verringerung des Leber- und Muskelglykogens, erhöhten Reststickstoff- und Kaliumwert im Serum, Senkung des Natrium- und Chlorniveaus, Adynamie usw.

Es erübrigt sich weitere Literaturangaben anzuführen, da die Klärung der Frage Aufgabe weiterer Experimente ist. Es sei jedoch noch erwähnt, dass die herabgesetzte Phosphorylierung, ausserdem dass sie auf die Reaktionsfähigkeit und Resistenz des Organismus schädigend wirkt, denselben auch gegenüber Bakterieninfektionen empfindlicher macht *(Hartmann u. Merle—Scott* (19), *Marmorston—Gottesmann u. Perla)* (13) und auch einige beim Fleckfieber und anderen Infektionskrankheiten wichtige Rolle spielenden physiologischen Funktionen empfindlich betrifft. Zahlreiche Verfasser berichten über die Tatsache, dass die Funktionsstörung der Nebennierenrinde auf die physiologische Wärmeregulation stark schädigend wirkt. Infolge der Umsatzverringerung nämlich ist bei einem solchen Organismus auch die chemische Wärmeregulation und auch die physikalische Wärmeabgabe gestört, der Organismus ist nicht imstande die Temperatur aufrecht zu erhalten und neigt zur Abkühlung. Es ist also offensichtlich, wie gross die Belastung ist, die das mit hohem Fieber einhergehende Fleckfieber, zusammen mit der unzulänglichen Funktion der Wärmeregulation dem Organismus aufbürdet.

Obwohl es gegenwärtig nicht zu unseren Aufgaben gehört therapeutische Schlüsse zu ziehen, müssen wir trotzdem auf den grossen C Vitaminbedarf des an Insuffizienz der Nebennierenrinde leidenden Organismus hinweisen *(Thaddea* u. *Hoffmeister)* (20). Auf Grund unserer Versuchsresultate zeigt sich bei Fleckfieber, insofern der beobachtete Ausfall der Phosphorylierung tatsächlich zentralen Ursprungs ist —, die Anwendung der Rindenhormonbehandlung, ferner sonstiger hierher gehörender therapeutischer Verfahren als angebracht.

ZUSAMMENFASSUNG.

Das Blut der Fleckfieberkranken zeigt eine starke Vermehrung des Histamingehaltes. Das Histaminplus entsteht während der Continua und erreicht während der Lysis stufenweise den Normalwert wieder.

Ebenfalls während der Fieberperiode ist eine beträchtliche Verringerung der Phosphataseaktivität zu beobachten, die mit dem Histamingehalt parallel stufenweise zur Norm zurückkehrt.

Die Adrenalinempfindlichkeit ist normal.

Der für die Erkrankung charakteristische gesenkte Blutdruck erreicht gleichzeitig mit der Normalisierung des Histaminwertes und der Phosphorylierung das physiologische Niveau.

Die Erscheinung zeigt sich für Fleckfieber dermassen charakteristisch, dass man durch sie auf das Stadium der Krankheit folgern und sie auch zur Differenzialdiagnose heranziehen kann.

Auf Grund der Versuchsergebnisse ist bei Fleckfieberkranken die Verabreichung von grossen Mengen Vitamin C und Rindenhormon angebracht.

Herrn Apotheker *Josef Berekméri* wollen wir für seine aufopfernde Hilfsbereitschaft, mit der er uns die für uns sonst unanschaffbaren Chemikalien zur Verfügung stellte und damit unsere Arbeit ermöglichte auch an dieser Stelle unseren besten Dank aussprechen.

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EXCRETION OF RIBOFLAVIN IN RATS AND PHOSPHORYLATION.

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If thiamin or riboflavin is given perenterally to organisms suffering from thiamin or riboflavin deficiency, less of these vitamins will be excreted than from the normal organism (1). If the vitamin dosage is repeated, the amount eliminated is greater. With repeated dosage the excretion continues to increase, until a certain state of equilibrium is reached, in which the organism behaves the same as the normal organism. If thiamin or riboflavin is repeatedly given parenterally to normal organisms in the same way, the excretion varies irregularly between wide limits, less or more, apparently following no sort of law. Only so much is clear, that after a certain time the excretion paradoxically decreases. The longer the vitamins are given the more pronounced the decrease in excretion (2).

In a case of thiamin hypovitaminosis, for example, excretion was as follows (2):

No of days	Thiamin, mg administered	Urine, ml	Thiamin excreted μ g
1	10	450	163
2	10	400	471
3	10	250	457
4	10	450	549
5	10	490	683
6	10	510	867
7	10	480	1190
8	10	480	1506
9	10	280	1382
10	10	320	1360

In riboflavin avitaminosis, on giving riboflavin every 2 hours, the excretion was as follows (3):

Dose	Riboflavin mg	Urine ml	Riboflavin excreted μ g
1	3,33	110	178
2	3,33	89	516
3	3,33	151	1170

Thiamin excreted by healthy individuals (4):

Dose	Thiamin mg	Urine ml	Thiamin excreted mg
1	2	86	572
2	2	124	400
3	2	142	586

Riboflavin excreted by healthy individuals (3):

Dose	Riboflavin mg	Urine ml	Riboflavin excreted μ g
1	3,33	92	1580
2	3,33	94	1541
3	3,33	96	1121

No explanation has so far been found for this behaviour as to excretion, because of which some doubt has been shed on the value of vitamin-tests. It is beyond question that some unknown factor affects excretion, aside from the organism's saturation, the function of the kidneys and possibly of the liver. Investigation of this unknown factor was the aim of the following experiments.

Both thiamin and riboflavin perform their functions in the organism in a phosphorylated state, that is, as phosphoric acid esters.

The phosphorylation of thiamin comes about through the cooperation of adenylic acid and adenosine triphosphate (Lipton and Elvehjem (5), Weil—Malherbe (6), in the following way: adenosine triphosphate + thiamin → adenylic acid + cocarboxylase. The phosphorylated thiamin is identical with cocarboxylase. An enzyme, *phosphorylase,* catalyses the binding to the phosphorus. This activity goes on in the intestinal wall during absorption but is carried out in most of the other organs, principally in the liver. In the urine only unphosphorylated thiamin is excreted, but we know that the tissues, principally the liver and the kidneys, can transform cocarboxylase into thiamin by dephosphorylation (Ochoa) (7).

Riboflavin is likewise a constituent of coenzymes. One type of such anzymes is riboflavin-phosphate, another is riboflavin-adenine-dinucleetide, a coenzyme containing riboflavin, phosphoric acid, ribose and odenylic acid. With a specific protein, called the apo-enzyme, these form the *yellow ferment,* long well-known. The phosphorylation of riboflavin is also carried out in the tissues, in the intestines, but principally in the liver. The phosphorylating capacity of human blood cells can be demostrated both in vivo and in vitro (Klein and Kohn) (8).

In the case of both vitamins mentioned, the effect of the vitamin is a capacity linked with phosphorylation. Hence one factor which affects the utilization of the vitamins, and thus their excretion, may be phosphorylation.

In the present experiments I investigated the excretion of riboflavin in rats. Isolated rats, in cages which collected their urine, were given certain daily quantities of riboflavin subcutaneously. In the urine collected in 24 hours the amount of riboflavin was determined on the basis of yellow fluorescence by the method recommended by Goth (9), using urine of the rats collected before the experiment as standard. Thus the spontaneous excretion of riboflavin deriving from the rats' diet could not be taken strictly into account, but the diet was uniform and the large doses of riboflavin given rendered negligible such quantities as tenths of ug.

As basic experiment each rat was given only riboflavin for at least a week, but in 5 animals the daily excretion was controlled for 4 weeks. The values determined were as follows:

No of days.	Daily dose of riboflavin γ	Excretion									
		Rat. 1.		Rat. 2.		Rat. 3.		Rat. 4.		Rat. 5.	
		Urine ml	μ g	Urine ml	μ g	Urine ml	μ g	Urine ml	μ g	Urine ml	μ g
1	200	30	12	35	86	15	20	15	41	16	121
2	200	12	75	20	152	21	101	23	62	20	143
3	200	21	120	31	122	18	144	25	79	21	156
4	200	25	103	24	96	30	140	24	108	20	100
5	200	30	81	15	104	22	92	20	69	27	90
6	200	34	68	19	116	12	67	12	88	25	84
7	200	34	64	18	92	26	72	15	44	33	64
8	200	39	53	39	82	25	53	12	40	30	60
9	400	25	169	40	245	25	157	15	161	44	214
10	400	20	145	30	191	22	166	27	221	25	178
11	400	38	117	29	185	36	116	15	196	15	134
12	400	30	120	45	100	35	150	30	108	27	232
13	400	32	159	36	125	26	84	44	91	33	167
14	400	39	87	32	83	38	81	29	64	20	119
15	400	27	92	30	90	24	134	53	82	24	125
16	400	26	90	25	110	19	125	30	92	21	102
17	400	32	112	24	150	16	120	30	101	22	115
18	400	52	75	52	142	14	101	39	68	22	130
19	400	25	103	50	96	30	108	32	55	32	122
20	400	23	120	42	87	32	140	15	76	35	96
21	400	27	96	40	82	35	92	32	76	25	87
22	400	25	83	45	73	20	67	35	62	23	89
23	400	28	67	49	102	26	83	30	78	33	92
24	400	42	82	27	87	37	82	35	82	34	69
25	400	25	74	38	101	35	80	28	76	25	76
26	400	35	102	27	92	22	96	27	54	50	72
27	400	38	76	30	76	22	80	27	52	45	63
28	400	35	82	30	100	38	67	28	62	27	87
29	400	22	63	29	58	36	63	22	60	28	82
30	400	19	74	33	52	34	72	13	61	22	62

As appears from the experiments, the daily excretion varied irregularly. It increased the first day or two, began to diminish already on the third and fourth days, and gradually — if not uniformly — continued to decrease. Doubling the dose decidedly increased the amount of riboflavin excreted, but the gradual decrease then also took place. All this corresponds to experiences in human beings and is contrary to the simple hypothesis that when saturation is reached the organism rejects the superfluous. If this were so, excretion would have to increase continuously up to an amount equivalent to the quantity taken in. The constant nature of the amounts of urine made it improbable that there should be laesion of the kidneys. We tried to find in phosphorylation an explanation for this behaviour as to excretion.

We investigated the excretion of riboflavin when the phosphorylating processes were impaired. According to the experiments of Verzár (10), *monoiodacetic acid* is capable of impairing phosphorylation. Of this we know, for instance, that it inhibits oxido-reduction in sugar consumption, prevents the transformation of phosphoglycerol-aldehyde into phosphoglycerol, as well as impeding the reaction which takes place between phosphoglycerol aldehyde and pyruvic acid, or between phosphoglycerol and pyruvic acid. According to Adler and Günther (11), monoiodacetic acid inhibits the dehydrolyzing of triose phosphate. *Phlorrhizin* inhibits the phosphrylation of glycogen, hinders the formation of phosphglycerol from triose phosphate, and prevents desintegration of adenosine triphosphate and the transformation of phosphate to hexosemonophosphate. Hübner and Verzár (12) created acid phosphoric esters of riboflavin artificially with an extract of intestinal mucous membrane. This phosphorylation could be inhibited equally by phlorrhizine and monoiodoacetic acid.

We induced chronic monoiodacetic acid poisoning using 0,015 mg monoiodacetic acid per g rat, 1/10th part of the quantity used in the acute experiment[1]. 5 rats, without regard to weight, were given an equal amount of monoiodacetic acid: 0,5 — 1,5 ml of a solution containing 2 mgr in 1ml.

According to these experiments the excretion of riboflavin gradually diminishes in rats poisoned with monoiodacetie acid, to a greater degree than in the rats of Group I. The connection of the decrease in excretion wit the monoiodacetic acid is shown by the fact that after ceasing to give the monoiodacetic acid, excretion increased again — if the animal was still alive (rats 6 and 10). We stopped giving the riboflavin too to rat No. 6 after the 35th day of the experiment. On that day there was still 30 μg riboflavin

[1] Grateful acknowledgement is made to Professor László Laszt of Friburg for his kind instructions.

The results of 5 experiments were as follows:

Day	Riboflavin γ g	Monoiodoacetic acid, ml	6. excretion		7. excretion		8. excretion		9. excretion		10. excretion	
			Urine ml	μ g	Urine ml	μ g	Urine ml	μ g	Urine ml	μ g	Urine ml	μ g
1	400		32	147	27	92	17	106	42	227	20	116
2	400		16	103	42	173	20	192	46	192	17	127
3	400		21	196	38	216	24	173	39	200	28	128
4	400		23	145	36	200	22	152	36	173	32	112
5	400		40	140	42	192	19	176	25	152	27	140
6	400		28	121	40	170	30	143	40	160	16	127
7	400		24	116	32	152	27	180	22	152	22	102
8	400	0,5	26	146	30	185	27	142	27	103	20	104
9	400	0,5	25	163	25	140	30	103	32	142	17	122
10	400	0,5	20	133	35	186	18	106	29	170	22	93
11	400	0,5	16	127	25	138	24	87	30	140	27	87
12	400	0,5	35	102	27	96	22	45	18	112	30	46
13	400	0,5	25	83	18	102	20	92	32	96	28	42
14	400	0,5	26	99	20	36	24	49	39	54	30	35
15	400	0,8	27	152	20	50	36	36	43	55	16	33
16	400	0,8	20	128	16	78	20	22	45	37	33	38
17	400	0,8	23	88	38	48	24	24	43	48	25	22
18	400	0,8	26	92	35	76	24	19	39	27	27	24
19	400	0,8	25	108	20	80	25	20	27	32	32	22
20	400	0,8	32	25	27	82	19	21	30	24	30	22
21	400	0,8	26	21	15	92	29	12	33	12	18	21
22	400	1,2	30	24	23	32	22	13			27	16
23	400	1,2	37	15	18	16	17				24	21
24	400	1,2	16	32	29	39	10				27	12
25	400	1,5	37	16	37	21	8				29	20
26	400	1,5	25	36	33	33					32	18
27	400	1,5	43	12	32	29					33	16
28	400	1,5	24	10	30	15					35	14
29	400	1,5	15	16	28	21					43	16
30	400	1,5	36	12	30	8					47	9
31	400	1,5	32	10	27	8					43	trace
32	400		27	16	28	12					31	48
33	400		23	80							29	75
34	400		30	97							30	106
35	400		27	112							42	91
36	400→	(none given to No 6.)	22	30							31	93
37	400		25	—							30	110
38	400		22	—							22	172
39	400										29	150
40	400										28	121
41	400										42	102
42	400										34	91

in the urine; on the following day no riboflavin was excreted. This experiment shows that it is not a question of the riboflavin being retained. Animals 7, 8 and 9 died on the 33rd, 26th and 22nd day of the experiment respectively. Animals Nos. 6 and 10 remained in health.

1 mg phlorrhizin was given for 1 week. During that time the rats' urine gave a strong reaction with Nylander reagent.

Day	Ribo-flavin µ g	Phlor-rhizin mg	Excretion					
			11		12		13	
			Urine ml	µ g	Urine ml	µ g	Urine ml	µ g
1	400		25	152	32	146	45	190
2	400		28	212	37	158	35	94
3	400		20	172	36	160	26	151
4	400		27	163	27	126	22	147
5	400		19	142	28	180	22	162
6	400		32	150	32	176	26	102
7	400		30	147	19	150	38	98
8	400	1	27	127	24	147	27	121
9	400	1	35	142	27	122	21	75
10	400	1	36	140	40	90	16	78
11	400	1	39	143	41	83	21	26
12	400	1	33	122	40	104	29	72
13	400	1	41	196	39	96	21	30
14	400	1	31	132	38	87	18	38
15	400		22	102	27	89	9	52
16	400		21	93	30	101	30	70
17	400		17	116	32	93	27	99
18	400		20	87	29	127	16	121
19	400		16	102	27	86	22	92
20	400		21	124	24	82	25	88
21	400		23	137	25	70	30	78

From this experiment it seems that phlorrhizin did not appreciably affect riboflavin excretion. In the 13th animal in the experiment, however,

Day	Ribo-flavin б g	Insulin unit.	Excretion			
			17		18	
			Urine	µ g	Urine	µ g
1	400		25	202	32	146
2	400		27	213	34	187
3	400		32	196	32	201
4	400		35	157	27	182
5	400		32	159	30	164
6	400		42	140	28	170
7	400		27	162	30	158
8	400	0,1	26	117	27	160
9	400	0,1	28	150	32	142
10	400	0,1	32	146	34	116
11	400	0,1	31	96	27	127
12	400	0,1	41	128	30	88
13	400	0,1	21	132	28	92
14	400	0,1	23	146	30	117
15	400		22	123	27	91
16	400		27	92	25	87
17	400		21	101	20	124
18	400		20	96	18	128
19	400		19	127	26	132
20	400		22	100	27	97

the excretion of riboflavin seems to have decreased during the dosage of phlorrhizin.

Summarizing the experiments so far: it appears that impairing phosphorylation (principally with monoiodacetic acid) diminishes the excretion of riboflavin.

After this we investigated the effect on riboflavin excretion of a substance which, according to experimental data (13, 14) increases the phosphorylation of carbohydrates: Insulin. As 1 unit of insulin per rat killed them, 2 rats were given 400 μg riboflavin daily with 1/10th unit insulin subcutaneously.

According to these experiments insulin had no effect on the riboflavin excretion of rats.

In a further series of experiments (4 rats) we investigated how adenosine triphosphoric acid affected the excretion. We gave this in the form of *Atriphos* (manufactured by the Hungarian Pharmaceutical Company, Ltd.) which, according to the manufacturers, contains 5 mg sodium salt of adenosine triphosphoric acid per ampulla. The rats were given 1 amp per day s c.

Day	Riboflavin μ g	Atriphos mg	Excretion							
			19		20		21		22	
			Urine	μ g	Urine	μ g	Urine	μ g	Urine	μ g
1	400		28	170	31	90	25	150	23	190
2	400		25	138	24	55	28	172	20	94
3	400		18	133	12	86	20	152	10	151
4	400		20	130	16	90	22	112	16	130
5	400		18	121	22	106	25	85	25	126
6	400		25	120	26	94	20	76	21	75
7	400		13	178	32	126	15	196	22	108
8	400	5	28	158	30	87	20	106	25	155
9	400	5	30	289	29	235	17	210	22	245
10	400	5	24	312	30	184	25	101	21	102
11	400	5	17	196	30	90	19	108	19	119
12	400	5	24	140	26	180	24	178	12	196
13	400	5	25	212	25	242	25	203	28	82
14	400	5	13	190	21	150	26	196	27	210
15	400		28	167	23	243	27	121	20	162
16	400		27	132	21	106	26	112	24	154
17	400		19	112	13	94	29	98	26	102
18	400		21	96	25	121	21	74	23	96
19	400		19	87	31	130	36	76	24	104
20	400		21	88	23	87	16	65	16	83

It appears definitely from the experiments that adenosine triphosphoric acid increases the excretion of riboflavin. After stopping the doses of adenosine triphosphoric acid the riboflavin excretion again diminished.

Hence, inhibiting phosphorylation diminishes riboflavin excretion, administration of adenosine triphosphoric acid increases it. From these two circumstances we can conclude that excretion depends on phosphorylation; that, if the phosphorylation of the riboflavin taken in diminishes, less of it will be excreted; if we facilitate the phosphorylation by administering adenosine triphosphoric acid, the excretion is greater. On the basis of this behaviour of the excretion the circumstance that the normal organism continually eliminates less riboflavin when it is repeatedly given can be explained as being that there is continually less phosphorylation of the incoming riboflavin. It can be supposed that the enormously large, non-physiological and superfluous vitamins getting into the organism in some way exhaust the phosphorylating apparatus, by tiring and using up either the phosphorylase ferment or the phosphorus available for that purpose. Those investigations according to which after administration of insulin the serum's inorganic phosphate-content decreases significantly in consequence of an increase in phosphorylation of sugar, speak in favour of this possibility (Bellinger and Hartmann (13), Harrap and Benedict) (14). Investigation now in progress will elucidate the behaviour of serum-P under the influence of riboflavin. But we first investigated how the excretion of repeated doses of riboflavin was influenced by other substances which also are phosphorylised in the organism. One group of rats was given a dex-

Day	Riboflavin μg	5 % dextrose ml	Excretion							
			23		24		25		26	
			Urine ml	μg	Urine ml	μg	Urine ml	μg	Urine ml	μg
1	400	5	22	102	24	137	25	180	22	147
2	400	5	18	86	14	119	16	161	18	102
3	400	5	20	99	23	96	28	148	19	83
4	400	5	18	47	23	52	24	100	30	72
5	400	5	30	32	22	57	23	48	28	53
6	400	5	30	42	25	42	16	42	27	72
7	400	5	27	50	20	60	16	43	25	96
8	400		25	78	28	130	15	183	19	104
9	400		16	124	18	116	25	162	18	127
10	400		17	137	14	202	20	112	27	133
11	400		22	119	23	193	26	267	32	192
12	400		35	190	27	170	30	115	35	143
13	400		14	200	25	156	25	96	33	152
14	400		15	172	35	186	16	88	29	107
15	400	5	20	67	27	170	27	36	27	96
16	400	5	24	30	20	108	28	29	19	54
17	400	5	18	58	15	32	19	52	18	42
18	400	5	26	42	23	46	18	28	19	50
19	400	5	17	22	13	45	20	32	23	42
20	400	5	22	46	35	52	32	30	25	40
21	400	5	23	53	32	27	28	16	27	42

trose solution in addition to riboflavin, another group thiamin (vitamin B^1). We supposed that the diversion of the phosphorus necessary for the phosphorylation of the dextrose or the thiamin would decrease the phosphorylation of the riboflavin given and, on the basis of the foregoing, the excretion.

4 rats were given 400 μg riboflavin daily for 1 week, together with 5 ml 5% dextrose (i. e., 0,25 g) s. c. Then, after a week without dextrose, another week with dextrose followed.

When the rats were getting dextrose they excreted decidedly less riboflavin than during the interval when they got none. It has long been known that sugar, and carbohydrates in general, increase the B-vitamin requirement. We considered the cause of this to be that the greater the consumption of carbohydrates the greater the need for the co-fermentum (thiamin, riboflavin, pyridoxin, etc.) figuring in carbohydrate metabolism. But the results of our experiments must, from the foregoing, be explained as a decrease in the phosphorylisation of riboflavin. We believe that among the causes of a hypovitaminosis created by administration of dextrose, is the fact that the phosphorylation of the dextrose, perhaps by P-consumption, crowds out the phosphorylation of riboflavin and other vitamins.

Administration of thiamin likewise diminished riboflavin excretion, as is seen from the following experimental results. The degree of decrease depends on the amount of thiamin given.

Day	Riboflavin μ g	Thiamin mg	Excretion					
			27		28		29	
			Urine	μ g	Urine	μ g	Urine	μ g
1	400		26	27	19	102	26	153
2	400		25	62	23	192	32	142
3	400		22	128	27	183	38	168
4	400		29	196	31	201	42	158
5	400		32	182	40	127	38	162
6	400		38	170	28	152	39	103
7	400		27	163	36	127	32	127
8	400	0,5	25	140	32	120	33	165
9	400	0,5	22	112	32	96	32	170
10	400	0,5	30	98	31	87	27	153
11	400	0,5	23	87	33	104	31	96
12	400	1	21	73	30	87	30	124
13	400	1	18	86	26	82	22	52
14	400	1	17	52	20	28	21	70
15	400	1	22	42	18	46	37	51
16	400	2	29	51	22	73	32	47
17	400	2	27	46	23	82	38	52
18	400		32	53	31	82	37	63
19	400		35	76	27	96	32	102
20	400		28	102	30	103	33	141
21	400		21	123	23	142	39	122
22	400		19	124	21	140	42	102

We explain the effect of thiamin in decreasing riboflavin excretion also as a repression of phosphorylation.

On the basis of these experiments, therefore, we consider that riboflavin excretion depends, aside from the organism's supply of it, on the phosphorylation. This does not diminish the value of load tests, as in an organism suffering from deficiency the role of phosphorylation is relegated to the background. The excretion of the first, or the first few doses of the vitamin depend primarily on the organism's supply of it. Excretion of further doses, however, is in proportion to the phosphorylation dilue. The load test can accordingly be evaluated only in case the individual examined had not received the vitamin before the test.

From our experiments it appears that the phosphorylation and the excretion — contrary to our former belief — run parallel: the more vitamin is phosphorylised, the more arrives in the urine (naturally dephosphorylated); the less is phosphorylised, the less is excreted. What happens in the organism to the non-phosphorylated vitamin, remains to be alucidated.

To verify our hypothesis, however, we are making the same experiments with thiamin. We are making determinations of phosphorus in the serum to ascertain whether there is a decrease of the inorganic P content after administration of the vitamins and, if so, how much; and to what extent this diminishes after giving insulin or dextrose *and* the vitamins. We are further investigating what other factors affect the phosphorylation and excretion (exstirpation of the suprarenals, administering of cortical extract, impairment or excitation of the RES, decrease or increase in basic metabolism, diuretics, etc.).

SUMMARY.

We investigated the excretion of riboflavin, given daily s. c, in the urine of rats. The elimination varied irregularly, decreased after a few days, and the more riboflavin the animals were given the less of it they excreted. The excretion of riboflavin decreased under the phosphorylation-inhibiting effect of monoiodacetic acid, was not changed by phlorrhizin; decreased in only 1 case. Excretion of riboflavin was augmented by adenosine triphosphoric acid. Administration of dextrose and thiamin reduced the excretion.

From these experiments we believe that riboflavin which was previously phosphorylated is excreted in the urine. (The kidneys dephosphorylate the riboflavin before its excretion.) Therefore the elimination taking place in the urine goes parallel with phosphorylation. The decrease

in excretion of riboflavin given over a longer period can be explained as due to exhaustion of the phosphorylation. Dextrose and thiamin are likewise phosphorylised; when they are given, less, riboflavin becomes phosphorylated, and therefore less is excreted.

Therefore, aside from the organism's supply of it, riboflavin excretion is affected by the degree of phosphorylation. The role of phosphorylation comes into the foreground only when the organism is no longer suffering from a deficiency. Load tests can therefore be evaluated, but only when none of the vitamin has got into the organism before the loading.

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THROMBIN PRODUCTION IN COAGULATION OF NATIVE PLASMA.

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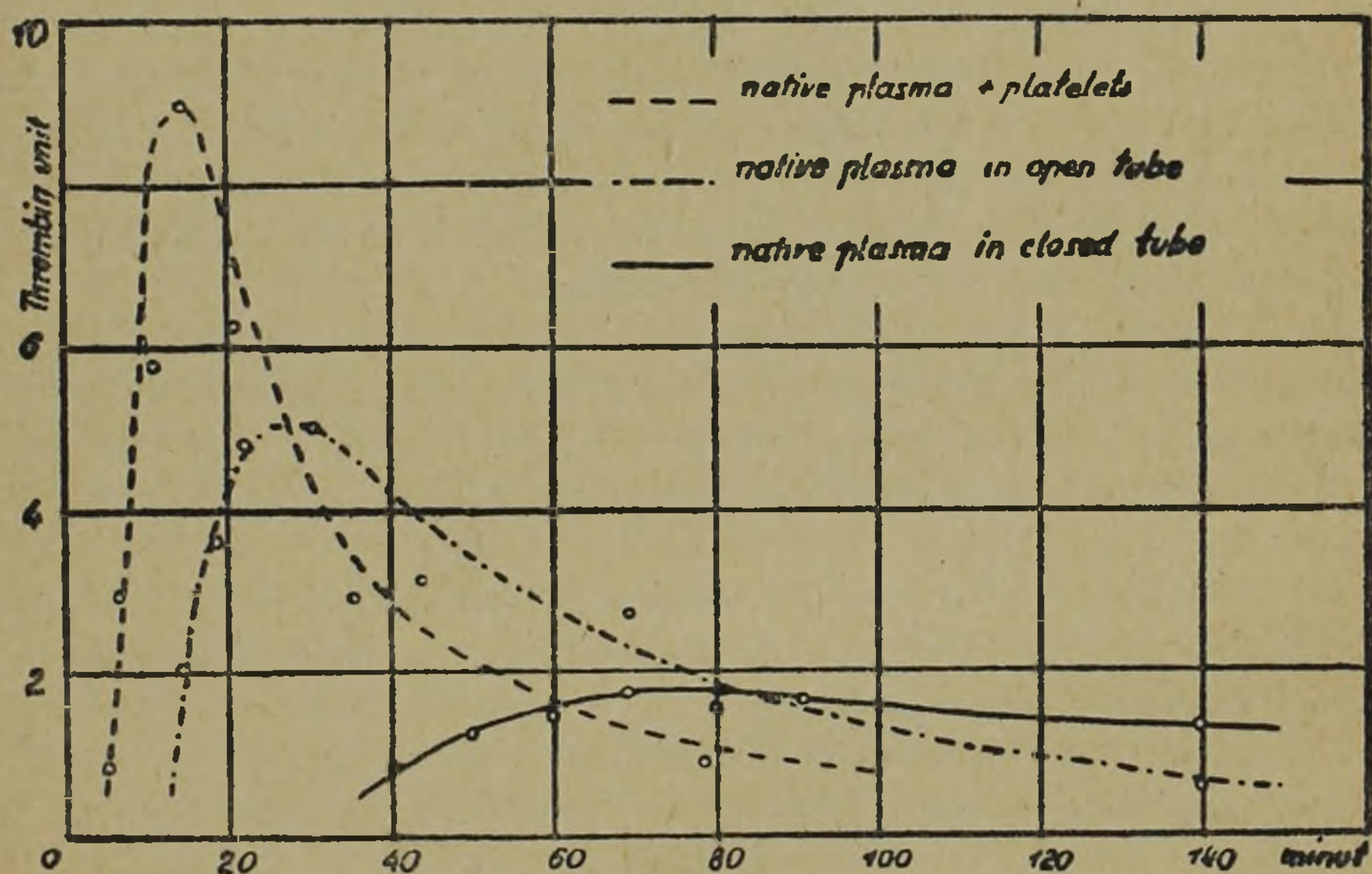
According to the opinion of the majority of investigators, blood coagulation is a catalytic — fermentativ process. Thrombin, the enzyme of blood coagulation is produced according to the rules of autocatalytic reactions. In blood, shed from an organism into a glass tube, thrombin formation starts abruptly, chain-reaction-like, after a few minutes of latency. Thus, besides causing the conversion fibrinogen into fibrin, thrombin also catalyses fresh thrombin formation. As shown by *Gerendás* (1), the largest quantity of thrombin, as a matter of fact, is formed after the clotting process itself is ended. Comparing fibrin separation with blood coagulation, this author examined and described in a previous publication (2), the speed of fibrin production in the native plasma. It was pointed out, that precipitation of fibrin in native plasma is essentially slower than complete blood coagulation itself and that the former undergoes changes, in a way typical for some pathological states. At the same time it was proved in these experiments, that the rapidity of the fibrin production in native plasma depends on its contact with alien surfaces (corpuscular parts of air, glass), but is independent of the quantity of produced fibrin. Data of numerous authors correspond with this fact, nevertheless it is remarkable that upon coagulation of the native plasma — i. e. if native plasma is poured from a celloidined tube into an ordinary glass tube — the formation of fibrin proceeds slowly and can be hastened by contact with a broader surface of glass and with the air in an open tube, as has been already demonstrated by the classic experiments of *Bordet* and *Gengou* (3). The question arises, whether the formation of thrombin is parallel with that of fibrin under these circumstances? It seemed worth while to follow this process and to examine the time-relations of thrombinproduction and in what extent is its quantity connected with the effect exercised by the alien surfaces as mentioned above.

If the amount of thrombin contained in plasma or serum has to be examined, the sample has to be added to a definite quantity of oxalated

plasma and the necessary time for clotting recorded. Following the principles described in a former paper (4) — native plasma was produced from human blood. This process consists of centrifuging blood in celloidin coated and corked glass tube for 30 minutes, with 5,000 revs. The blood does not coagulate for a long time in this celloidined container and native plasma can be obtained by centrifugation. In this process — besides employing a completely dehydrated celloidin solution — it is of utmost importance to prevent contamination with corpuscular parts of air as far as possible. Native plasma being pipetted into an ordinary glass tube, precipitation of fibrin starts after a while. During this process at fixed intervals 0,1—0,1 c. c.-s to 0,3 c. c. of oxalate plasma from the same individual were added and the time of coagulation measured. The amount of thrombin, measured by this method was expressed in special units, introduced by the author: one unit equals the quantity of thrombin being capable of coagulating 0,1 c. c. of oxalate plasma in one minute. The changes of thrombin level determined by this procedure during the clotting of native plasma are demonstrated in the diagram.



Proceeding from above observations the thrombin content of native plasma was determined while clotting in an *open* glass tube and in an airtight *closed* tube as well. In the first case about 2 c. c. of native plasma were placed in an open centrifuge tube of 10 c. c. capacity wherefrom at each given interval 0,1 c. c. was sucked up. In the other case, however,

equal samples (few tenths of a c. c.) of native plasma were measured out of graduated pipette into each test tube. These were immidiately corked and at different intervals successively 0,1 c. c. was sucked up from each tube and given to 0,3 c. c. of oxalate plasma. In the course of this procedure it was easy to observe precipitation of fibrin as well and notice the moment, after which no fibrin precipitation took place anymore. At first the native plasma was absolutely transparent, then in a while turned opaque and from this time on the clot had to be compressed first, before plasma i. e. serum for the tests could be pipetted. In case all fibrinogen was not yet converted into fibrin, the remaining plasma coagulated anew after every exprimation i. e. until all fibrin had been removed. After this no further coagulation occured, thus the thrombin content of the serum could be determined.

According to the present findings the quantity of thrombin increased in every examined case as long as postcoagulation was to be seen and reached its peak when after exprimation of the clot, coagulation was brought to standstill i. e. when all the fibrin had been removed. From that moment on the quantity of thrombin in the serum decreased.

To facilitate explanation the results of only a single test executed in the described way, where shown in the diagram. Essentially all tests, carried out in series, have ended with identical results, a differences were found only relating to the moment, when thrombin production started and reached its peak value, but the general shape of the curves showed close resemblance with that in the diagram.

Comparing the figures, obtained by coagulation in corked tubes with the results of the open tubes, it was found that production of thrombin in the closed tubes starts later, the increase of thrombin is very alow and its highest value was considerably lower than in the case of plasma, coagulating in an open tube.

Simultaneously the quantity of all precipitated fibrin was determined by gravimetrical method, both in the open and closed tubes and absolutely identical results were obtained i. e. a small amount of thrombin converts all the fibrinogen contained in the plasma, to fibrin, exactly so as a greater amount, however the procedure was prolonged by a smaller amount.

It was found remarkable that in native plasma at the first appearance of fibrin it has not been possible to detect thrombin with the above described method. This is probably due to the fact, suggested by *Laki* (5), that at first only a small amount of thrombin is available, which possibly being completely adsorbed by fibrin cannot be separated from fibrin by simple exprimation.

Thrombin formation was further accelerated and the produced quantities increased, if platelets were added to the native plasma. Upon producing native plasma platelets are generally to be found in form of aggregated cuticula on the top of the red cell layer. Therefore to obtain platelets the blood corpuscules were washed off the cuticula. The coagulation of the native plasma containing platelets starts abruptly and the thrombin figures increase rapidly. Thrombin maximum is coinciding in this case with the completion of fibrin precipitation.

The accomplished experiments prove, that this quantity of thrombin and the rapidity of its formation depend on thromboplastic effects. These conclusions drawn from native plasma coagulation: Blood from venesection was poured into two ordinary glass and two celloidin coated tubes. Thereafter one of the glass and one of the celloidined tubes was corked, leaving the two others open. Keeping the tubes at an indoor temperature, the moment of coagulation was recorded. Blood coagulates most rapidly in the open glass tube, somewhat later but often almost simultaneously in the corked glass tube (in 10 to 12 minutes). In the opened celloidined tube coagulation was slowed down (20 minutes) and was much slower (120 minutes) in the corked celloidined tube. Samples of about 5 c. c. were used in tubes of 12 c. c. capacity. Coagulation in the celloidined tubes started on the surface and took a long time, whereas beginning and termination of coagulation in glass tube fell close together and the both samples coagulated in the same time. These observations correspond with hes results of the above described experiments. Plenty of thromboplastin was produced in these glass tubes by the destruction of the platelets on the glass wall, thus the effect of corpuscles in the air has to be considered as negligable. Therefore clotting has been rapid, nearly identical in open and corked tubes. In the celloidined tube, where — as generally known — the destruction of platelets and thus the release of thromboplastin is delayed, instead probably corpuscular elements of the air take over their role. Consequently under such circumstances coagulation depends on what extent blood comes contact with air.

The fact, that coagulation starts always on the surfaces exposed to air and the previous finding (4), that native plasma coagulates in the same time in a corked and in an open tube provided filtered air, stresses the importance of corpuscular parts of air in the process.

On the basis of these facts the question arises, whether an alien surface is a physiological factor in blood coagulation? Because this is the case in every bleeding occuring on the surface of the body, however, it is well known that blood found by the surgeon in the cases of hemorrhages in cavities of the body is preserved in a liquid state. As no alien surface,

capable of exerting a thromboplastic effect, is available, in the lack of desintegrated platelets, the small amount of thromboplastin produced by the eventual destruction and decomposition of tissues, is unable to cause clotting, due of its small quantity. This a similar phenomenon to the one which can be observed sometimes, when, in order to prevent coagulation, less oxalate or heparin than necessary is added to the blood, or if these are mixed too slowly with blood. In this case, to some extent, thrombin is produced, coagulation starts and a small amount of fibrin appears, the whole blood, however does not coagulate, not even after hours. Thrombin produced in the beginning, as mentioned before, gets absorbed to fibrin and unable to take part in the further coagulation process.

SUMMARY.

Thrombin production during coagulation in native plasma is depending on the effect of thromboplastic substances (platelets, glass, corpuscular parts of air). Both the quantity of thrombin and fibrin during coagulation is increasing simultaneously and thrombin production culminates upon the termination of fibrin precipitation. At the beginning of fibrin production thrombin could not be detected by the described method. The absolute quantity of the precipitated fibrin is quite independent of the thrombin level that is minute amounts of trombin are as capable of converting the entire fibrinogen content of plasma into fibrin, as more substantial amounts, however, the speed of the conversion will differ with the available amounts of thrombin, thus ultimately depending on thromboplastic factors. The thromboplastical effect of the so called "alien surfaces" is probably indispensable to all blood coagulation.

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