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

FUNCTION OF THE HEALTHY AND THE DISEASED KIDNEY.*

BY ISTVÁN RUSZNYÁK.

In accepting the honour conferred to me by the Medical Association I find myself in a difficult position. I have to recall the memory of *Frigyes Korányi*. I am afraid that there are by now very few among us to whom this great physician means not just a name but a personal event. I am one of those fortunate ones who had the chance to attend his lectures as a medical student, and now, on looking back to his epoch, I ask myself what has been left from the activity of this great physician to his successors.

The work created by an artist or a writer does not perish after his death. The great statesman can determine the fate of his country for generations. The results of the scientist's research are still active when the developments of science have advanced far beyond them. But what remains of the work of a great physician?

To this question the life of *Frigyes Korányi* gives the answer. The great physician does not merely heal, for if this were so, his memory would only be cherished by the patients he had cured and maintained by the therapeutic methods he had develóped; the truly great physician trains whole generations of doctors and forms a school. The main significance of the school is not merely that the master passes his knowledge on to his pupils, but principally that he inoculates his way of thinking into them. This characteristic way of thinking is also the essential feature of Korányi's school. It began with *Frigyes Korányi*, was continued by his son, *Sándor Korányi*, and today it is still a living, progressive community. One of the most important features of this school is, presently too, the spirit which tries to transform medical science to an exact science, by applying the results and methods of the more exact sciences to the solution of its problems.

The greatest success of this effort has been recorded in the field of renal pathology, and it is for this reason that I took up this subject for my lecture. It is a well known fact that all exact knowledge of renal function and its disorders derive from the discoveries made by *Korányi*'s school, first of all *Sándor Korányi*. This knowledge started from the definition and measuring of renal function. Now, it would perhaps be useful to recollect the circumstan-

* Frigyes Korányi Memorial Lecture read at the Budapest Medical Association, on May 14th, 1948.

Fig. 1.

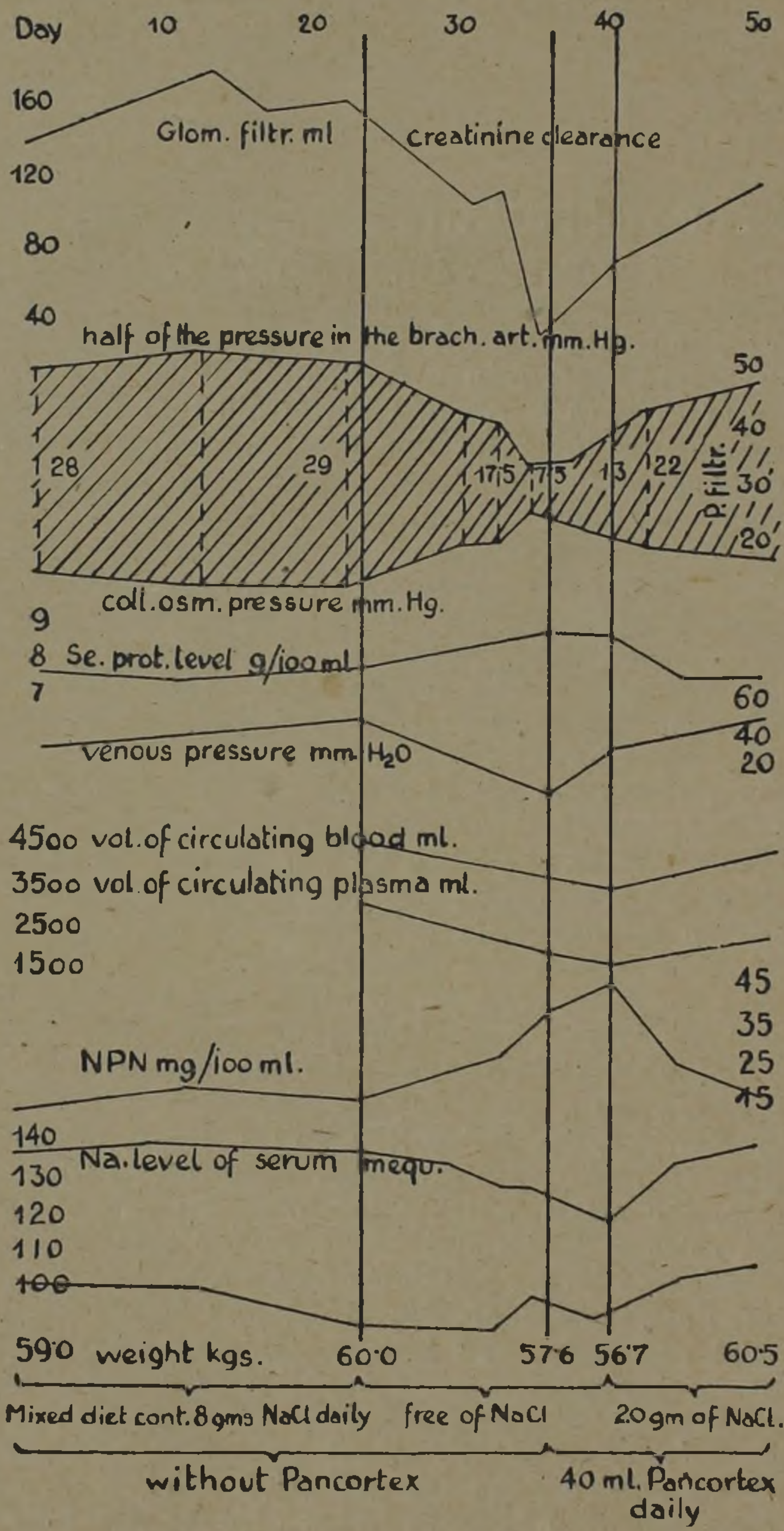

Figure 1.: Glom. filtr. ml. Creatinine clearance. Towards P. art. brach. Hg. m.m. 4500 circulating blood, ml; 3500 circulating plasma, ml 59.0 body weight, Kg. Varied diet, 8 gr NaCl daily. Diet without NaCl, 20 gr NaCl daily. Without Pancortex, 40 ml Pancortex daily.

ces under which the functional pathology of the kidney has been developed.

Sándor Korányi started his fundamental researches known the world over nearly 60 years ago. It was in 1890 that, during a gynecological operation, the surgeon injured the right ureter and stitched it to the abdominal wall. The fistula thus formed was extremely disagreeable to the patient and the surgeon applied to *Korányi,* who was then a young assistant at the Internal Department whether it would be advisable to remove the right kidney. After a long and careful examination *Korányi* revealed that the urine escaping from the fistula was more dilute and of a more constant composition

Fig.2

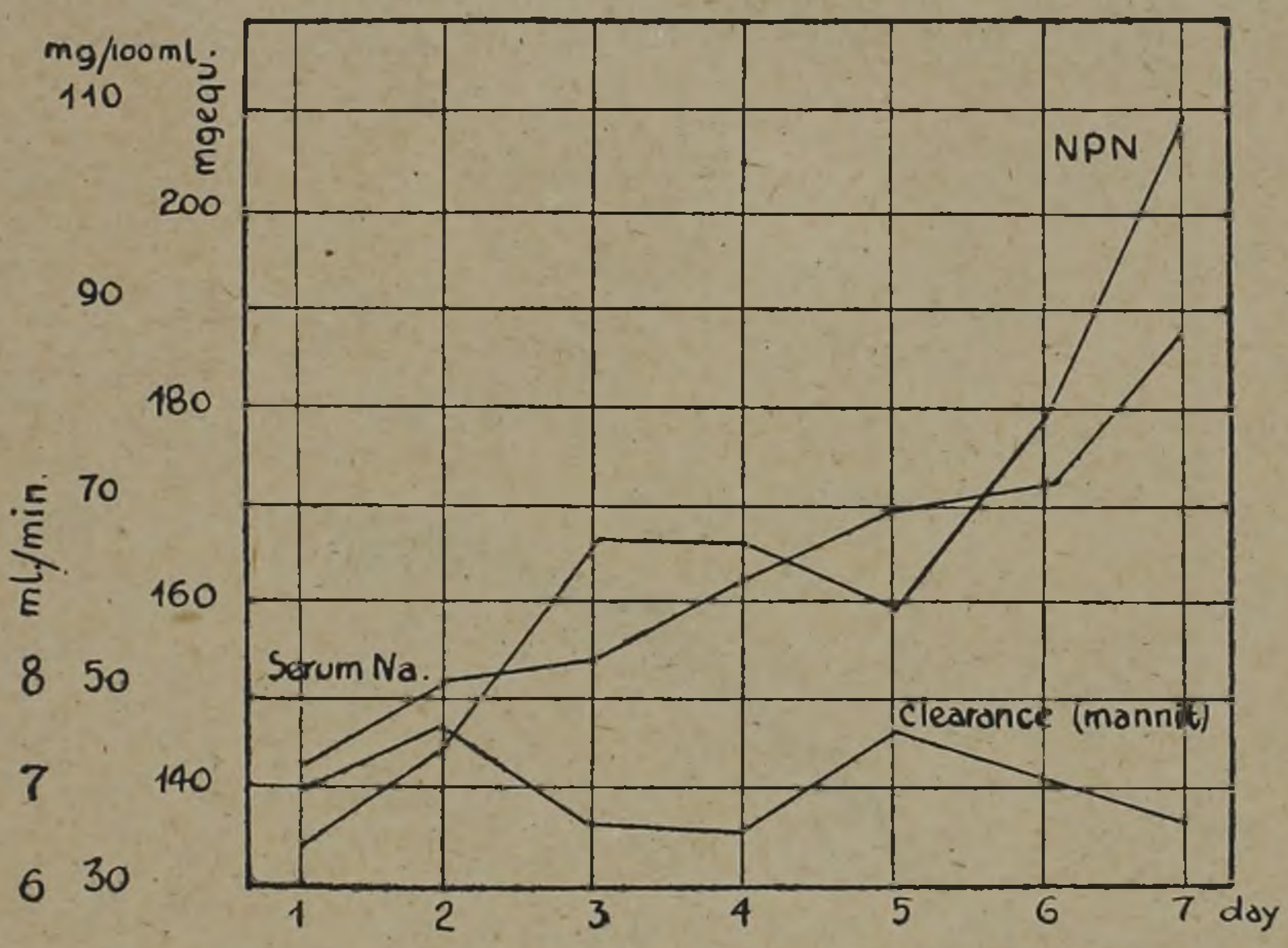

Figure 2.: ml/min.

than that of the bladder. From this he concluded that the right kidney was diseased. His presumption was ascertained by the operative autopsy. This observation was the basis of his later investigations resulting in the conclusion that the disorder of renal function particularly manifests itself in the incapacity of the diseased kidney of secreting a urine of varying concentration. The impairment of this function of the diseased kidney has been termed hyposthenuria and its failure asthenuria. (Volhard's term, isosthenuria, originated in the misunderstanding of etymology.) In asthenuria the composition of urine is much the same as that of albumin-free blood plasma. There is no space here to engage myself in the results of these investigations which elucidated the correlations of salt and fluid retention, and the mechanism of oedema formation. However, it

should be mentioned that the most important tests of renal function, the concentration and dilution test, have been devised by two of *Korányi*'s pupils, *Kövesi* and *Illyés*. The French authors *Albarran* and al. applied these tests later, on the basis of the publications of the Hungarian scientists.

The recent advances in the knowledge of renal function have been developed chiefly by two authors. One of them is *Richards* who tapped, by means of a micromanipulator, the glomeruli and various parts of the tubules of amphibian kidneys. The old presumption that in the glomeruli utra-filtration occurs and the composition of the filtrate is, as regards crystalloids, the same as that of the plasma has been proved hereby. In the tubules, a massive reabsorption of fluid takes place and it is here that glucose disappears from the ultrafiltrate, like salt and one part of the urea. He has learned that sugar reabsorption is accompanied by phos-

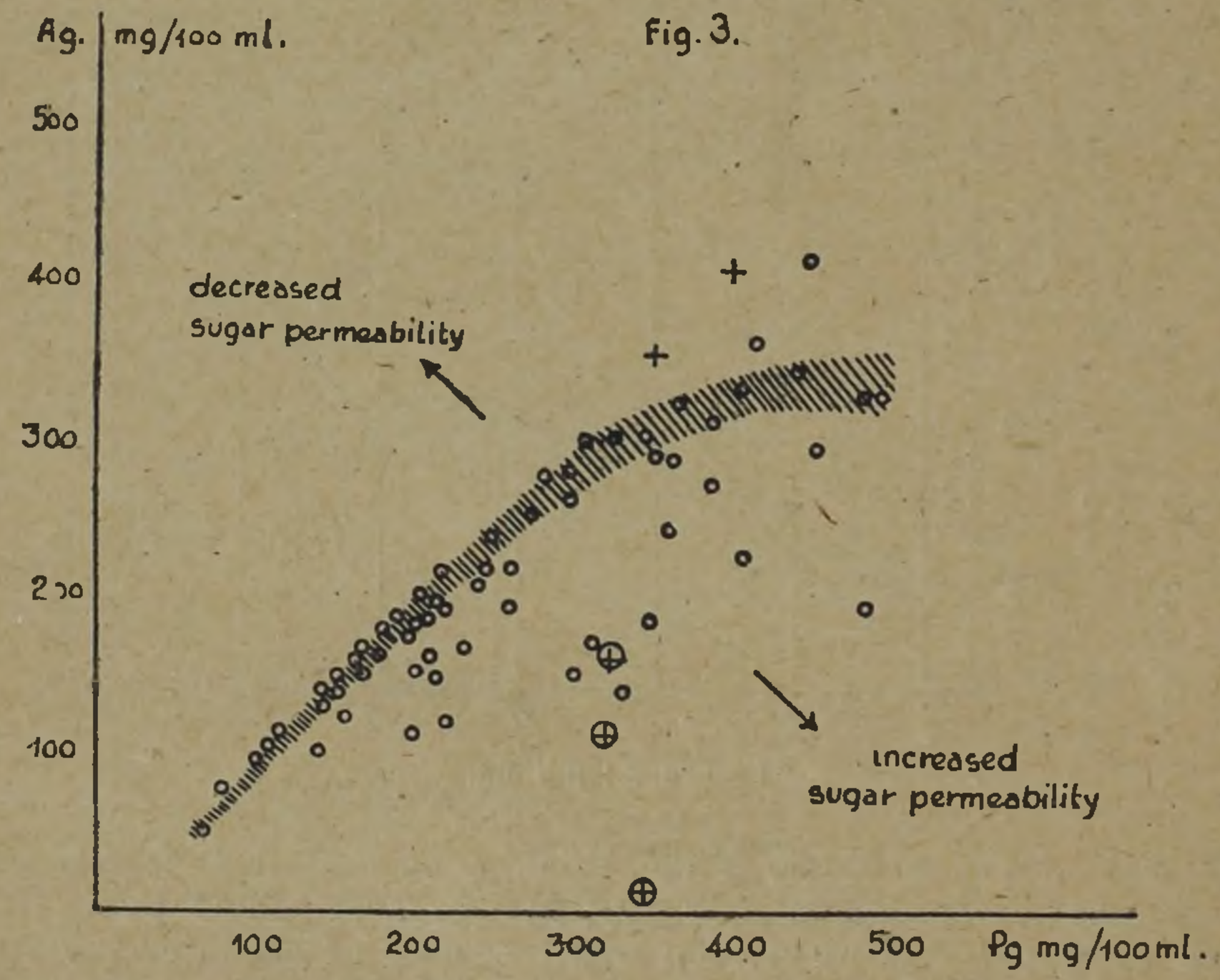

Fig. 3.

Figure 3.: Decreased sugar filtration. Increased sugar filtration.

phorylation, a process which can be impeded by phlorrhizin whereby phlorrhizin-glycosuria may ensue. *Richards* demonstrated further that certain substances are actively secreted by the tubular epithelium.

The other author to whom we are greatly indebted is *Van Slyke*. He endeavoured to find a mathematical form for the evaluation of renal function. He was preceded by *Ambard* whose formula, does, however. from a mathematical point of view, not yield more

than the determination of nonprotein nitrogen in the blood. Van Slyke has introduced the concept „clearance“ by which the quantity of plasma is meant which contains so much of a substance as secreted by the kidney in one minute. Therefore, one speaks of different kinds of clearances: urea, creatiuine, inulin, diodrast, penicillin, paraaminohippuric acid, glucose, chlorides, sodium clearance, etc.

I do not intend to discuss wellknown details. Only the fact should be mentioned that diodrast and similar substances are, unless their concentration in the plasma is high, completely excreted. Therefore, the diodrast, paraaminohippuric acid, etc. clearance are equal the quantity of plasma streaming through the kidneys in one minute (renal plasma flow). In this manner, from the clearance and the haematocrite value, the quantity of blood perfusing the kidneys in one minute can be estimated. It has been shown by

Fig.4.

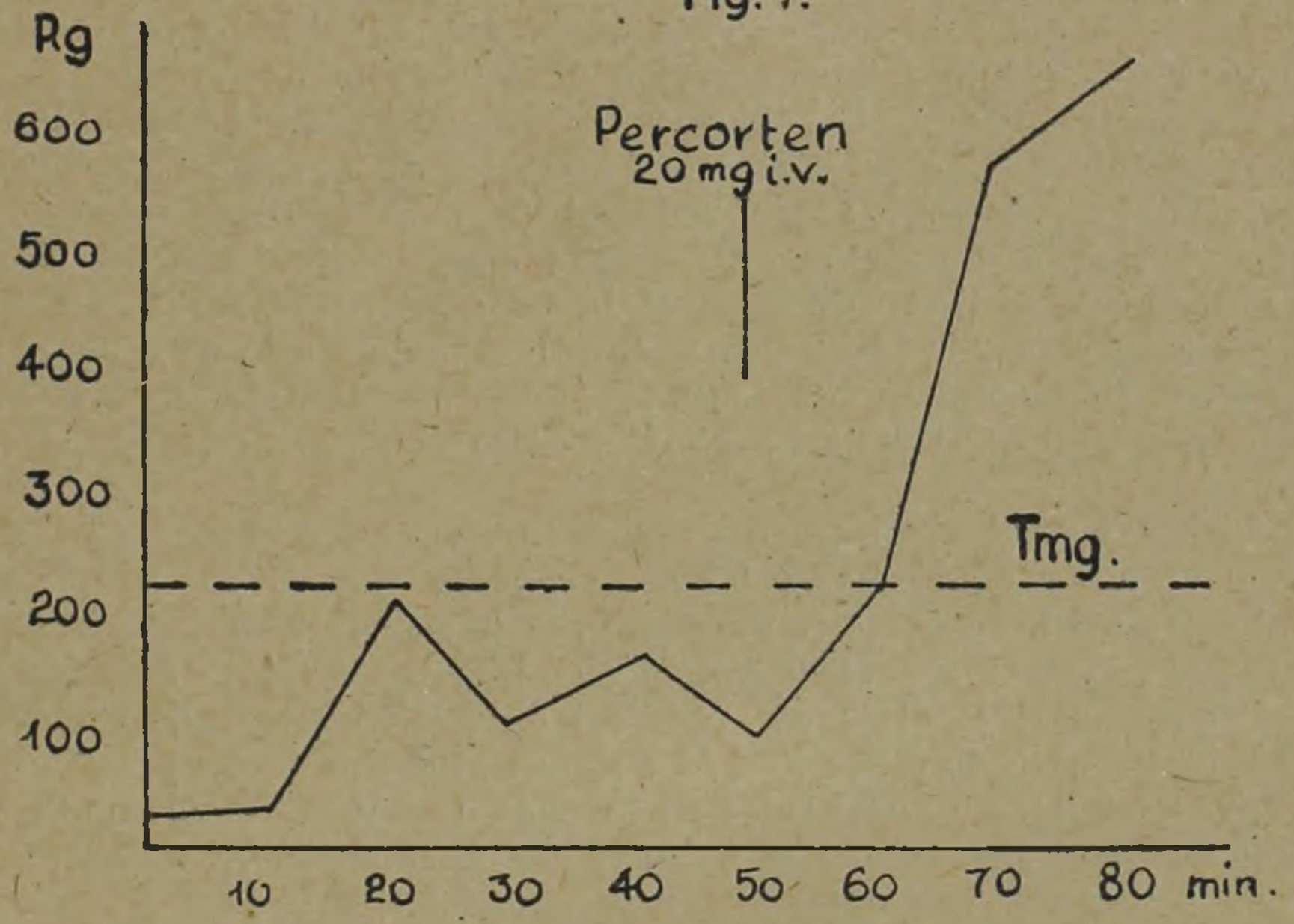

Figure 4.

Rehberg's investigations that inulin, mannite, endogenous creatinine, etc., are excreted exclusively through glomerular filtration; in the tubules they are neither secreted nor reabsorbed. They are called glomerular clearance substances. Thus the inulin-clearance, e. g., means the total of glomerular filtrate per minute. In adult, this amounts to 120—130 ml. From the quantity of the glomerular filtrate and the concentration of various substances in it which is equal their concentration in the plasma, it has been learned that about 99% of the filtrated water is reabsorbed in the tubules and of 120 ml filtrated per minute only 1—2 ml are excreted. All substances

of a lower clearance, e. g., inulin or endogenous creatinine, are reabsorbed whereas those with a greater clearance are also actively secreted in the tubules. In a given case, reabsorption or secretion can be quantitatively determined by simple calculation. It has been found that the increase of the concentration of these substances is, only to a certain limit, followed by the increase of their absorption and secretion, for the active secretory capacity of the tubules is limited. This value termed „Tm" is already known for many substances. For example, a healthy kidney cannot reabsorb more sugar than about 320 mg per minute, no matter how high the blood sugar level is.

It is an interesting and practically important fact that the upper limit of reabsorption can be influenced by certain substances. E. g. penicillin excretion diminishes if the kidneys have, at the same time, to excrete much hippuric acid. *Mosonyi* and his coworkers (II. Int. Dpt. Budapest University) have found that the effectiveness of a penicillin cure can be augmented by keeping the patients at a diet of oat flour. At this diet much hippuric acid is formed which inhibits the excretion of penicillin.

As to the functional changes of the diseased kidney, it has been soon found that, as it was to be expected. in glomerular alterations filtration is very much reduced. If this decrease is very extensive e. g. the creatinine or mannite clearance falls below 30—35 ml, nitrogen retention and azotaemia occurs. (In severe cases the clearance may fall as low as to 1—2 ml). Azotaemia is now generally considered to be due to inadequate glomerular filtration. However, azotaemia can, as it will be shown, arise through another mechanism also.

It has been known since a long time that a kind of azotaemia occurs with intact kidneys. This form was called by *Blum* „azotaemie par manque de sel". Presently, it is more often termed hypochloraemic azotaemia. *Kerpel—Fronius* was the first to demonstrate that, in these cases, it is not reduced chloride content of the blood which is decisive, but the loss of sodium and the accompanying exsiccosis. *Gömöri* and *Frenreisz* have shown that animals made azotaemic by the ligature of the pylorus can be kept alive much longer if they are given physiological salt solutions than by hypertonic salines though the hypochloraemia ceases after the injection of the latter also. *Gömöri* and his colleagues succeeded in elucidating the origin of hypochloraemic or exsiccosis azotaemia. Fig. 2 shows the changes found in a patient suffering from Addison's disease. When deprived of salt the patient's condition grew worse, the arterial blood pressure decreased on account of the fluid loss, the protein content of the serum and, at the same time, the colloid osmotic pressure, increased. The sequel of this condition was a reduction of the filtration pressure in the glomeruli, the filtration rapidly decreased as it could be seen from the low creatinine clearance. This is the mechanism of the increase of azotaemia. The administration of cortex hormone and salt resulted in a regression of the symptoms.

By means of the clearance method *Gömöri* and *Greiner* examined the glomerular filtration in recumbent and erect position. The filtration is invariably reduced in erect posture the difference is, however, more marked in orthostatic albuminuria than in healthy people. The explanation of this phenomenon can obviously be found in the fact that the lumbar lordosis attending orthostatic albuminuria causes stagnation in the renal veins whereby the filtration diminishes. These investigations represent a confirmation of *Jehle*'s view that orthostatic albuminuria be due to lumbar lordosis.

TABLE I.
Creatinine clearance.

	Lying	Walking	Change (in %)
Mean value, from 7 normal individuals	134	119	—17
Mean value, from 6 individuals with orthostatic albuminuria	130	73	—44

The magnitude of normal filtration raised the idea that diuresis (be it water-, salt-, or any other kind) was due to the increase of clearance. It has been learned that, in adults, this is not the case. In infants and rabbits diuresis is really the result of increased glomerular filtration. In dogs and in adult human beings the cause of increased water secretion is not an increase of filtration but a decrease of tubular reabsorption. As I have already mentioned, normally about 99% of the filtrate are reabsorbed and only the remaining 1% forms the 1—2 lit. of urine daily excreted. If tubular reabsorption decreases e. g. by 1%, i. e. to 98%, the daily quantity of urine is doubled. It is already common knowledge that 80% of water reabsorption is an obligate process whereas 20% is controled by the activity of the posterior lobe of the hypophysis. If this hormone is absent e. g. in diabetes insipidus that part of the reabsorption regulated by the hormone is missing whereas the „obligate" reabsorption is unchanged. Thus the maximum diuresis cannot exceed this 20% i. e. about 40 liters of urine per day. Following water ingestion the production of the hormone decreases and so does reabsorption whereby diuresis results. At the same time, the action of the cortical hormone exerts a greater influence on the proximal tubules in which more salt will be absorbed. This latter is an important mechanism because, otherwise, the ingestion of water or tea would result in an immense loss of salt.

On the basis of the above said it could be expected that morbid changes in the tubular epithelium would result in an impaired absorption i. e. diuresis. In fact, the first stage of mercury chloride and cyanide poisoning is attended by an increase of diuresis. In *Starling*'s preparation it was also found that if the blood used for the perfusion was cooled the kidney responded with a greater excretion of urine. In tubular diseases there is, however, no diuresis present,

on the contrary, oliguria or anuria ensues. As shown by *Frey* and our own examinations, in nephrosis the clearance is normal while the reabsorption of water and salt is definitely greater than the normal. One of *Richard's* observations is very interesting: he produced anuria in frogs by poisoning them with corrosive sublimate and showed by puncture that the filtration was normal in the glomeruli but the filtrate was thoroughly absorbed in the tubules. Being unlikely that the diseased, especially necrotic, epithelial cells could function better than the healthy ones the increased reabsorption occurring in tubular diseases should be regarded as a passive process. Let us realize that filtration and passive reabsorption take place in all normal peripheral capillaries, the site of filtration being the arterial and that of reabsorption the venous section. Under normal conditions, the whole filtrate returns passively into the circulation. In the kidney, filtration occurs in the glomerulus. The site of reabsorption is, in contrast to the conditions existing in other parts of the organism, lined with living epithelium the function of which results in that only 98—99% of the filtrate are absorbed while the rest is excreted. It is easy to see that, in degeneration or desquamation of the epithelium, a process similar to that in the capillaries may occur the whole filtrate being passively absorbed and anuria ensues.

It seemed necessary to investigate the effect of diseases of the renal epithelium on the function of the organ. *Földi* and *Szabó*, my co-workers, produced acute and chronic lesions in the tubules. If they compressed one of the renal arteries for two hours and then released it, they found the tubular epithelium more or less severely damaged as a sequel of lack of oxygen. The function of the healthy kidney was normal. The injured kidney showed typical functional changes according to whether the injury was relatively slight or severe.

TABLE II.

Dog's kidney after a 2-hour constriction (severe lesion).

	Intact side	Ischaemic side
V ml — — — — — — — —	3.8	1
Clear. inulin. ml — — — — —	34	3.6
Clear. p-amino hipp. ac. ml — — —	70	2.8
U_{Cl} mgr % — — — — — — —	25	340
P_{Cl} mgr % — — — — — — —	400	400
U_N — — — — — — — — —	530	52
P_N — — — — — — — — —	50	50

V = Quantity of urine/min.
U_{Cl} = chloride concentration of urine
P_{Cl} = chloride concentration of plasma
U_N = nitrogen concentration of urine
P_N = nitrogen concentration of plasma

After a slight injury the paraaminohippuric acid clearance became equal to the inulin clearance, i. e. the secretion of this substance had completely ceased; it was only filtered. If the injury was greater the paraaminohippuric acid clearance was, like in *Bobey's* experiments, lower than the inulin clearance, i. e., it had been partially absorbed. This observation harmonizes with a case of *Chasis*. He found in severe sulfonamide poisoning that, at the peak of the illness, paraaminohippuric acid was reabsorbed, when improvement began it was filtered without absorption and during convalescence secretion was again resumed. It is not only the paraaminohippuric acid which can be absorbed in tubular lesions. *Richard's* demonstrated in 1938 that in uranium poisoning creatinine was partly reabsorbed. *Földi* and *Szabó* found in uranium poisoning that creatinine, paraaminohippuric acid, chlorides, and nitrogen, underwent absorption to the same extent, a fact favouring the view that a passive absorption took place. In such cases, the resemblance of the composition of urine and blood is due to the fact that reabsorption is passive, an unchanged ultra-filtrate is absorbed by the tubuli and the different substances are not distinguished by the epithelium.

TABLE III.

Passive rediffusion of sugar in uranium poisoning.

Clearance.

Time (Min.)	i	c	PAH	N	g.	
10	5.4	5.3	4.7	5.1	5.2	
20	5.8	5.2	4.6	3.5	3.7	
30	3.3	3.1	2.6	2.1	2.0	0.5 gr
40	3.7	4.0	3.3	2.7	2.7	← phlorrhizin
50	3.6	3.4	3.0	2.1	2.1	

i = inulin
c = creatinine
PAH = paraaminohippuric acid
N = nitrogen
g = glucose

In such cases, glucose also appears in the urine, what *Hetényi* was the first to demonstrate. Normally, the absorption of sugar is an active process which can be inhibited by phlorrhizin; in uranium poisoning phlorrhizin does not affect the limited but entirely passive process.

The increased passive rediffusion due to the destruction of the tubular epithelium may be accompanied by the impaired secretion of nitrogen compounds whereby tubular azotaemia can develop. Years ago I demonstrated that non-protein substances containing nitrogen can pass the diseased mucosa of the bladder whereby a cystogenic azotaemia may result. In the cases of tubular

azotaemia it is the diseased tubular epithelium that permits increased reabsorption. Therefore, the earlier conception that azotaemia is invariably associated with reduced clearance cannot be maintained. This has been proved by my co-workers *Bálint*, *Hársing* and *Lenner*. They found in rabbits that a high increase in the sodium chloride content of the blood was followed by azotaemia in spite of normal or even increased clearance. Histological examination showed, in such cases, severe damages in the tubular epithelium.

In the following the results of those investigations related to the problem of glycosuria will be discussed.

It is known that phlorrhizin causes glycosuria in the healthy kidney but not in the diseased one. This phenomenon has been applied to the diagnosis of unilateral renal diseases. Phlorrhizin is undoubtedly a poison to the kidney: it inhibits phosphorylation, a pre-requisite of tubular reabsorption. At first sight, it is difficult to understand that a poison is more effective on a healthy kidney than on a diseased one. My colleague *Földi* found the solution of this problem. His experiments showed that there was so little

TABLE IV.

Nephrolithiasis l. s.

		right	left
C	— — — — — —	43	14
Fg	— — — — — —	48	15.5
Eg	— — — — — —	5.5	0
Rg	— — — — — —	42.5	15.5

C = creatinine clearance
Fg = filtrated glucose
Eg = excrcted glucose
Rg = reabsorbed glucose

glomerular filtrate in the diseased kidney, i. e., so little glucose got into the tubules that they could reabsorb this minimal quantity. An additional factor was mentioned above: in tubular diseases the reabsorption of sugar is passive, hence phlorrhizzin has no effect upon it.

Another question was related to the observation that, in certain diseases such as renal disorders, arteriosclerosis, etc., glucosuria is missing though the blood sugar is occasionally high. Under normal conditions, glucosuria occurs when the sugar content of the blood rises above 170 mg. percent.

This value is called the sugar threshold of the kidney. In the cases mentioned missing glucosuria associated with an elevated blood sugar level was attributed to a rise of the threshold. Our investigations have made it clear that in these cases the glomerular

TABLE V.

Cases of kidneys not filtrating sugar.

Number	Pg	C	Fg	Eg	Rg
1	350	5.	17.5	0	17.5
2	420	1.4	6	0	6
3	260	3.7	9.9	0	9.9
4	347	16	55.5	0	55.5

Pg = Plasma sugar concentration
C = Creatinine clearance
Fg = filtrated sugar
Eg = excreted sugar
Rg = reabsorbed sugar

filtration is so slight that, in spite of the high blood sugar level, the quantity of sugar arriving at the tubules does not exceed the normal one. This quantity is, however, reabsorbed.

Glucose excretion is not a function of any hypothetical threshold. It depends on whether or not the quantity of glucose filtrated exceeds the reabsorbing capacity of the tubules. If the blood sugar rises and more glucose is filtrated more will be reabsorbed until the blood sugar exceeds 170 mg%. Then the tubules can no longer keep pace with the supply and the glucose is partly excreted. At a blood sugar value of about 700—800 mg% the tubules perform a reabsorption equaling their maximum capacity. This is about 320 mg/min. (Tmg). From these it follows that the expression „sugar-threshold" has a dynamic meaning. In glycosuria, the sugar threshold rises as the blood sugar does until the reabsorbing capacity of the tubules can meet with the increased supply. My colleagues *Szenes* and *Földi* have termed this dynamic threshold values „aglycusoric-blood-sugar-level". By this term that blood-sugar level is meant above which, in a given hyperglycaemia, sugar is excreted. Being able to estimate, by clearance examinations, the quantity of the filtrated, reabsorbed, and excreted sugar, the aglycosuric blood sugar level (Ag) can be calculated by the following simple formula:

$$Ag = Pg - \frac{P}{U} Ug,$$

where Pg is the sugar content of plasma, Ug that of urine, P means the creatinine concentration of the plasma, U that of the urine.

If individuals having intact kidney suffer from a diabetic or artificial hyperglycaemia their aglycosuric blood sugar level bears a relation to the various blood sugar concentrations which can be recorded. Of course, this curve cannot rise above a certain level (Tmg). If the Ag is above the curve, i. e. it corresponds to a higher sugar threshold the renal function is, according to the above

said, no longer intact. Below the curve are recorded those cases in which the sugar threshold is, according to the old conceptions, low, i. e., the cases of diabetes renalis and those treated with phlorhizin.

TABLE VI.

Low Ag.

Number	Pg.	Ug.	Found Ag.	Normal Ag.
17	390	4400	177	242
27	480	8100	188	334
29	139	1000	99	143
33	300	6400	147	258

Pg. = sugar concentration of plasma
Ug. = sugar concentration of urine

By determining the aglycosuric blood sugar level cases of diabetes mellitus were found in which the sugar excretion was greater than it was expected. In these patients diabetes mellitus was associated with renal diabetes. *Földi, Szabó* and *Zsoldos* have devised the equation of the curve also:

$$Ag = 0.00121\ Pg^2 + 1.3\ Pg - 23.5\ (\pm 14)$$

with the aid of which it can be established whether the aglycosuric blood sugar level shows a significant divergence from the normal one. Of course, the direction of the deviation can also be stated.

Szenes has pointed out that a similar hypothetical blood level can be computed for other threshold substances also. By this method. *Földi* and *Zsoldos* have stated that the threshold of sodium or chlorine is reduced by novurit.

Further investigations were concerned with the maximal reabsorption of sugar: Tmg. Considering that absorption is a process accompanied by phosphorylation we studied the effect of the adrenal cortex hormone on Tmg. This hormone has a stimulating effect on phosphorylation. Its administration resulted, in animal experiments, in a great increase of Tmg. Thus, the value of Tmg is limited by the phosphorylation capacity of the kidney. Interestingly, this effect of the hormone does not ensue in animals suffering from alloxan diabetes, either because the kidney is damaged by alloxan, or because phosphorylation is, as stated by *Laszt*, so intensive in diabetes that it cannot be further increased.

Still a few words should be said about the results of *Trueta* and al. *Bywaters* and *Beall* observed during air raids on London that severe bruising of the extremities was frequently followed by oliguria, and even anuria. Trueta and his coworkers carried out animal experiments to reveal the mechanism of this anuria. They found, after crushing of the extremities of the animals, extensive vasoconstriction (reflex?) in large areas far from the injury, and in the kidneys too. Later, a similar effect could be elicited by stimulating the sciatic nerve or the hilus of the kidney with faradic

current, further by injecting pituitrin or staphylococcus toxin to the animals, etc. The changes of the circulation resulted in a peculiar picture in the kidneys. The ischaemia resulting from vasoconstriction was restricted nearly exclusively to the cortical substance. There was no circulation in the glomeruli. At the same time, there was an enormous hyperaemia in the medullar substance, the vasa recta and the so — called juxta — medullar glomeruli lying at the border of the cortex were markedly congested. The essential change of the circulation consists in that blood is diverted from the cortical glomeruli and flows through the large juxtamedullar glomeruli, like through a short circuit, to the vasa recta, and into the veins. The great reduction in the number of active glomeruli accounts for the oliguria or anuria. The so-called reflex anuria occurring in human pathology e. g. in renal stones is due to this mechanism. The rare pattern called bilateral cortical necrosis of the kidney also belongs to this group. Recently, *Gömöri* recognized this condition, which was formerly a post-morten finding, in a living patient.

My co-workers, *Gömöri, Földi* and *Szabó*, have partly checked partly completed the work of *Trueta* and his co-workers from a functional viewpoint. First they confirmed *Trueta*'s results by morphological methods, then they have demonstrated that cortical ischaemia is attended by reduced glomerular filtration and the quantity of blood perfusing the kidneys diminish. The phenomenon can well be explained by the reduced number of active nephrons. Hereby, functional evidence has been added to *Trueta*'s morphological results. Interestingly, they observed that. in cortical ischaemia, the sugar absorption pertinent to one nephron was increased. The phenomenon is still unexplained. It may be due to medullar hyperaemia or to the larger size of the juxtamedullar nephrons.

Obviously, the majority of the new facts discovered by the investigation of renal function are based on the clearance method. Therefore, attention should be paid to the sources of error of this method. Of course, the importance of suitable chemical methods need not be emphasized. On the other hand, the exact collection of urine and the determination of its quantity minutely is frequently missing in the experiments reported. The smaller the quantity of urine the greater this error wherefore it is wise to perform experiments as far as possible with an abundant diuresis and, to obtain, by repeating the experiment, data of several periods. There is still a question of principle which is still more important. The whole clearance method is built up on the hypothesis that the so-called clearance subtances — i. e., inulin, mannite, endogenous creatinine, etc. — are exclusively filtrated and not subject either to secretion or to reabsorption. It is a question, however, whether this is true in pathological cases particularly in tubular diseases. It has been shown that the necrosis of the tubular epithelium leads to increased, in our opinion passive, rediffusion. The question is whether this is not applicable to the clearance materials too, and, if so, the basis of our calculation changes. It is not easy to decide

this problem. It is generally thought that, in passive diffusion, various substances pass the membrane at different speeds, according to their molecular weight, the smaller molecules passing more rapidly than the greater ones. In fact, if inulin and other substances having small molecules such as thiosulphate, creatinine etc., are intraperitoneally injected the samples taken after a time show that the quantity of inulin has less decreased than the concentration of the other substances. From this it may be inferred that in passive rediffusion of clearance substances their clearance will not be equal and the substance being absorbed at a greater speed will have a lower clearance. A similar phenomenon occurred in our experiments on animals, in severe uranium poisoning in which the

TABLE VII.

Uranium intoxication in dogs.

Clearance

i.	t.	c.	PAH.	N.	Cl.	g
0.78	0.89	0.60	0.54	0.56	0.46	0.56

i = inulin
t = thiosulphate
c = creatinine
PAH = paraaminohippuric acid
N = nitrogen
Cl = chloride
g = sugar

reabsorption of creatinine was demonstrated by *Földi* and *Szabó*. Strangely, the clearance of thiosulphate having a small molecule was equal that of inulin. My co-workers (Gömöri, Földi, Komáromi and Szabó) could, in their examinations concerning renal diseases of man, not yet demonstrate the separation of clearances and the passive rediffusion of these substances. Nevertheless, it is advisable to work with at least two substances at the same time.

TABLE VIII.

Threefold examination of clearance in renal diseases.

Case	i.	c.	t.
1	3	3.2	3
2	7.3	92	73
3	114	119	85
4	1.1	9	9
5	10.3	122	131

i: inulin c: creatinine t: thiosulphate

The results quoted in this lecture supply an undoubted evidence for the contention that the knowledge of renal function has made an enormous progress during the last decade. At Sándor Korányi's time only a sum of renal functions could be determined. Presently, the details of the function are accessible to our methods. The question does, however, arise whether these results are relevant from a practical point of view. Huxley, the great biologist, has said that the goal of life is action, not knowledge. Action means in medicine curative work. Therefore, the statement made by Bright over 100 years ago is still a lecture to be read with a slight melancholy: it is humiliating to realize how little can be done in curing renal diseases, despite the fact that we have been engaged in them for over 10 years.

No doubt, the therapy of renal diseases has also made a great progress after Bright, however, there is a disproportion between the progress of therapy and theoretical development. It was a frequent event in the history of medicine that therapy was preceded and guided by theory. S. Korányi started from the conception of molecular diuresis, created a functional point of view and produced hereby a basis on which the dilution and concentration tests and functional therapy could be built up. Certainly, the better the healthy and pathological processes are understood, the surer the hands interfering with them. I hope that the progress of science will soon result in advances referring to the field of action i.e. curative medicine.

FROM THE II. MEDICAL CLINIC OF THE UNIVERSITY OF BUDAPEST
(DIRECTOR: PROF E. HAYNAL).

RECENT PENICILLIN RESEARCHES.

BY LÁSZLÓ MOSONYI.

(Presented at the session of the IV. Section of the Hungarian Academy of Sciences the 23 February 1948.)

The most practicable way of increasing the effectiveness of penicillin is by reducing as far as possible its rapid excretion. For this purpose a barrier should be erected to inhibit its passing through the tubular cells of the kidneys. The first experiments referring to this problem were carried out by *Rammelkamp* and *Bradley* with the dye diodrast, then the method of *Beyer*, *Woodward* and their co-workers based on paraaminohippuric acid became prevalent. In fact, an efficacious level of penicillin could be maintained for a longer time by this method (for 4—5 hours instead of 3). We have employed also the principle applied to the investigations of *Beyer* and his colleagues. First we tried to eliminate the drawbacks of the drop infusion of hippuric acid as employed by the American authors (e. g.sclerosing of the vessel wall may occur due to the continuous action of concentrated solutions). With this in view we abandoned the injections and tried to bring about an increase in the synthesis of endogenous hippuric acid. The animal experiments of *Hara* show that an ample content of animal protein and fat reduces the process whereas vegetable protein promotes this synthesis. We therefore composed a diet which was maintained for the duration of the experiment. The caloric requirements were for the most part covered by substances of vegetable origin whereas animal proteins and fats were reduced. Starting from the high hippuric acid content of horse urine which is increased still more by pure oat nutrition, we used oatmeal in large quantities in our experiments. Hippuric acid synthesis is also increased by certain fruits, such as prunes. Our diet contained about 40 g protein, 30 g fat and 200 g carbohydrate, and, to give the necessary amount of calories, we supplemented it with as much bread and cakes made of oatmeal as desired. By determining the Ha content of the urine the extent of hippuric acid synthesis could be checked. First the Ha content of the urine of the experimental subjects was daily determined for several days. In 10 individuals having an intact kidney the excretion of endogenous Ha was from 0.6 to 1.62. Then they were given the diet described above for two days and the Ha of the urine collected in 24 hours was determined. It appeared from our results that the Ha

excretion increased to many times the normal and usually reached the maximum on the second day. The slow digestion and assimilation of vegetable proteins so evidenced thoroughly corresponded to our needs, as the Ha level could thereby be maintained by the diet. While the diet is being maintained for more than two days the quantity of Ha excreted no longer rises after the third day but it remains. with small oscillations, at the same level. In the course of this diet a series of determinations were performed after the intramuscular injection of 20.000, 60.000 and 120.000 units of penicillin. Our data showed that the excretion of penicillin could be to a great extent retardated by this diet and, consequently, the penicillin level considered effective (above 0.05 unit pro ml) persisted for a longer time. The daily administration of 4×60.000 units seemed the most suitable from the aspect of both convenience and economy. *(Mosonyi, Oblatt* and *Surján.)*

Having worked out our dietary procedure, its difficulties had also to be taken into account (for example, to make the oatmeal palatable, special culinary tricks were necessary). We experimented also with chemical materials. *Beyer* and his co-workers observed a new and usefull detail in this field: they could slow up penicillin excretion by about 80% by using caronamide. It is to be taken every hour, or every two hours, in a relatively large quantity; that signifies its disadvantage, besides that during the treatment irritation of the kidneys may ensue. We found scarcely any drawback, however, of the use of pyramidon, which *Rosenthal* (personal communication) had already mentioned to increase the effectiveness of penicillin by raising its serum level about one and a half times. In clearing up the mode of action of pyramidon an important feature seems to be the fact that the penicillin administered can be nearly quantitatively found in the urine. Thus the rise in serum level may be due to the fact that, owing to the condensation of the vessel wall caused by pyramidon, penicillin excretion is inhibited in those parts of the organism where it is more rapidly disintegrated. Our hypothesis is confirmed by data which show that the higher serum level can be maintained, regardless of the quantity of pyramidon, if it is given repeatedly — whereas no stronger effect can be obtained by a high single dose. Through the single dose, the effect on the vessels can evidently be brought about only once *(Mosonyi* and *Ducks).*

On the basis of our experiments *Fleming*'s observation that in kidney diseases the excretion of penicillin is retardated may also be relevant. We found a rise in the serum level only when there was, at the same time, an increased hippuric acid content in the urine, i. e. the excreting capacity of the tubular cells was disturbed from other aspects also. If this disturbance increased to complete permeability, as for example in nephrosis, retention of penicillin could not be observed.

In the series of side-effects we distinguish the phenomena which occur through the physical, chemical or other direct action

of the penicillin molecule from those which are the results of prolonged or repeated dosage. It is a condition of direct penicillin action that the drug come in contact with the various sensitive parts of the organism in an adequate concentration. In this respect first of all the fact is relevant that penicillin is a weak acid and its high concentrations may cause the chemistry of the tissues to undergo a change. In the organs, penicillin is found in different concentrations. As stated by *Struble* and *Bellows*, the greatest quantities accumulate in the kidney, then in the oral mucosa and the bile. We examined also the changes in the pH of the saliva. These examinations performed with the saliva of 22 patients showed that, during the treatment, the chemical reaction shifted towards acidity *(Mosonyi, Surján* and *Szécsey)*. Direct effects of penicillin are further: change in the coagulability of the blood *(Mosonyi, Pálos* and *Komáromy)*, and epileptiform cramps arising through excitation of the central nervous system. A direct and lethal effect was shown in the guinea pig experiments of *Miescher* and *Böhm:* they demonstrated that after the administration of 50.000 units of penicillin per kg. body weight the animals died of suprarenal haemorrhage and symptoms of general collapse. Their experiments showed that the severe toxic effect did not develop in animals insensitive to vitamin C deficiency. Direct effect was also shown in our own experiments on isolated surviving frog heart. High concentrations of penicillin resulted in an immediate but reversible decrease in the contractions of the heart (Fig. 1).

Fig. 1. The reversible effect of 2000 O. U. of penicillin on the contractions of surviving frogheart in acid medium. (pH 6,5)

The question whether high concentrations of penicillin come in a contact with the various tissues depends not only on the structure of these tissues but also on the physico-chemical properties of the penicillin. Strikingly, bacteria found sensitive to penicillin displayed an unimpeded growth in the valvular vegetation of patients who had been treated for endocarditis lenta with penicillin for a due time with adequate doses. In order to find an explanation for this phenomenon we studied the diffusion capacity of penicillin. In accordance with the physiological conditions the diffusion was examined in the fibrin layers formed by the mutual action of fibrinogen and the thrombin produced by *Laki* and *Geren-*

dás. Our results showed that penicillin was, in a concentration of 0.5 units per ml (i.e. in a concentration persisting after an intramuscular injection for a few minutes only), incapable of penetrating a fibrin layer of 3 mm thickness. Further experiments carried out in test-tubes proved beyond doubt that penicillin may be adsorbed by colloids; substances reducing the surface tension (e.g. Na dehydrocholicum) inhibited the adsorption and the penicillin already adsorbed could be washed out of the precipitate by Na dehydrocholicum whereby its original efficacy had been restored *(Mosonyi, Held* and *Kocsán).* These results show that, although the ideal aim is a constant serum level to meet with various strains dividing at different times, still, it is advisable to give a high concentration of penicillin from time to time intravenously whereby deep tissues and the inner parts of vegetations become accessible to adequate concentrations of the drug.

Other clinical symptoms directed our interest to some indirect effects of penicillin treatment. These symptoms pointed to the deficiency of water-soluble vitamins. In fact, avitaminoses can hardly occur with our mixed diet, nevertheless disorders of the vitamin balance (hypovitaminoses) can be demonstrated by tolerance tests. In our experiments changes in the balance of the vitamins B_1, B_2, C, and nicotinic acid were demonstrated in patients treated over a long time with several million units, by the tolerance tests devised by *Magyar, Góth, Pearson* and *Winegar.* This statement refers mainly to vitamin C inasmuch as the dichlorphenol-indophenol reduction time of the urine (which is proportional to the vitamin C content) began to increase after a dose of about 1,000.000 units and on further increase of the quantity of penicillin showed a rise parallel with it. When the average reduction time of 1.5—2,0 minutes increased to over 10 minutes the deficiency could be balanced with 120 ctg. of vitamin C, and by giving 30 ctg. vitamin C daily no further change could be observed in the reduction time, i.e. the vitamin C balance was restored. The results of the vitamin C tolerance test carried out with *Góth*'s method likewise showed a significant correlation with the penicillin treatment: in 8 cases this value diminished to 60% of the normal. A decrease of similar degree was seen in 11 cases in regard of vitamin B_2. We found less marked differences with vitamin B_1 and nicotinic acid though a decrease could be stated in these cases also. Vitamin deficiency symptoms occurred also in healthy rats treated with penicillin *(Mosonyi* and *Oblatt).*

Ellinger and *Shattock* observed symptoms of nicotinic acid deficiency after the per os administration of penicillin; they explained it by an action of penicillin destroying the saprophytes which carry out the synthesis of nicotinic acid in the alimentary canal. For this purpose we began a series of bacteriological examinations with persons ingesting penicillin. Our results did not in the least prove a sterilization of the alimentary canal: we observed at most a disappearance of the haemolytic type of streptococci,

but the coli strains remained in an unchanged quantity. Sometimes, a change occurred in their biological features e. g. there appeared strains disintegrating proteins but such a shift as would impair vitamin synthesis either quantitatively or qualitatively was not observed.

We think the vitamin deficiencies to occur through a disorder in the equilibrium of the oxydizing systems (of which the vitamins themselves and their disintegration products form a significant part) by the penicillin treatment. To confirm this assumption we performed direct examination of cell respiration and quantitative determinations with the various oxydizing systems. The examination of brain and heart tissue in a Warburg apparatus during the treatment did not disclose any appreciable difference wherefore we turned to the chemical examination of the various components of the cell respiration apparently undisturbed as a whole. The most important was the examination of the glutathione-redox system deriving from the basic amino-acid of the penicillin molecule i. e. cystein. By applying *Bansi & Rohrlich*'s method it was found that, following a single penicillin injection, and during a constant treatment, the reduced glutathione content of the serum rose to one and a half times to twice the normal value. These results explain our earlier hypothesis that the organism tries to compensate for the increase of the glutathione-redox system by a parallel increase of systems serving similar purposes. It is a sign of this effort that hypovitaminoses occur and can be demonstrated by tolerance tests as a sequel of the greater vitamin-avidity of the tissues *(Mosonyi* and *Oblatt)*.

Clinically, one of the most characteristic symptoms of an apparent state of B_1 avitaminosis is a more frequent heart beat. Though it was shown in the experiments mentioned that hypovitaminotic symptoms do occur, still the tachycardia suddenly observed after the onset of penicillin treatment cannot always be explained by vitamin deficiency, as in many cases the food is entirely satisfactory and neither the amount of penicillin taken nor the duration of the treatment could have occasioned the accelerated heart action. According to *Libbrecht* and *Danielopolu*, glutathione increases cholinesterase activity, that is, it shifts the vegetative innervation towards the adrenergic state. With regard to our preceding data indicating that the administration of penicillin leads to an increase in the glutathione content of the serum, the tachycardia may be explained by the adrenergic state.

There is still another phenomenon to be mentioned of the difficulties encountered in combating decompensation tachycardia. This chiefly refers to patients treated for a long period. The significance of this fact lies not only in the increased frequency of the heart function representing an overload but in the general observation that endocarditis lenta cannot be successfully treated with penicillin without the restitution of compensation. We tried to determine in healthy pigeons whether the difficulty of bringing about

compensation was due to the mutual antagonism of penicillin and digitalis or to influences created in the organism independently of the chemical structure of the drugs. At this item, we had also to answer the question whether or not the decrease in the efficacy of digitalis were a manifestation of B_1 hypovitaminosis due to the penicillin treatment, considering *Wenckebach*'s finding that in beriberi the heart does hardly react to digitalis. On the other hand, the lipoids of the organism, chiefly the cholesterol, likewise increase in B_1 hypovitaminosis *(Beznák)* whereby a partial absorption of the digitalis glycoside in the lipoids can occur. In the experiments performed by the technique of *Hanzlik, Méhes* and *Péter*, 34 pigeons were given penicillin parenterally once or twice a day, in quantities corresponding to a daily human dose of 500.000 units. Though we were careful to see that the food of both groups was adequate, part of the animals were also given a daily injection of 1 mg vitamin B_1, to prevent hypovitaminosis. Vitamin C was unnecessary, pigeons being capable of synthetizing this vitamin. At the beginning of the experiments and after three weeks treatment the sensitivity of the animals to digitoxin and digitalin was examined. We found that the animals given vitamin B_1 also behaved quite differently from those treated exclusively with penicillin. There was less reaction among the latter to toxic doses of digitalis (because much of the glycoside was absorbed by the cholesterol-plus brought about by penicillin), whereas they reacted to small doses, instead of the expected vagus effect occurring in healthy animals and in those treated with penicillin + vitamin B_1, with a shift towards an adrenergic state, i. e. with accelerated pulse, elevated P and ST in the ECG and a reduction of the conduction time. These differences may account for the difficulties of restoring the compensation in patients treated with penicillin and show that the simultaneous administration of vitamin B_1 is advisable *(Mosonyi, Szendei* and *Porszász)*.

In the serum of 6 of 11 patients treated for a long time with penicillin an increase of cholesterol content was established. Thus the above mentioned statement of *Beznák* may hold true of our material.

The increase of cholesterol content can be brought into a correlation with another, likewise indirect, penicillin effect i. e. the sensitization phenomena associated with penicillin treatment. The greater part of the protein of fungi has an antigen character and, penicillin being a product of the fungus species penicillium notatum, its injection or reinjection immediately after the interruption of the treatment, may produce various sensitivity symptoms (urticaria, muscular pains) though it was proved, that penicillin sensitivity has nothing to do with penicillium spores. Earlier investigators believed the phenomenon to be caused by impurities in the penicillin preparations, but as there was no decrease in the number of the above phenomena after the marketing of crystalline penicillin (according to *Gordon* allergic reactions occur with the fre-

quency of 1 in 1500—2000), it became evident that the antigen effect was due to the penicillin molecula itself. We succeeded in 1946 in obtaining a direct proof of this presumption, by bringing about the Praussnitz—Küstner reaction with the serum of patients showing allergic symptoms after the administration of 21,000.000 units. Systematic examinations of the blood, however, proved that sensitization was invariably produced by penicillin treatment (eosinophilia occurred without exception after about 4—5 million units). Nevertheless, these phenomena yield, during the treatment, to a spontaneous desensitization. The balance of the vegetative nervous system also changes — this is confirmed also by our observations that Pirquet's skin test repeated during penicillin treatment results in a decrease of the hyperaemic area and, in some cases, the test becomes negative during penicillin treatment. All these allergic symptoms may, independently of the mycotic origin of the drug, be attributed to the vitamin deficiency occurring with penicillin treatment whereby the state of the vegetative nervous system may be essentially influenced *(Mosonyi* and *Oblatt)*. Probably, the great sensitivity to penicillin observed by *Miescher* and *Böhm* in animals incapable of vitamin C synthesis is due to the increased vulnerability of the vegetative nervous system.

Greater disorders of the vitamin balance may lean to the lesion of the peripheral nerves especially their inflammation; as seen from the results of the histamin hyperaemia test devised by *Lewis* and *Haynal* such lesions never occur with penicillin treatmeat.

In the last experiments the mechanism of penicillin action was examined. As mentioned, a rise in the reduced glutathione level of the serum could be detected after the administration of penicillin. An increase of this or related substance, containing an SH-groups, occurs also in test-tubes if penicillin is added to a bacterium culture. The increase being independent of the sensitivity of the bacteria to penicillin, the phenomenon is considered non-specific. In pure morphologic examinations *Holzer* and other authors have observed that penicillin has rather an enhancing effect on the growth of bacteria. This observation is in accordance with the increase of glutathione. Evidently, this substance representing a redox-system stimulates the metabolic processes. Besides this, being the chief constituent of the protein enzymes papain and cathepsin it provides for the disintegration of the amino acids necessary for the nourishment of bacteria. Similarly, the data of *Szold* and *Zádor*, and *Sankovits* and *Korossy*, are also in accordance with our observations. They found that the action of penicillin is promoted by methylene blue and inhibited by substances having an energetic oxydizing and reducing effect. Owing to its low redox potential glutathione fits, electrophysically, into this system.

In explaining the second phase of penicillin effect, the creation of specific bacteriostasis, it is again the increase in the quantity of glutathione to be dealt with. As shown by *Fildes*, a minimum of glutaminic acid is necessary for the multiplication of bacteria. This

is synthetized by bacteria on the surface of their bodies. In view of the fact that one third of the molecule of glutathione is supplied by the glutaminic acid synthetized, at least partly, by the bacteria and extracted from the surface of their bodies (as shown by our test-tube experiment) and, on the other hand, the thesis that the surface of spherical bodies is relatively the smallest in comparison with their volume, it is understandable that cocci are more sensitive to penicillin whereas bacilli having a relatively large surface are less exposed to such outside influences as e. g. the antibiotic action of penicillin.

In the course of our investigations we were concerned with several chapters of penicillin research. A review of our results proves that the solution of an apparently theoretical problem frequently represents, beside a contribution to the building of science, also a relevant progress in practical therapeutic.

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FROM THE II. MEDICAL CLINIC (DIRECTOR PROF. E. HAYNAL),
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TACHYCARDIA AND SENSITIVITY TO DIGITALIS DURING PENICILLIN TREATMENT.

BY LÁSZLÓ MOSONYI, ÁDÁM SZENDEI AND
JÁNOS PORSZÁSZ.

Among the many therapeutic effects of penicillin one of the most significant is the successful treatment of subacute bacterial endocarditis. According to *Christie*'s results, of 61 „lenta" patients treated with penicillin 58 have been cured. But *Christie* emphasizes that, during the whole course of treatment, great importance must be attached to digitalis, for the chances of recovery are less in decompensated patients. Other authors, whose results were not so excellent have also stressed the fact that the success of digitalis treatment is a very essential element in perfecting the cure. But in the question of decompensation the new studies of *Fiese* are also noteworthy, as he points out that, contrary to previous opinion, in the fatal issue of the majority of subacute bacterial endocarditis cases the death can be ascribed to heart failure.

Our experiences obtained with 14 cases of endocarditis lenta show that a dose of digitalis effective in other cases of heart failure is not sufficient given simultaneously with penicillin to restore compensation; in spite of the disappearance of septic symptoms (fever, embolism) the pulse rate often cannot be reduced to below 90—100, even by large doses of digitalis. Of the 14 patients there were altogether 3 in whom digitalis had a lowering effect on the pulse rate, and one of these later died of coronary thrombosis. In 8 patients the tachycardia was constant; in 3 cases, the pulse accelerated in spite of strophanthin injections given daily for weeks, until death ensued. During the same time, in patients treated with moderate doses of penicillin for ailments other than those of the heart, the pulse rate usually decreased parallelly with the fever. Patients suffering from subacute bacterial endocarditis were given 25—30 million units, in other diseases (sepsis, abscess of the lungs, pneumonia) an average of 3—4 millions. Those patients who were given *greater* quantities of penicillin for other diseases likewise became tachycardiac, or remained so. E. g. the pulse rate of one of our patients having an abscess of the lung who had been given 11.800.000 units of penicillin had a pulse rate constantly above 100 during a three months treatment, though he was kept in bed and had, with the

exception of the first four days, no fever. Neither the physical examination, nor the ECG or other methods could reveal a change which would have explained this tachycardia. On a control examination made three months after the penicillin treatment was over the patient was thoroughly well, with a pulst rate of 72. Christie's findings are, therefore, interesting for us also from another point of view, namely that the tachycardia of penicillin-treated patients is more difficult to combat than usually.

In order to examine whether penicillin really diminishes the effect of digitalis or the phenomenon is due to some other change in the patient's organism, the disease, or its progression, animal experients were carried out. The most important problem to investigate was the mutual effect of digitalis and penicillin in healthy individuals. In accordance with *Hanzlik* pigeons were employed as experimental animals. Their sensitivity to digitalis can be rapidly established by the number of vomitings occurring as an effect of the glycoside given intravenously. Before and after the administration of digitalis the condition of the heart was observed with ECG as *Méhes* and *Péter* did it.

In these experiments *Méhes*' technique was applied: 0.2 mg of Digitoxinum cryst. Merck per kg body weight was dissolved in alcohol and injected into the wing vein of the pigeon. Vomitings were counted for 6 hours after the injections. ECGs were taken with needle electrodes from the right wing and left leg of the pigeon before and 15 minutes after the injection. The pigeon lay on its back, its head was covered. Thereafter, for three weeks, the animals were given daily 7.000 units of penicillin per kg body weight. This corresponds to a daily dose if 500.000 units for a man of 70 kgs, that is, the dose usually employed in subacute bacterial endocarditis. In general we gave the penicillin in two doses each day, thereby differing from the usual 3-hourly dosage. On the basis of newer experiences in this department an absolutely even serum level for a good therapeutic effect is not aimed at. Besides the 3-hourly dose of 40.000 units, twice a day a larger dose (on an average 120.000 units) is usually injected into the patients, in the hope of a more energetic anti-bacterial action. On the other hand, it appears from the investigations on the diffusion of penicillin of *Mosonyi, Held* and *Kocsán*, that penicillin getting from the blood stream into the tissues preserves a higher level for a longer time and does not decrease like in the blood. In another communication we have reported that a diet containing vegetable protein (oat flake) but poor in animal protein and fat, considerably increases the synthesis of hippuric acid and parallel with this, penicillin remains in the blood for a longer time at a higher level. Our pigeons were also fed mainly oats. This diet undoubtedly increased the formation of hippuric acid and the related ornithuric acid in their organisms. On the basis of these facts it seemed warranted to assume that, if an even serum level could not be maintained by 1 or 2 injections daily, penicillin was constantly present in the animals and thereby a

method was offered to observe the action exerted on healthy tissues.

A part of our animals were given 0.1 mg vitamin B_1 daily during the penicillin treatment, in view of the fact that after a protracted penicillin treatment disturbances in the household of watersoluble vitamins, demonstrable by tolerance tests, may arise *(Mosonyi* and *Oblatt)*. At this item an early observation of *Wenckebach* should be quoted who found that, in beri-beri, the heart is less sensitive to digitalis.

In pigeons the above mentioned nauseating effect of digitalis is, according to *Hanzlik*, due to a reflex mechanism evoked by the digitalis glycoside getting into the liver. *Méhes*'s pigeons vomited on an average 12 times in 6 hours after the injection, our animals did 10 times. The proximity of the two numbers proves that, at least at the beginning, the experimental conditions were identical. *Méhes*' animals living on polished rice vomited after three weeks on an average only twice after the administration of digitoxin, most of them not at all. In our experiments the average number of vomitings of the penicillin-treated animals was 8, after a digitoxin injection to animals treated with penicillin + vitamin B_1, eleven. This difference was too little to draw conclusions as to differences in nauseating effect, it may, however, be claimed that penicillin-treatment does not bring about changes resembling vitamin deficiencies due to diet.

We tried to clear up the condition of the heart muscle which could be directly investigated by analysis of the ECG findings. In the pigeons' normal ECG three well defined waves were distinguished in accordance with *Hanzlik* and *Méhes*, which were designated P, S and T waves. Regarding the action of digitoxin on the normal ECG of pigeons opinions differ, but, as stated by *Dock*, *Stockton*, *Wood* and *Hanzlik*, 1/4th of the nauseating dose causes bradycardia, prolongation of the conduction time and flattening of the ventricular waves especially of the T wave. All these symptoms can be brought on by stimulation of the vagus but they cannot be regarded as a pure vagus effect, because after cutting through the nerve this type of ECG cannot always be obtained by stimulation of the peripheral stump *(Kahn)*. *Méhes* and *Péter* found that after the injection of a nauseating dose the pulse rate considerably diminished. However, detailed study of their data showed that the decrease was considerable in two of five animals only, i.e. No. 2 and 7, in one the pulse rate first increased, in two it was about constant. The analysis of *Méhes* and *Péter*'s data is very important for us, because, in all of our pigeons treated with the same doses and method, an *increased* pulse rate was observed, the average increase being 64%. It is unlikely that the missing bradycardia were due to the fact that our animals were already more bradycardiac at the beginning of the experiment than those of *Méhes* and *Péter*, which had an unusually frequent pulse rate.

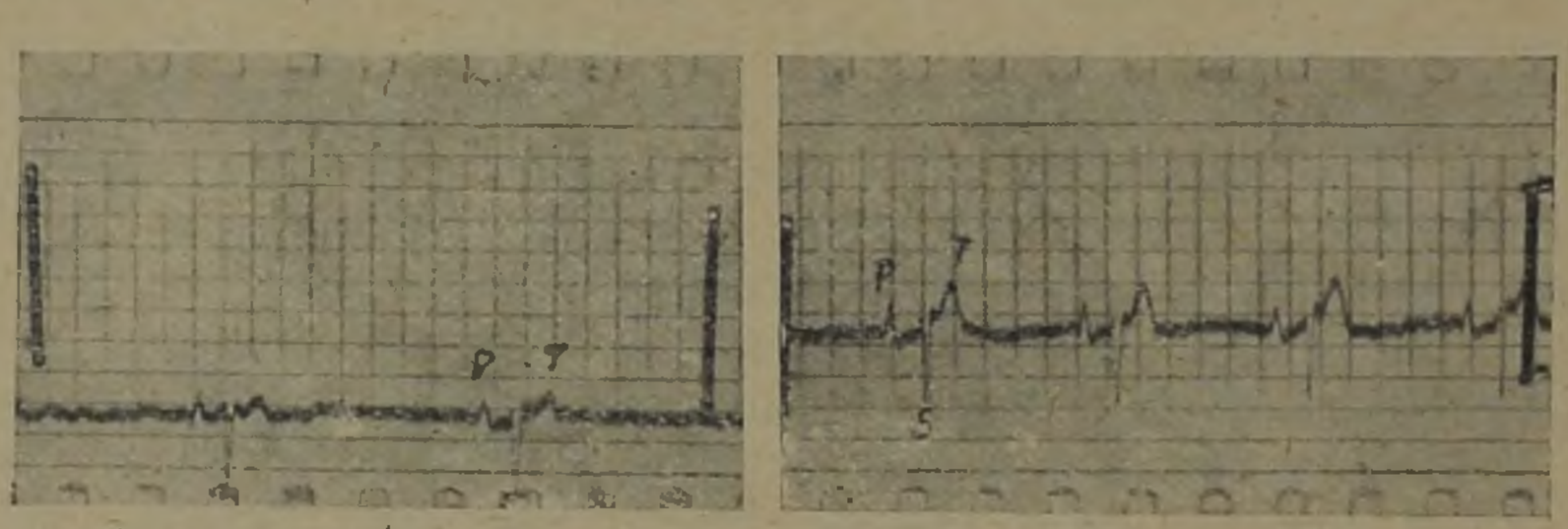
TABLE I.

Comparison between data before and after treatment with penicillin.

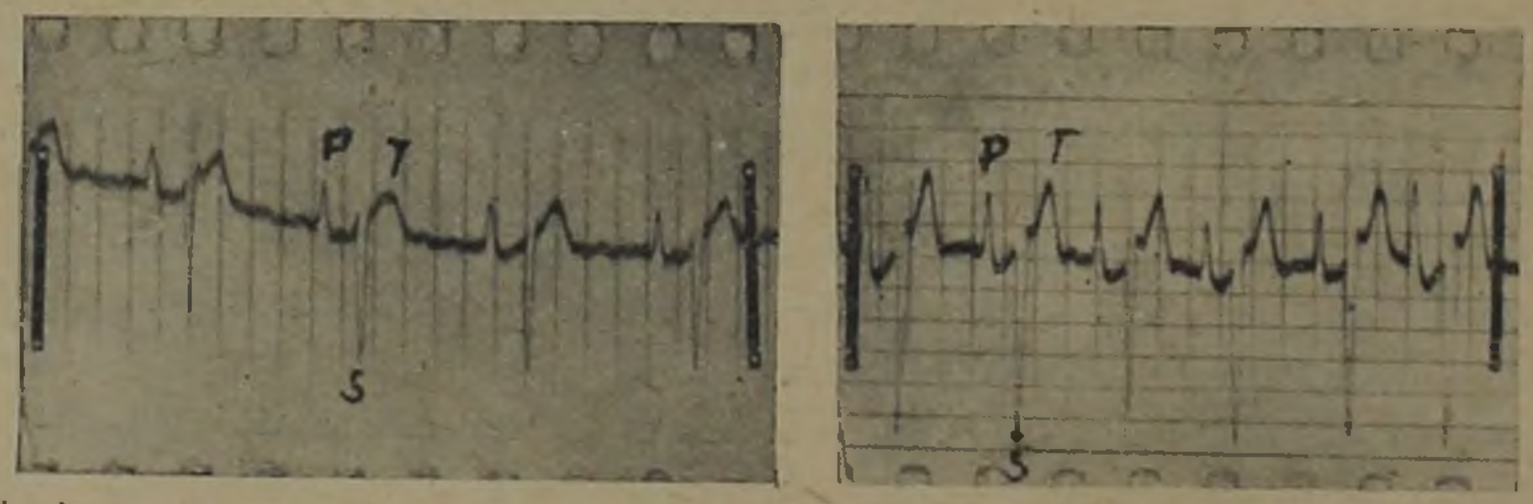
	Number of pigeons	weight (mean)	pulse rate (mean)	P–Q (second)	P	S (mean mm)	T
Before treatment	15	281 g	155±18	0,04	1,7	4,8	1,5
After	9	269 „	220±9	0,03	1,9	5,8	3,1
After treatment with penicillin and vitamin B_1	9	295 „	185±7	0,045	1,7	4,8	1,6

Digitoxin sometimes increases the pulse rate in man too. *Oettel* called this phenomenon „paradoxical digitalis effect". In such cases the conduction time decreases, the P, Q, R and T waves grow and the S—T interval is elevated. According to *Oettel*, this occurs in carditis, coronary sclerosis, Grave's disease and toxic states of the heart (for example, in diphtheria); it points to an increased excitability of the sinus node. In this sense it may be assumed that, the dose used in our experiments being much higher than that which produced bradycardia in the experiments of the American authors mentioned, the toxic dose of digitalis resulted in a paradoxical digitalis reaction in our healthy animals. (The lethal dose of digitoxin is somewhat higher i. e. 0.34 mg per kg body weight.) The fact also that a few minutes after the injection the pigeons seemed very ill speaks in favour of the above assumption. They buried their heads in their breast feathers, their bodies were swollen, they trembled and behaved apathetically to environmental stimuli. The average pulse rate of *Méhes* and *Péter*'s pigeons was 213, that of our pigeons 155. That of 8 beri-beri animals was 153, the average of 5 treated with penicillin and vitamin B_1 was 130. As it may be seen the changes produced in our pigeons were contrary to those in *Méhes* and *Péter*'s experiments. Their vagotonic beri-beri animals did, however, react to digitoxin with a considerable acceleration of the pulse amounting to 40—60%, in one case even to 86%. It seems therefore that, besides other factors, vagotonic condition promotes the paradoxical reaction. In our experiments the rise in the pulse rate of the sympathicotonic animals treated with penicillin only was more or less lower than that of the normal animals whereas the pulse rate of the bradycardiac pigeons treated with penicillin and vitamin B_1 markedly increased (by 98%). Thus, digitoxin acts in the dosage employed in a different way on animals treated with penicillin only and those treated with penicillin and vitamin B_1, these latter being vagotonic. Two of our animals, which died a short time (72 minutes) after the digitoxin injection, showed as a maximum of toxic effect a pulse acceleration of 135%. Our observations can therefore be interpreted as follows: in the pigeons treated only with penicillin a toxic dose of digitoxin brought on a mild paradoxical reaction, either because a smaller quantity of digitalis

reached the heart or the heart muscle (sinus node) was less sensitive. The former assumption (i.e., that the digitalis exerted a lesser effect in the organism) was also supported by our observation that the penicillin-treated animals were fresh after the digitoxin injection and but little indisposed even when they got to the vomiting period. The unchanging number of vomitings did, on the other hand, prove that the mechanism of the reflex running through the vagus was undisturbed. The different responses of the conduction time in the two groups were in accordance with this: in animals treated only with penicillin the conduction time was longer (vagus effect of digitalis) while in the other group (untreated and penicillin vitamin B_1 treatment) the paradoxical reaction resulted in the abbreviation of the conduction time. The dissociated behaviour of the heart muscle and the vagus are, in our view, relevant facts which should be commented later. The fact that the supply of vitamin B_1 abolishes the difference between treated and untreated animals confirms our earlier experience that penicillin treatment produces disorders in the vitamin balance. However, the disorder of the vitamin C household was in our human experiments still greater and it is true, that *Galli, Bruneo* and *Martolini* could protect guinea pigs against the lethal dose of digilanide with vitamin C, we considered it superfluous to examine the effect of vitamin C because pigeons being capable to synthetize all vitamins but B_1, are sensitive to vitamin B_1 only *(Funk)*.

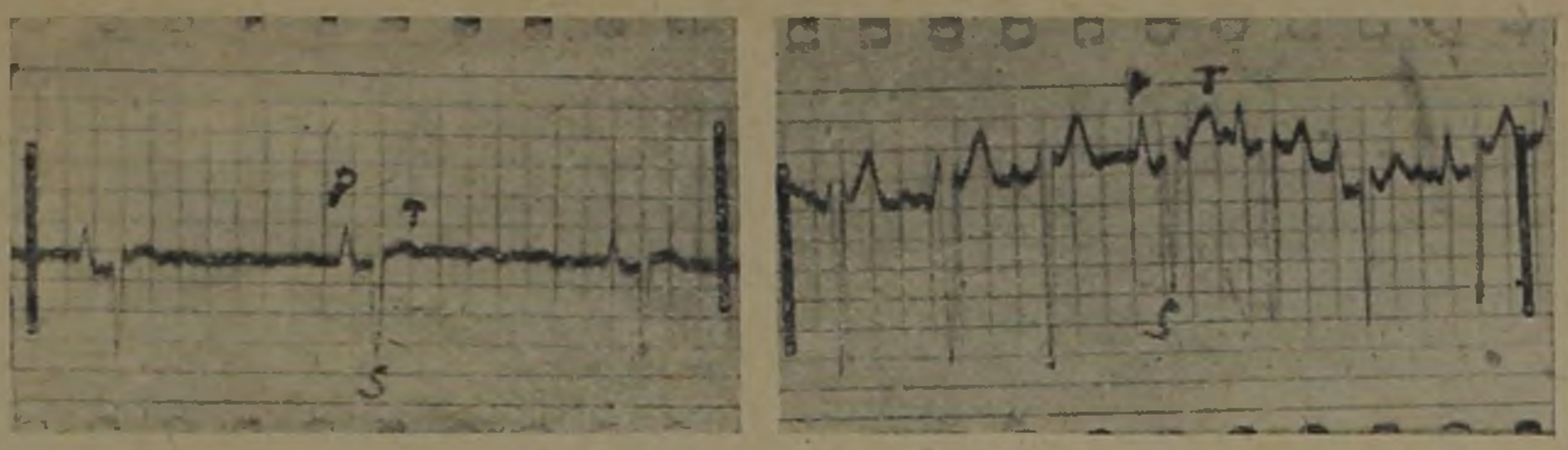


Paradoxical reaction to high dosis of digitalis in the ECG of normal (non-treated) pigeon. $S_1 = 4.5$ mm, $S_2 = 6$ mm, $T_1 = 1, 5$ mm, $T_2 = 3$ mm.

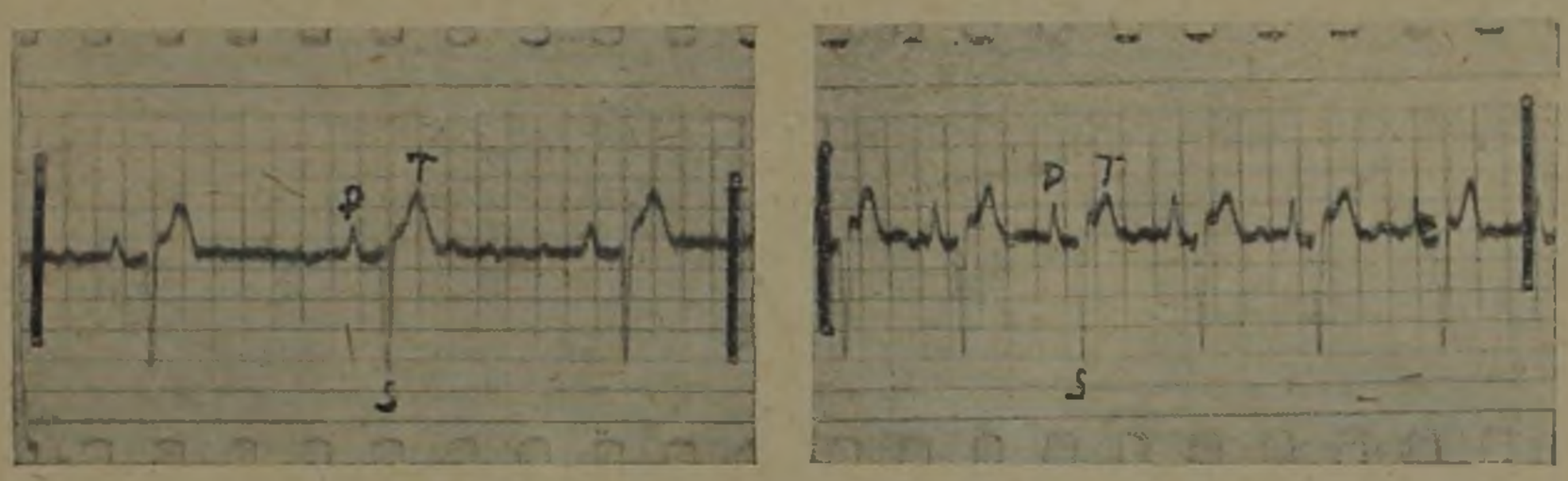


Similar response to digitalis in the ECG of penicillin treated pigeon, complemented with vitamin B_1 treatment.. $S_1 = 8$ mm, $S_2 = 12$ mm, $T_1 = 2$ mm, $T_2 = 5$ mm.

Fig. 1. a.



Maximal sympathicotonic response to digitalis in a pigeon dead 72 minutes after the injection of digitoxin. $S_1 = 6$ mm, $S_2 = 14$ mm, $T_1 = 0{,}5$ mm, $T_2 = 3$ mm.



Response to the same dosis of digitalis in a pigeon treated with penicillin solely. $S_1 = 9$ mm, $S_2 = 8$ mm, $T_1 = 4$ mm, $T_2 = 3$ mm.

Fig. 1. b.

TABLE II.

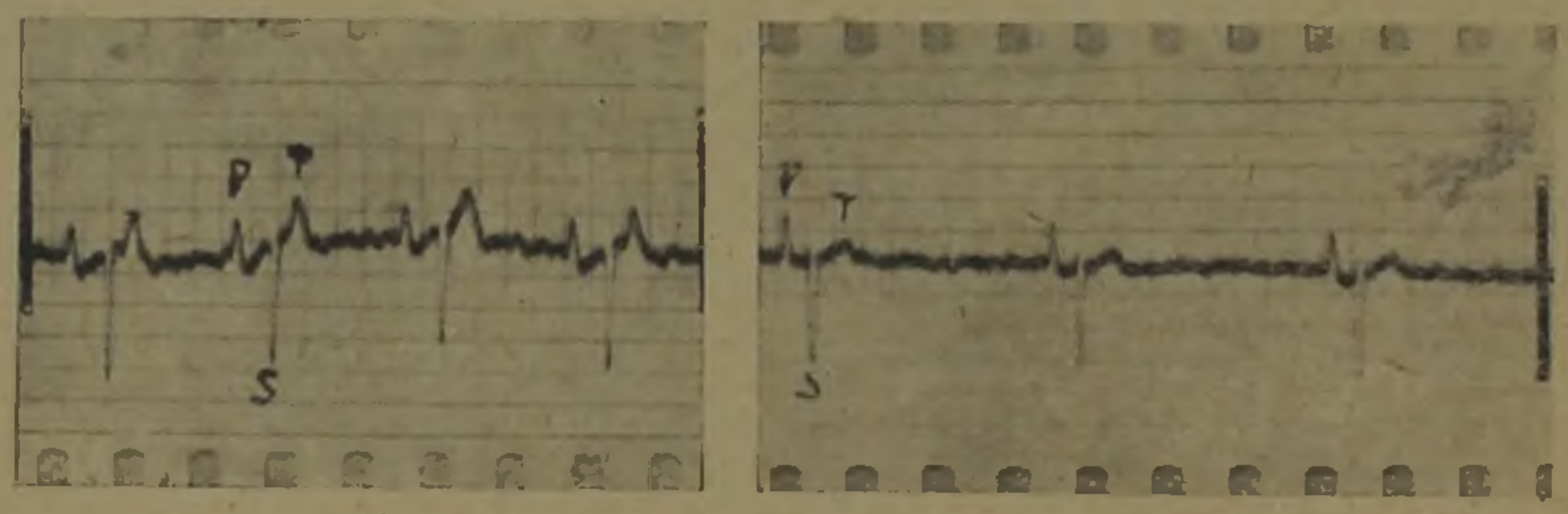
Experiments with digitoxin.

	Number of pigeons	Weight (mean g)	Pulse a)	Pulse b)	increase in %	P—Q sec a)	P—Q sec b)	P mm a)	P mm b)	S a)	S b)	T a)	T b)
Untreated	15	281	155±18	240±13	+55	0,04	0,037	1,7	2,2	4,8	7,0	1,3	2,5
Treated with penicillin	5	281	190±16	292±10	+53	0,03	0,035	1,9	2,2	5,5	4,9	3,4	1,6
Treated with penicillin and vitamin B_1	5	297	130±15	257±20	+98	0,04	0,04	2,6	2.8	4,5	6,1	1,5	2,7
Pigeons intoxicated and dead following injection of digitoxin	I.	243	140	340	+143	0,04	0,025	2,0	2,5	4,0	8,0	1,0	3,0
	II.	210	140	320	+128	0,03	0,021	2,0	2,5	5,0	12,0	0.5	4,0

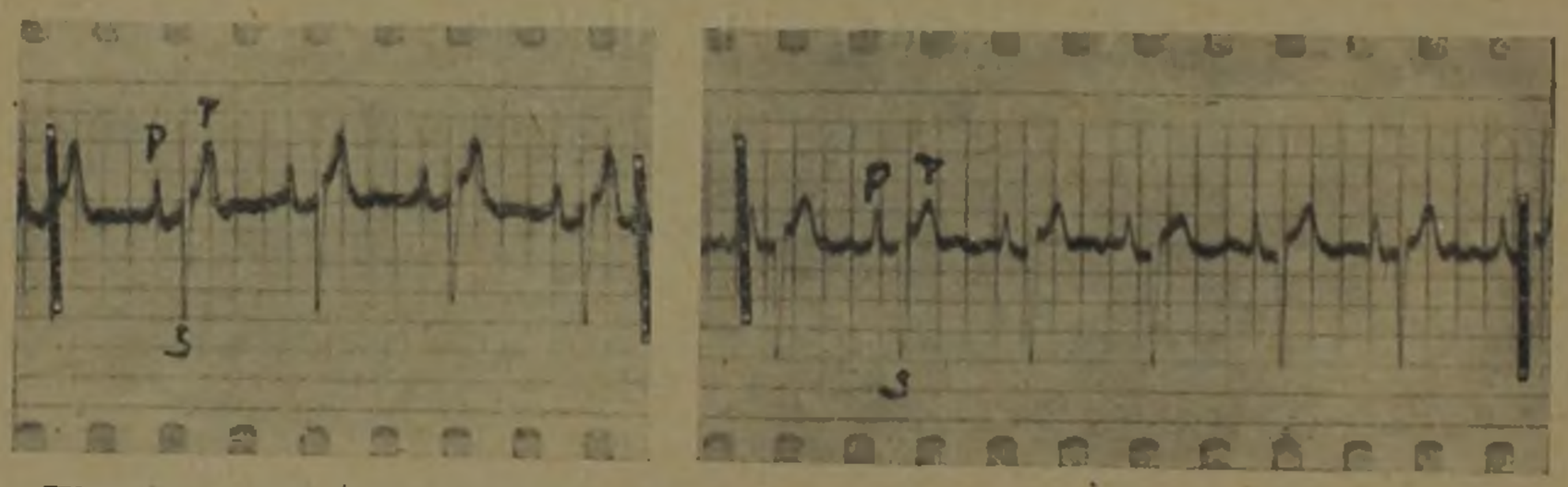
In column *a)* there are data collected before the injection of digitoxin, in column *b)* those after the injection.

In another series 4 pigeons were treated in the above manner with penicillin and 4 with penicillin + vitamin B_1. At the end of three weeks the comparison of the electrocardiograms of the two groups showed again that the animals treated only with penicillin seemed more sympathicotonic in view of frequency, conduction time

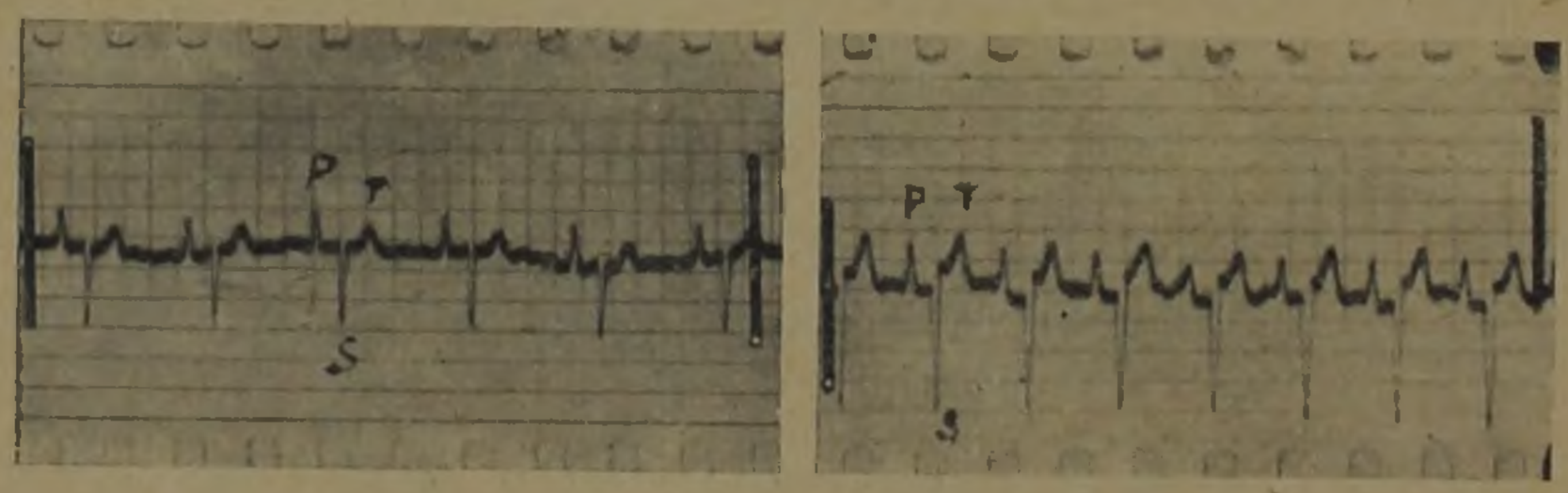
and size of ventricular oscillations (Table III). Then a lower, presumably non-toxic, dose of digitalis than that used in the previous experiments, namely 0.225 mg digitalin (digitoxigenin) per kg body weight were given the vomitory dose of which was stated to be about 0.3 mg per kg body weight by *Méhes* and *Péter*. 15 minutes after the injection the electrocardiograms taken from both the untreated and treated animals exhibited normal reaction to digitalis, like in the experiments of *Méhes* and *Péter*, except the conduction time which was abbreviated. The animals treated only with penicillin responded, regarding frequency, with the paradoxical effect even to this small dose; in any case, however, the prolongation of the conduction time, decrease in the height of the T wave, and, as a new detail, the depression of the S—T interval, were signs of a normal rather than paradoxical reaction. (Fig. 2.)



Normal response to non-toxic dose of digitalis on the ECG of pigeon
$S_1 = 7$ mm, $S_2 = 6$ mm, $T_1 = 3$ mm, $T_2 = 0{,}5$ mm.



The response to the same dose of digitalis after treatment with penicillin. $S_1 = 8$ mm, $S_2 = 9$ mm, $T_1 = 4{,}5$ mm, $T_2 = 2$ mm. Pulse rate accelerated.



Response to digitalis after treatment with penicillin and vitamin B_1
$S_1 = 5$ mm, $S_2 = 3$ mm, $T_1 = 0{,}5$ mm, $T_2 =$ neg.

Fig. 2.

TABLE III.

Experiments with digitalin (digitoxigenin).

	Number of pige-ons	weight (meang)	pulse a)	pulse b)	change %	P—Q a)	P—Q b)	P mm n)	P mm b)	S mm a)	S mm b)	T mm a)	T mm b)
Treated with penicillin	4	263	260±12	330±17	+27	0,05	0,055	1,5	1,5	6,4	7,5	2,8	1,5
Treated with penicillln and vita-min B1	4	278	235±16	220±15	—7	0,058	0,052	2,3	1,5	5,6	4,3	2,0	1,4

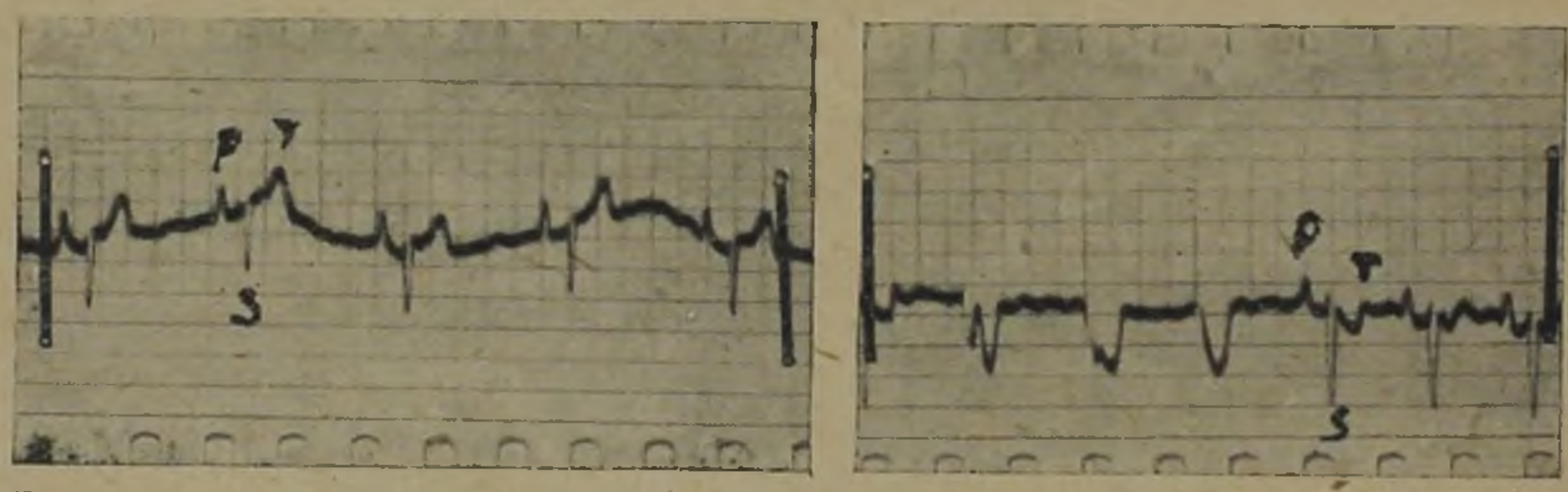
The tachycardia appearing during the penicillin treatment may be an effect of increased activity of cholinesterase. *Mosonyi* and *Oblatt* have shown that the glutathione content of the serum increases after a single dose of penicillin. On the other hand, *Libbrecht* considers glutathione as the activator of cholinesterase. It can be easily supposed on this basis that the increased cholinesterase activity produces a condition similar to sympathicotonia.

Our data have shown that the myocardium characterized by the rate and intensity of the heart beats and the vagus function characterized by the conduction time react, in animals treated with penicillin, differently to both high and low doses of digitalis. If small doses of digitalis produce a paradoxical effect it may be due to the pathological condition of the heart muscle brought about, in the view of *Oettel*, by toxic injuries. No other influence having taken place. we consider the penicillin treatment producing this toxic effect. In the case of fractionary doses of digitalis, the paradoxical effect occurs only in the heart muscle (sinus node) where the capacity of reaction is changed, while the processes under the direct guidance of the vagus take place normally; in the case of a toxic digitalis dosage, the paradoxical reaction extends to this area also.

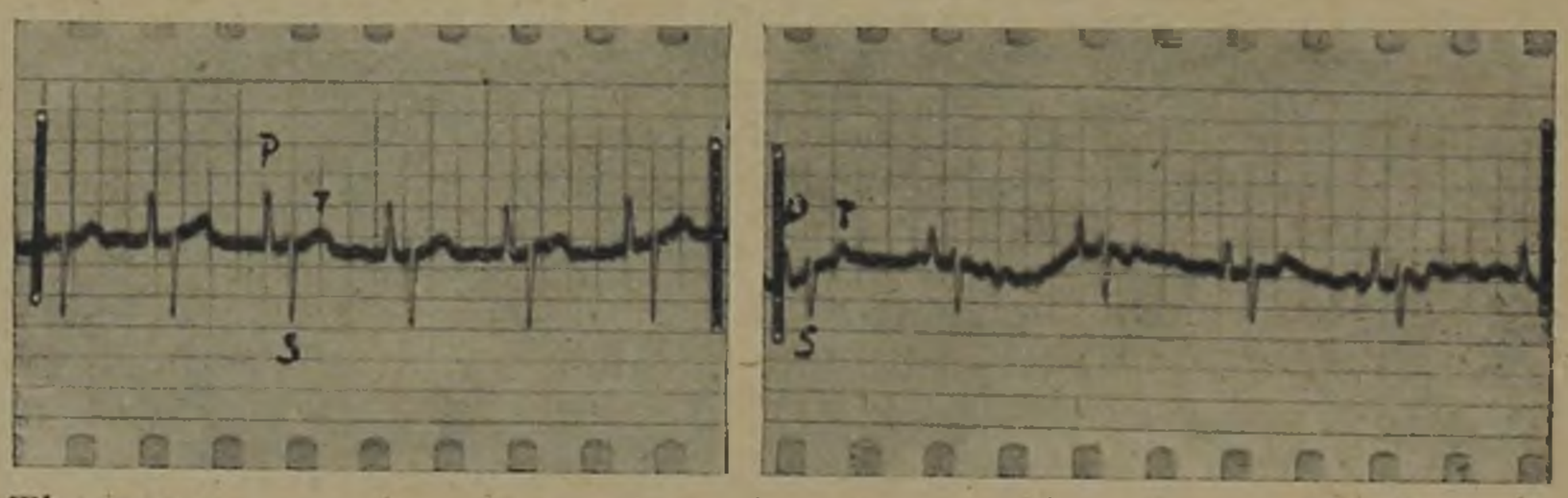
To explain the facts the question has been raised why did less glycoside get into the heart of animals treated with penicillin? *Méhes* and *Péter* found in the organs of the animals suffering from beri-beri an increased cholesterol content. They stated that the glycoside was partly absorbed by cholesterol. Obviously, in our experiment there was no such factor as beri-beri, but the mild disturbance occurring in the vitamin B_1 balance could have been responsible for the increase in cholesterol. Besides this, it has been shown in one of our pevious experiments that repeated administration of penicillin elicits a sensitization of the organism which can, as claimed by *Beznák*, give rise to an increase in the cholesterol content of the tissues.

In *Danielopolu*'s experiments, the increase of cholinesterase reduced the effectiveness of digitalis. On evaluating our experimental results this factor must not be disregarded. The activation of cholinesterase following the increase of the glutathione and the absorption of digitalis glycosides developed partly through the increase of lipoids. Hereby, the tachycardia observed in peni-

cillin treatment and its resistance to digitalis can be explained. Therefore, one need not seek for the cause of the changes occurring after the cessation of septic symptoms, nor is *Honigmann* and *Karns* theory on the stiffness and failure of the valves due to cicatrisation necessary. The changes ensuing in the penicillin-digitalis relation are, at least partly, reversible as seen from our experiences made on patients free from heart ailments. Figure 3 is a record of ECGs of one of our pigeons. Initially, the usual penicillin treatment and a small dose of digitalis resulted in a pronounced vagus effect (extra-systoles) and a partial paradoxical reaction (accelerated pulse). Then we injected 1 mg vitamin B_1 daily for 10 days and no penicillin; the previous dose of digitalis did, after 10 days, evoke a complete paradoxical reaction. It is probable that the restoration of vitamin B_1 equilibrium arrested the pathological increase of cholesterol and the quantity of digitalis used the second time reached the heart muscle and exercised its toxic effect without any diminution. It may be assumed that, if the heart has the time necessary for complete regeneration the other part of the process (pathological excitability of the heart muscle) will also decline.



Response to small dose of digitalis in penicillin-treated pigeon. $S_1 = 4$ mm, $S_2 = 5$ mm, $T_1 = 2$ mm, T_2 flattened, series of extrasystoles.



The same animal after 10 days treatment with vitamin B_1. $S_1 = 4$ mm, $S_2 = 8$ mm, $T_1 = 0{,}5$ mm, $T_2 = 3{,}5$ mm.

Fig. 3.

SUMMARY.

1. A quantity of digitoxin used according to the experiments of *Méhes* and *Péter* exerted a toxic effect in pigeons and brought about a „paradoxical digitalis reaction". Also electocardiographic changes like those observed by *Méhes* and *Péter* occurred if 2/3rds if this dosage were used.

2. In comparison with untreated pigeons those treated with penicillin seemed to become sympathicotonic. This is seen from the accelerated pulse, the decrease of conduction time and the increase of some of the ECG waves. The invariable emetic effect of the digitalis parallelly with the often diverse behaviour of the conduction time speak in favour of the intactness and excitability of the vagus function. Evidently, the acceleration of the pulse is due to the cholinesterase activating effect of the serum-glutathione content, increased during penicillin treatment.

3. The animals treated with penicillin are less sensitive to small and toxic doses of digitoxin than the untreated animals or pigeons which were given vitamin B_1 simultaneously with penicillin. This decrease of sensitivity is virtually due to the fact that less glycoside arrives to the heart.

4. Be granted that animal experiments rarely apply to the different conditions existing in the human organism especially under pathological circumstances, it seems however, on the basis of the above experiments, probable that the accelerated pulse often experienced in penicillin treatment can, to a certain degree, be attributed to a shift towards sympathicotonia, and the decreased capacity to react to digitalis is due, aside from this sympathicotonia, to the organism's greater capacity to absorb the glycoside, when it is sensitized and contains more lipoids (cholesterol). The increase of cholinesterase may also play a rôle in this process by hindering the effect of digitalis. During penicillin treatment, tachycardia following digitalis therapy represents a paradoxical reaction of the heart muscle previously exposed to toxic injury.

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FROM THE II. MEDICAL CLINIC OF THE UNIVERSITY OF BUDAPEST
(DIRECTOR PROF. E. HAYNAL).

THE SIGNIFICANCE OF OXIDATION IN BLOOD-CLOTTING.

BY L. A. PÁLOS.

(Presented at the session of the IV. Section of the Hungarian Academy of Sciences the 23 February 1948)

A significant step in clearing up the mechanism of blood-clotting is the knowledge of the thrombin-inactivating capacity of blood serum, as this radically modifies our conception of clotting. The earlier idea was that abnormal *thrombin formation* was one of the most important causes of disturbances in blood-clotting. From recent investigations the importance of *thrombin inactivation* has also become evident. The role of the latter in blood-clotting can be proved by experimental data, and these data admit of the assertion that coagulation is regulated by the correlation of the formation and disappearance of thrombin. Some time after coagulation the thrombin diappears, no matter in what excessive quantity it was present. *Moravitz* has, without having known the mechanism of the process, called this transformation metathrombin formation, meaning that ineffective metathrombin is formed of active thrombin. The investigations of *Rettger, Landsteiner* and *Landsberg* have, however, shown that thrombin loses its efficacy by binding to serum proteins, probably to serum albumin. The experiments of *Lenggenhager* represented another step forward. He recognized the continuous character of thrombin destruction, its important role in blood-clotting, and could also demonstrate thrombin inactivation by a reaction he worked out.

The investigations of *Gerendás* cleared up the mechanism of thrombin inactivation still further. By his simple and quick method the degree of thrombin inactivation can be exactly measured and conclusions can be drawn as to the state of coagulability of the blood. In his opinion, two factors play a part in thrombin inactivation: the one has an adsorptive, the other a fermentative effect. The two factors can be separated from each other. The ferment effect can be eliminated by warming to 65—70° C, the adsorptive one by the action of alcohol or chloroform.

With *Gerendás'* method the degree of thrombin inactivation can be estimated in the following way. Oxalated blood is mixed with thrombin of known activity in a test tube. The juice is expressed of the clot and, at certain intervals (0.5, 1, 2, 5 minutes) sam-

ples of the juice are taken with pipets and added to oxalated ox plasma. The juice containing the thrombin brings about clotting of the ox plasma, the later the sample taken the slower. This means that thrombin is constantly used up by the system present in the juice. Thrombin inactivation is expressed by the clotting time of the ox plasma.

The following method was used. to find a correlation between thrombin inactivation, blood clotting and oxidative processes.

First the relation between thyroid activity and blood clotting was studied, assuming that the tendency to haemorrhages observed in hyperthyreosis and the cause of the increased coagulation present in hyperthyreosis were due to differences in thrombin inactivation. A special impulse was given by a case of ours, observed with *Bozóky*, where a tendency to spontaneous thrombosis which had existed for years and resisted to various therapeutic procedures including heparin has been completely cured by thyroid tablets. The clotting time of 10 seconds rose to 4.5 minutes and became constant at that level.

The experiments were done with rabbits. The thyroid was removed and the change in thrombin inactivation was examined. Initially, it increased in all operated animals and began to fall about 6—8 weeks afterwards, generally reaching its minimum in 10—14 weeks.

I expressed the degree of thrombin inactivation by the so-called coagulation (C) factor obtained through dividing the clotting time of the control animal by that of the experimental animal and taking the average of the estimations. In this manner, the degree of thrombin inactivation can be expressed by a single figure. The factor C is, though not mathematically exact, sufficient for orientation. Normally $C = 1$. If $C > 1$, the inactivation is diminished, blood is more coagulable; if $C < 1$, thrombin inactivation is increased and congulation is protracted.

The data obtained in animal experiments have been completed by investigation on patients.

Increased inactivation was found in hyperthyreosis. In hypothyreosis it was reduced. like in the thyroidectomised rabbits. Correspondingly, clotting time increased in the first case and diminished in the latter.

As early as at the first experiments it seemed probable that the changes of oxygen consumption exert an influence on thrombin inactivation. In hyperthyroidism, more oxygen is required, far above the normal, oxidations accelerate. In hypothyroidism, less oxygen is needed and less oxidation takes place.

The experiments carried out on rabbits have shown that oxidations bear a relation to thrombin inactivation and blood clotting.

Rabbits were put into a case in which vacuum of any degree could be established. The animals were kept for hours at an atmospheric pressure corresponding to the height of about 5000 meters. Due to the O_2 deficiency the thrombin inactivating capacity of the

blood of the experimental animals diminished, i. e. the coagulability of the blood increased.

If the air was changed, for one group of the animals by overpressure, and for the other by a slow and uniform O_2 current (in the latter case CO_2 does not accumulate) thrombin inactivation increased and the coagulability of the blood diminished.

In the vacuum increased CO_2 pressure, reduced atmospheric pressure and lack of O_2 may exert a combined effect. As to the effect of CO_2 on clotting opinions are divided. *Wiedenbauer* (1942) attributed an energetic inhibiting effect to CO_2 whereas *Stuber* and *Lang*, further *Gerlóczy* and *Szőke*, claim that it increases coagulability.

My previous results being in contrast with those of *Weidenbauer*, the effect of CO_2 on blood clotting was examined. Rabbits were given CO_2 through inhalation until the typical deep breathing occurred. Then the inactivation of thrombin and the clotting time of the recalcified blood was determined. In repeated experiments the decrease of thrombin inactivation (and the increase of coagulation) was greater than in my previous experiments. The examination of the effect of CO_2 *in vitro* revealed similar results.

These *in vivo* experiments show that coagulability is a constant feature of blood. Any attempt to influence it artifically is met with the defensive manifestations of the organism. As an effect of O_2 thrombin inactivation may temporarily decrease. In vacuum, after thyroidectomy or after thiouracil treatment, the decrease in thrombin inactivation is preceded by its transitory increase. These phenomena may be considered compensatory effects of the organism, which is capable of compensating, for a short ime, for the effect causing the change. Little changes may occasionally be attended by overcompensation. This observation is in complete harmony with the effect of thrombin injected intravenously. The latter gives rise to intravasal coagulation and sudden death of the animal. On the other hand, if diluted thrombin is injected in small quantities and slowly compensation or even over-compensation takes place: instead of clotting coagulability rapidly decreases. *Gerendás* and *Csapó* have also found that reduction in coagulation is, in this case, due to the increase of thrombin inactivation.

Considering that in the *in vivo* experiments the compensatory effects might act disturbing on the results, it seemed necessary to examine a condition in which the lack of O_2, being a constant factor, could not be due to compensation. To this purpose I investigated the thrombin inactivation of the blood of patients having suffered for a long time from dyspnoea. In these patients the coagulation and the decrease in thrombin inactivation were approximately parallel with the degree of cyanosis.

In hyperthyreotic patients it was remarkable that those who partook of a thiouracil treatment showed a reduced thrombin inactivation and increased coagulation even before the hyperthyroidism ceased. According to *Paschis*, *Cantarow* and *Tillsen*, thiouracil

has a paralysing effect on cytochrom-oxidase and thereby on the synthesis of thyroxin. Both phases of the process are associated with oxidation whereby the change in blood-clotting may be explained.

The mechanism of the effect exercised by thiouracil on blood--clotting could not be thoroughly cleared. Nevertheless, the experiments carried out with *Komáromy* proved that, in rabbits treated with 0.5 g thiouracil daily by a tube for weeks, the initial increase of thrombin inactivation is followed by its decrease and the blood of the animal becomes highly coagulable.

The correlation of oxygen consumption and clotting served as basis for the idea that respiration or its changes might have an effect on thrombin inactivation or blood clotting.

Oxygen is assured for the organism through respiration and the capacity of the circulating blood to carry oxygen, but is was supposed that these two relevant automatic processes have, besides assuring gas metabolism, also a practical relation to one another. It cannot be disputed that the organism requires several factors to regulate the fluid state of blood. The claim that one of these is oxygen or oxidation, is proved also by the experiments described and others carried out to this end.

I consider as an important proof of my statement that the coagulation, or thrombin inactivating capacity of the arterial and venous blood, differ from one another. To prove it in human subjects and under experimental conditions blood was withdrawn from arteries and veins coursing by each other and their thrombin inactivating capacity was determined.

It has been invariably found that thrombin is more rapidly inactivated by arterial than by venous blood. The difference could be shown in all experiments.

Then hyperventilation experiments were carried out. A needle was introduced into an uncompressed vein of the arm and left in place for the whole experiment. It must be emphasiized that it is hardly possible to bring about, by forced breathing, an oxygen saturation in the arteries the effect of which would extend through the tissues to the veins and cause a greater difference than usually. Another source of error is the defensive apparatus of the organism by which it strictly controls its optimum coagulation, like the pH of the blood.

In the course of the experiments there was found an optimum of time following ventilation. Blood taken at that time showed always increased inactivation. If this moment is missed no change can be observed because the compensation has already set on and it is impossible to bring about, by hyperventilation, an oxygen saturation which would overcome the compensation and cause a change.

These results should be completed by the experiments concerning the correlation between ether or chloroform narcosis and blood-clotting. My goal was to demonstrate that these drugs are capable of diminishing the thrombin inactivating capacity of blood not only

in vitro but also *in vivo*. In fact, the thrombin inactivating capacity of the blood diminishes during inhalation narcosis. Interestingly, if the narcosis was deep the thrombin inactivation remained lower for several days. This change was attributed to a decreased cell metabolism i. e. reduction of the oxidation processes brought about by the narcosis.

These experiments also seem to confirm the idea that respiration also plays a role in assuring the fluid state of blood.

Our results referring to blood-clotting bear a relation to the problem of thrombosis also. It is no longer doubted that the appearance of a thrombus is first of all due to a shift of the composition of the blood towards clotting. This change may be occasionally associated with a predisposition and give rise to thrombosis. Our investigations so far on thrombosis patients have always shown a lowered thrombin inactivation. So there is a hope that the factors influencing inactivation will be discovered and the problem of thrombosis will, thereby, be brought nearer to its solution.

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FROM THE 1 ST MEDICAL CLINIC OF BUDAPEST UNIVERSITY.
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EFFECT OF KETOSIS AND VITAMIN B_1 ON THE ACTIVITY OF THE ANTERIOR LOBE OF THE PITUITARY.

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(Awarded with the Ede Szenger prize of the Hungarian Medical Association.)

The anterior lobe of the pituitary produces hormones stimulating other endocrine glands. Aside from its theoretical significance, the discovery of this fact has meant a marked progress in the treatment of endocrine diseases. The introduction into therapy of the gonadotropic hormone of the anterior pituitary lobe was particularly relevant. In menstrual disturbances, the application of the gonadotropic hormone was a progress, as inactivation of the ovary could be prevented. The administration of oestrin or progestin is, in fact, a substitution. The prevailing drawback of substitutional therapy is that, sooner or later, it leads to the inactivation of an organ while its substance is partly functioning. The gonadotropic hormone stimulates the ovary, its application is, however, from the aspect of the anterior pituitary lobe, substitutional therapy. Therefore, in the treatment of diseases resulting from decreased activity of the pituitary any procedure stimulating its anterior lobe would be advantageous. The removal of certain endocrine glands would be such a procedure (castration, thyroidectomy). Evidently, this method cannot be employed. Stimulation of the pituitary can occasionally be brought about by X-rays irradiation but, as to the existence of a stimulating dose, even the opinion of the roentgenologists is not uniform, X-rays being as yet applied for the inactivation of this gland only. The publications on the stimulating effect of short-wave treatment cannot be considered convincing so far either.

It may be stated therefore, that no procedure has been known which promotes the activity of the hypophysis.

The search for such a procedure was the aim of our experiments in 1937. An interesting report served as starting-point. *Barborka* found that 30% of the women kept at a ketogenic diet became amenorrhoic. He considered this phenomenon a result of vitamin B_1 deficiency and gave his patients brewers' yeast while the diet was maintained; their menstruation re-appeared.

The fact that a symptom disappears after the administration of a drug is by no means a proof of a causal connection between the symptom and the drug. The correlation between ketogenic diet and

amenorrhoea was considered to be hormonal rather than vitaminogenic. This view was supported by the experiments of Burn and Ling, and of Hoffmann and Anselmino; they isolated a fraction from the anterior lobe of the pituitary which raised the ketone-body content of the blood. Ketogenic diet is, aside from its stimulating effect on the production of ketogenic hormone in the anterior pituitary lobe, considered to stimulate some of its other functions also.

The urine of women kept on ketogenic diet was injected into immature female mice. In the majority of cases complete maturation of follicles occurred indicating that the urine contained per litre at least 333 mouse units of prolan A. The same phenomenon could be demonstrated with the urine of men kept on a ketogenic diet, with the urine of ketonuric diabetic patients and such as suffering from starvation ketosis.

Figure 1. shows the uterus and ovaries of a mouse treated with the urine of a normal individual. Figure 2. shows the uterus and ovaries of an immature mouse injected with the urine of the same woman kept on ketogenic diet for 10 days. The cornua are edomatous, thick, like the intestinal loop seen in the middle line. The ovaries are enlarged, hyperaemic.

Microphotographs pertaining to another experiment can be seen in the following Figures (3. 4, 5, 6, 7).

Figure 3. shows the ovary of a normal immature animal. Only primordial follicles can be seen. Figure 4: ovary of an animal treated with urine prior to the ketogenic diet: immature, slightly enlarged follicles. Figure 5: Injection of urine after 5 days of ketogenic diet: mature follicles. Figure 6: Injections started 2 days after the period of diet: increased follicular maturation, no Graffian follicles. Figure 7: Treatment started 12 days after the diet: normal ovary.

Figure 8 shows the ovary of a mouse treated with the urine of a ketonuric diabetic man: complete follicular maturation can be observed. After the ketonuria had been relieved by diet and insulin, only primary follicles could be seen in the ovaries of animals treated with the urine (Figure 9).

It should be emphasized that only the amount of prolan A was increased in the urine; haemorrhagic spots or corpora lutea never could be detected in the ovaries of the mice.

Of late, *Behrenstein* (Switzerland) has confirmed these results (personal communication). He found 280 mouse units of prolan A in the urine of a subject suffering from starvation ketosis. In a paper published in the Schweiz. Med. Wochenschrift (1947 No. 8) *Kousmine* (Lausanne) reported two cases, in which severe asthma was attended by an excessive excretion of prolan A and acetone.

On the basis of these experiments the amenorrhoea of women kept on ketogenic diet has been attributed to the ketosis having a stimulating effect on the prolan A production of the anterior lobe of the hypophysis. Thus prolan A is in excess of prolan B and the

sexual cycle is held up in the proliferation period, the result being that menstruation does not take place.

At present, ketogenic diet is the only procedure by which the anterior lobe of the hypophysis, i. e. its prolan A production, can be stimulated. Numerous examples could be found which show that the organism makes use of this possibility. Years ago the author was called to a 17 year old girl suffering from a mild influenza. During the examination he became aware of the smell of acetone. On being questioned the girl's mother said that every now and then the child kept days of fasting which often led to disputes between them. The girl pleaded that at such times she felt very well. In the ovaries of mice treated with her urine matur follicles were found (Figure 10). The test repeated with a urine taken during the acetone-free period was negative. (Figure 11).

It is a well known fact that the animal organism also employs ketosis when a maximum degree of sexual function is needed. Certain species of fish take no nourishment for a time during spawing. Many theories have been set up to account for this. The author believes that, by temporary fasting, ketosis is developed which enables the animal to achieve a maximum degree of sexual function.

Other data also are known which prove that the effect of ketosis stimulating the pituitary has already been employed in medicine. In *Bergmann—Staehelin's* textbook, chapter on Endocrinology, *Marx* writes that patients suffering from Simmonds' disease improve if they are kept fasting for a day or two. They feel hungry and begin to eat spontaneously. „...One has the feeling that through the fasting we break down a childish spite..." The explanation is naive but this therapeutic measure proves the possibility of using ketosis to increase the activity of the hypophysis, a fact which has been recommended by the author in a paper published in 1940.

Presumably, ketosis may also play a role in the regulation of pituitary activity under physiological conditions. *Szarka* has called attention to the fact demonstrated by him in 1927 that the ketone-body content of women's blood shows cyclic changes. He found the maximum values during the first and second day of menstruation, that is the maximum ketone-body content of the blood coincides with the time when the prolan A producing activity of the anterior pituitary lobe begins. This interesting observation speaks in favour of our earlier assumption.

The experiments described have left open the question of the mechanism through which brewer's yeast inhibitis the amenorrhoea due to ketogenic diet. To answer this question, experiments were performed with the urine of women in menopause who excreted much prolan A and with that of a young woman in whom increased prolan A excretion was brought about by ketogenic diet. These women were given 50 mg vitamin B_1 daily for 10 succesive days i m. During the period of vitamin administration and on the 14 th and 20th day immature female mice were repeatedly treated with their urine. As figure 12. shows, advanced follicle maturation

occurred in the ovaries. Figure 13. shows the ovary of an animal treated with a urine taken on the 3. day of vitamin B_1 administration: the intensity of maturation has decreased. Similar conditions were found on the 5. day (Figure 14). On the 8. day (400 mg of the vitamin) only primary follicles were present (Figure 15). On the 14. day (4 days after stopping the administration of vitamin) Graafian follicles again appeared (Figure 16). The patients' hot flushes ceased during vitamin B_1 treatment but recurred again, one week after the last dose of vitamin.

As it may be seen, vitamin B_1 inhibits the prolan A production of the anterior pituitary lobe. Its action is similar that of the follicle hormone. This similarity was also manifested in the effect exerted on the hot flushes in menopause.

The question referring to the mode of action of bewer's yeast counteracting the amenorrhoic effect of ketogenic diet has, on the basis of above experiments, been answered as follows. If vitamin B_1 is administered simultaneously with the ketogenic diet to healthy mature women no change occurs in the normal cycle, because the overproduction induced by the ketogenic diet of prolan A is balanced by the inhibiting effect of vitamin B_1. Thus no essential change is produced in the activity of the anterior lobe of the hypophysis.

In further experiments made in the society of Eilzabeth *Winkler* we investigated whether the inhibiting effect of vitamin B_1 on the prolan A production is accompanied by histological changes in the hypophysis.

First the experiments were made on mature female rabbits. The hypophysis of 3 normal female rabbits were examined to establish the proportion of the different kinds of cells in the anterior lobe. (Mallory stain as modified by *Farkas* was applied.) The number of chromophobic and eosinophil cells was found to be about equal, while the number of basophils was about 3% (Figure 17.). The data found correspond with those of *Farkas* and *Berblinger*.

After this, mature female rabbits were given daily 10 mg vitamin B_1 s.c. for different periods. The results can be summarized as follows: In the pituitarity of mature, isolated female rabbits the number of eosinophil cells multiplied at the expense of the chromophobes, that of the basophils diminished. The ratio chromophobe: eosinophil shifted from the normal 1:1 to 1:2. The great increase in acidophil colloid is considered to be paricularly characteristic. The anterior pituitary lobe shows a marked eosinophil hyperfunction the signs of which were, on the one hand, ageing eosinophil cells with pycnotic nuclei and vacuolized thin protoplasm (Figure 18.), on the other hand remarkably many young cells with a loose protoplasm abundantly filled with large eosinophil granules (Figure 19.).

Though the results of animal experiments cannot be applied to human beings these experiments indicate with a certain probability that the inhibition by vitamin B_1 of the prolan A production is brought about by the eosinophil hyper-activity suppressing the basophil function.

The hypophysis of animals castrated at maturity shows a picture of basophil stimulation, and the great amount of colloid present is considered to be quite as characteristic as the castration cells (Figure 20.). If the animals are given vitamin B_1, either directly after castration or weeks later, the basophil stimulation presumably due to castration and the eosinophil one caused by vitamin B_1 appear together: besides the great quantity of basophil colloid much fuchsinophil colloid can also be seen partly separately, partly mixed, in the spaces between the pseudoacini, in the capillaries and the larger sinusoids. This picture was so characteristic that it could, even with low power magnification, be said at first glance that the animal had been castrated and given vitamin B_1. A particularly great number of vacuolized eosinophil cells can be seen in the anterior pituitary lobe of animals subjected to castration + vitamin B_1 therapy (Figure 21.).

In these experiments, the weight increase of the castrated animals stopped when vitamin B_1 was administerd, then it showed a downward tendency, like under the effect of follicle hormone. Really, the effect of vitamin B_1 and that of follicle hormone resemble one another in many aspects. An essential difference, however, is that follicle hormone causes the tissue changes of castration-hypophysis to disappear whereas the vitamin is incapable of preventing the development of the castration-hypophysis, nor does it cause a regression of the changes already developed. The mechanism of the effect and the point of attack of vitamin B_1 do, therefore, differ from those of the follicle hormone.

The results of the experiments may be summarized in that vitamin B_1 produces hyperactivity of the eosinophil cells in the anterior pituitary lobe of mature female rabbits.

The fact that eosinophil adenomes are accompanied by increased bone growth whereas the pituitaries of McDowell's dwarf mice are deficient in eosinophil cells, speak in favour of the role of eosinophil cells in growth-hormone secretion. Therefore, the idea was raised that growth-hormone secretion might be increased by vitamin B_1, through stimulation of the eosinophil cells.

In further experiments the mode of action of vitamin B_1 on the pituitary and on the ossification of the long bones of immature female rabbits and rats, or those which reached maturity during the experiment, was investigated as well as the problem whether the possible changes observable could be brought into a causal correlation with the injection of vitamin B_1.

The experiments were divided into two groups: rabbit and rat experiments.

The rabbits received 10 mg, the rats 5 mg vitamin B_1, for different periods. In these experiments the pituitaries were worked up in serial sections. From each rabbit hypophysis 160—250, from each rat 50—100 horizontal sections were made. All sections were examined and the ratio of the different kinds of cells estimated. Further

the distal epiphysis of the femur and the proximal one of the tibia, as well as the ovaries, were worked up for histology.

Figure 22. shows the distal epiphysis of the femur of a rabbit given altogether 490 mg vitamin B_1: large marrow cavities, strong, thick, abundantly calcified bone trabeculae, a rich osteoblast border, a lamellar structure around the bone cells, were found. The difference is particularly striking if it compared with the microphotogram from a control animal of equal weight (Figure 23.).

A peculiar disorder of the enchondral ossification was found in the femur of one of the rabbits after the administration of 490 mg vitamin B_1 (Figure 24. and 25. low and high power magnification). The bone trabeculae of this animal proved to consist of osteoid tissue were short, their ends were gross, gnarled and terminating with a sharp line.

In the other two cases a few trabeculae of somewhat uneven thickness and poor calcification of the bone were found. In these cases the ossification was of atrophic type, while in the first case it was hypertrophic.

A further question is whether changes can be demonstrated in the hypophysis of these animals. According to earlier examinations. in the anterior pituitary lobe of mature, older rabbits the number of chromophobic and eosinophil cells is about equal, the number of basophil cells is few, and an almost exclusively basophil colloid formation is present.

The structure of the pituitary of immature, or recently matured, female rabbits differs in several points from the above. In these animals the number of chromophobic cells is usually greater than that of the eosinopils. There is a difference in the colloid formation also. While in older, mature animals almost exclusively basophil colloid is to be found, in the young animals at the borderline of maturity usually a little fuchsinophil colloid also can be demonstrated in the anterior lobe. This difference is undoubtedly due to the increased growth-hormone secretion (i. e., increased eosinophil activity) permitting these young animals increased growth. The increased formation of eosinophil colloid, and retrograde changes observed in the eosinophil cells, point in this direction (Figure 26, 27.).

So it is not easy to recognize the stimulating effect of vitamin B_1 on the eosinophil function in rabbits at the borderline of pubescence, but it can undoubtedly be recognized. Figures 28. and 29. show the anterior and posterior lobes, respectively, of the pituitary of the animal in which the severe ossification disturbances of atrophic character were found. In this hypophysis the number of eosinophil cells is not greater than in the control, but among the eosinophils there are many regressive forms which indicate increased secretory activity, like the migration of fuchsinophil colloid into the posterior lobe.

The pituitary of only one rabbit showed basophil stimulation as an effect of vitamin B_1, and, strangely enough, this animal

displayed the ossification disturbances characteristic of hypertrophy which have been mentioned. This phenomenon is not believed to be a fortuitous coincidence.

Marked disturbances of enchondral ossification were found in half the rats treated with vitamin B_1. Figure 30. shows the epiphysis of a control rat. There are no signs of disturbed ossification. Figure 31. is of the epiphysis of an animal treated with vitamin B_1. In this the cartilage cells of the deeper layers are edematous, they have ceased to be arranged in regular columns. Flattening of the marrow cavities, swelling and paucity of the bone trabeculae mean deficiency of lime in the cells which has led to swelling of the bone cells and the appearance of lime-deficient osteoid lamellae. In one animal (Figure 32.) the increased pressure in the metaphysis of the tibia also produced bending of the thickened, lime-deficient bone lamellae. Swollen, lime-deficient epiphyseal bone splinters can be seen in figure 33. deriving from another experiment, with swollen bone cells. The bone splinters seem inflated. Many giant osteoblast cells appear in the emerging marrow cavities, especially in the marginal parts beside the bone trabeculae.

In connection with the disturbed ossification just described, the question arises whether we are not dealing with some form of spontaneous rachitis, relatively common in rats. The fact that no trace of rachitis could be observed in the control animals proves that the ossification disorder seen in the vitamin B_1-treated animals was not due to deficient diet but to the B_1 hypervitaminosis induced.

In three out of ten animals treated with vitamin B_1 the number of chromophobic cells and eosinophils in the anterior pituitary lobe was equal, in one the number of eosinophil cells was greater than that of the chromophobes. In 6 out of the 10 cases, strong eosinophil hyperactivity, many piknotic-nuclear eosinophil or fuchsinophil cells were found, their protoplasm turned into fuchsinophil colloid indicating an abundant production of the latter. In these cases fuchsinophil colloid was found in abundance among the fibres of the posterior lobe also.

The treated animals gained more weight than those used for control. There was no essential difference in their growth.

The ovaries of the animals treated with vitamin suggested a very important connection. In the ovaries of 6 out of 10 treated rats corpora lutea were found, whereas out of the control animals only one had a corpus luteum.

The mechanism of the luteinizing action of vitamin B_1 was not clear but it was not considered a chance finding. *Lajos* writes in a summarizing abstract recently published, that eosinophil cells produce prolan B, the luteinizing hormone. The same is to be found in *Selye*'s Textbook of Endocrinology. If this is true it can be understood why vitamin B_1 had a luteinizing effect in our rat experiments. It increases the activity of the eosinophil cells whereby it leads to increased secretion of prolan B. The result is increased luteinization of the follicles.

In this connection interesting experiments are being performed at the II. Gynecological Department by *Csillag* and the author of this paper. It was thought that, if ketogenic diet increases the prolan A production and vitamin B_1 the secretion of luteinizing hormone by stimulating the eosinophil cells, the injection of the urine of individuals kept on ketogenic diet into immature female mice or rats, followed by the administration of vitamin B_1, would elicit a positive Aschheim-Zondek reaction. Up to now we have performed this experiment with the urine of one woman kept on ketogenic diet. There were no blood spots but the corpora lutea were easily perceptible to the naked eye on the enlarged, livid ovaries. The result of the gross examination has been proved by histology. These experiments will be continued.

Figure 34. shows the ovary of an immature rat to which the urine of a woman kept on ketogenic diet has been injected: increased follicle maturation can be seen. Figure 35.: the animal treated with the same urine was given daily 7 mg vitamin B_1 for two days after the treatment with the urine. A large and a smaller atretic corpus luteum can be seen. Figure 36. and 37. show the same corpora lutea under higher magnification. In figure 38. a normal primordial follicle from the same ovary can be seen.

Clinical experiences have shown that the various functions of the anterior pituitary lobe undergo a parallel change under certain physiological conditions, or in certain diseases. During the experiments palpitation and tremor of the hands were encountered in a part of the experimental subjects kept on ketogenic diet for a protracted period; one of the patients complained of tightness in her neck. In four patients of *Berger* and *Vajda* kept on ketogenic diet, palpitation was attended by an increase of the basal metabolism ($+26$ to $+40\%$). Probably, ketogenic diet enhances the thyreotropic activity of the anterior pituitary lobe.

It has long been known that, after a diet rich in fat, or after fasting, the blood-sugar curve goes higher up than the normal one and its descending section is protracted. It is assumed that sugar tolerance is impaired by diet or starvation. However, little is known of the mechanism of this phenomenon. On the basis of the correlations between ketogenic diet and anterior pituitary lobe, it was considered worthwhile to extend our researches to the possible correlation between ketogenic diet and diabetogenic activity of the anterior pituitary lobe. These experiments were also carried out with Elizabeth *Winkler*.

Preliminary experiments showed that the blood sugar curve became diabetoid in the course of a ketogenic diet. Figure 39. demonstrates this effect. This is the blood-sugar curve of a woman suffering from hypophyseal infantilism. It is perhaps a little higher than usually, but in two hours it returns to the initial level. Therapeutic experiments were carried out on this patient for nearly two years with ketogenic diet. On one occasion, after three months of therapy a three months' pause was maintained. A dextrose tole-

rance test performed then showed a marked diabetoid curve (I). Dieting a week it became still more pronounced (II). After another week the blood sugar increased after the administaration of dextrose from 100 mg% to 282 mg%, after 2 hours it was still 257 mg% (III) and glucosuria occurred. The blood sugar curve of this patient was diabetoid in character for years, in any case without diet. By the ketogenic diet a prediabetic condition was produced.

The results of these experiments are not new as it is a well-known fact that a diet rich in fat makes the blood sugar curve higher and more prolonged. Nevertheless, they represent new contributions if they are considered as links in a chain of correlations recognized as generally valid: ketosis of whatever origin is a strong stimulus to the function of the anterior lobe of the hypophysis. It has been assumed that diabetogenic effect of the ketogenic diet is brought about through the mediator role of the anterior pituitary lobe. To prove this, further experiments were necessary.

Regarding that the diabetogenic substances isolated from the urine have been shown to be identical with those deriving from the anterior pituitary lobe the diabetogenic peculiarities of the urine of a subject kept on ketogenic diet were investigated in rabbits. Irrespective of the details of the experimental method, it is to be noted that experiments were always carried out in three groups: 1. The blood sugar incerasing action of urine was investigated in longlasting experiments (diabetogenic substance) and 2. in experiments of short duration; 3. further it was investigated whether it suspends the hypoglycaemic effect of insulin (contra-insulin hormone). The urine was given to the animals i. v.

In one of the longlasting experiments the rise in the blood sugar was 49% if the urine was taken before the diet, and 67% if urine after the diet was injected. In the short experiment no significant difference could be detected in the blood sugar of animals treated with the urine taken previous to, or after, the diet. While the urine taken before the diet did not diminish the hypoglycaemic effect of insulin, that taken by the end of the ketogenic diet had a cosiderable contra-insular effect.

Since the patient's blood-sugar curve did, after a time, show constant diabetoid characteristics the urine taken before the diet could not be cosidered as normal either. The unusually high blood-sugar produced in the pre-diet phase of the experiment is thought to be related to the increased diabetogenic activity of the anterior lobe. This is the patient who was being kept on ketogenic diet for about two years, the interruption of which for shorter or longer periods was, however, no longer followed by the reduction of its stimulating effect on the anterior lobe.

In another case the amount of the carbohydrate metabolism hormone was increased. While the blood sugar of the animals treated with pre-diet urine rose from 124 mg% to 146 mg% and fell to 101 mg% after 2 hours, the rise in the blood-sugar of the

animals treated with urine taken during the diet was considerably higher and protracted; it mounted from 139 mg% to 202 mg%, and after 2 hours it was still 150 mg%. On repeated injections in the short experiment the rise in the blood sugar was usually less. That this phenomenon was not due to a decrease of carbohydrate metabolism hormone is clearly shown by the fact that in the rabbits which had never been treated before the injection of this urine produced as high, or even higher a rise in the blood sugar, as the first injection did in the rabbits in the protracted experiment.

Experiments so far had shown that ketosis increased several functions of the anterior pituitary lobe (secretion of prolan A, thyrotropic hormone). Obviously, vitamin B_1 inhibited not only the prolan A production but also other functions of the anterior lobe.

Investigations being carried out with *Megyesi* to reveal the correlation between vitamin B_1 and ketogenic function are still in progress but it has already been shown that the ketogenic function, i.e. the specific dynamic effect of the protein, may be reduced by vitamin B_1.

Many investigators have studied the correlation between vitamin B_1 and carbohydrate metabolism and it is, considering the simple and fairly uniform experimental methods used, surprising how diverging the data are. No doubt, in a part of cases vitamin B_1 has an insulinlike effect. However, those researchers who observed an improved tolerance in diabetes during vitamin B_1 therapy pointed out that, owing to an unknown cause, aneurin was ineffective in many cases of diabetes. The results being not uniform, their explanation related to the mode of action of vitamin B_1 do also differ.

The problem was considered approachable on the basis of the above experiments. It was thought that vitamin B_1 would inhibit not only the secretion of prolan A but the diabetogenic function of the anterior pituitary also.

Among the mechanisms maintaining normal carbohydrate metabolism the balance between insulin and the diabetogenic hormone is perhaps the most important. According to this mechanism diabetes mellitus is the consequence either of diminished insulin production, or of increased production of diabetogenic hormone. It is in this sense that *Hetényi* speaks of the dualism of diabetes. The insulin sensitive diabetic patient is insulin sensitive because he has no, or little, diabetogenic hormone. The insulin resistant patient has much contra-insular factor. As a measure of insulin sensitivity *Hetényi* uses, like *Himsworth*, the I:D quotient. The meaning of this quotient may be seen from the following graphs. Graph 1 (Figure 10.) gives the blood-sugar values within 1 hour after the administration of 30 g dextrose per os. The other curve was obtained by giving the experimental subject i. v. 5 U insulin per m^2 body surface directly after the consumption of the dextrose. If *i* is subtracted from the D area the pure insulin effect is obtained; dividing this by the D area, the quotient I:D results which, therefore, equals

$\frac{D-i}{D}$. Naturally, the greater the effect of insulin the greater the I:D quotient i. e. the individual under testing is more insulin sensitive.

After this introduction the working hypothesis should be reported on the basis of which the experiments were started. It was assumed that the blood sugar lowering effect of vitamin B_1 was mediated through the hypophysis by inhibiting the production of contrainsular hormone in the anterior lobe. Therefore, in cases of constant insulin secretion the insulin effect can be increased by eliminating the contrainsular factor. The result will be a decrease in the blood sugar.

If vitamin B_1 acted by way of over-production of insulin it would reduce the blood sugar in any case. But if, according to our hypothesis, the vitamin acts through the hypophysis, a difference will be found according to wether the vitamin is administered to an insulin-sensitive or an insulin-resistant person. There being little or no diabetogenic hormone present in the case of insulinsensitivity, the vitamin, having nothing on which to act decreasingly, will not raise the I:D quotient. In case of insulin resistance there is much contra-insular factor, i. e. a point of attack for the vitamin and the I:D quotient will rise. That is, the insulin-resistant individual becomes insulin-sensitive by the vitamin.

From these theoretical considerations other conclusions also can be drawn. In analogy to the I:D quotient a B_1:D quotient can also be established which is the measure of vitamin B_1 sensitivity i. e. insulin resistance, like the I:D quotient is a measure of insulin sensitivity. The B_1:D quotient is obtained if the dextrose vitamin B_1 curve area is subtracted from the area of the dextrose curve and divided by the area of the dextrose curve.

If our assumption is correct a reverse relation exists between the I:D and the B_1:D quotients: where the I:D quotient is high the B_1:D quotient is low and vice versa.

Taking all this into consideration, our experimental procedure was arranged as follows: 4 tolerance tests were carried out with each experimental subject. The first experiment was a simple tolerance test with 30 g dextrose given per os. Blood sugar was determined in the fasting subject, then after 15, 30, 45 and 60 minutes. In the second experiment the ingestion of 30 g dextrose was followed by the i. v. administration of 5 U insulin per m^2 body surface; in the third one, per os 30 g dextrose, insulin and 100 mg vitamin B_1 i. v. were given. Finally, in the fourth experiment 30 g dextrose and 100 mg vitamin B_1 were given. In all experiments the blood sugar determinations were made at 15 minute intervals for 1 hour.

22 experiments were performed on 20 patients in the society of *Gábor* and *Megyesi*. The results are briefly summarized as follows.

There were 3 cases in which the anterior pituitary lobe was in a state of increased activity. One of them was the hypophyseal infantile woman already mentioned in whom the ketogenic diet given for two years turned the clinical pattern of hypopituitarism to hyperpituitarism. The second was a diabetic woman in the polyprolan stage of the menopause, the third a woman suffering from spontaneous hypoglycaemia in whom hyperpituitarism was artificially induced by transplanting calf hypophysis. All three conformed completely to our working hypothesis, as vitamin B_1 considerably increased the sensitivity to insulin and the vitamin itself caused a considerable lowering of the dextrose curve.

The third case should briefly be reported. A woman of 57 years had suffered for years from occasional attacks of unconsciousness. She first reported in this department in 1943, when a diagnosis of spontaneous hypoglycaemia was made. In October of last year she again appeared. She had also epileptiform attacks several times a day. During the attacks her blood sugar dropped even below 30 mg%. 4 experiments were carried out on this patient. Her dextrose curve was fairly low (Figure 41. I.) As the second curve shows there was a pronounced insulin sensitivity, which was reduced by vitamin B_1. The vitamin itself did rather raise than lower the dextrose curve.

First, the patient did not consent to an operation. The attempt to lower the production of insulin having failed, the augmentation of the diabetogenic factor had to be induced, to bring the patient into „Supergleichgewicht". This was thought to be brought about by the transplantation of calf hypophysis. Professor *Verebély* was kind enough to perform the operation.

A week after the operation the previous experiments were repeated (41. II.). As may be be seen, the patient's insulin-dextrose curve had changed quite surprisingly, as the previously insulin-sensitive patient had become insulin resistant: her blood sugar not only did not decrease under insulin, but mounted decidedly. Vitamin B_1 reduced this insulin resistance to a great extent. This effect of the vitamin can be read from the 4th curve. The patient greatly sensitive to insulin and resistant to vitamin B_1 became after the transplantation of hypophysis insulin resistant and vitamin B_1 sensitive.

9 cases were found which did not conform to our hypothesis; they were sensitive to vitamin B_1, yet the vitamin did not raise the I:D quotient. It was interesting that in all 9 cases some sort of disorder of the hypophyseal-hypothalamus system could be demonstrated. The cause of this phenomenon is not known so far.

The patients resistant to vitamin B_1 behaved in a way corresponding entirely to our working hypothesis: the vitamin did not influence the I:D quotient.

The last item of our hypothesis that high I:D quotient is associated with low B_1:D quotient has not been fully confirmed by the experiments, but the cited cases of hypophysis transplantation

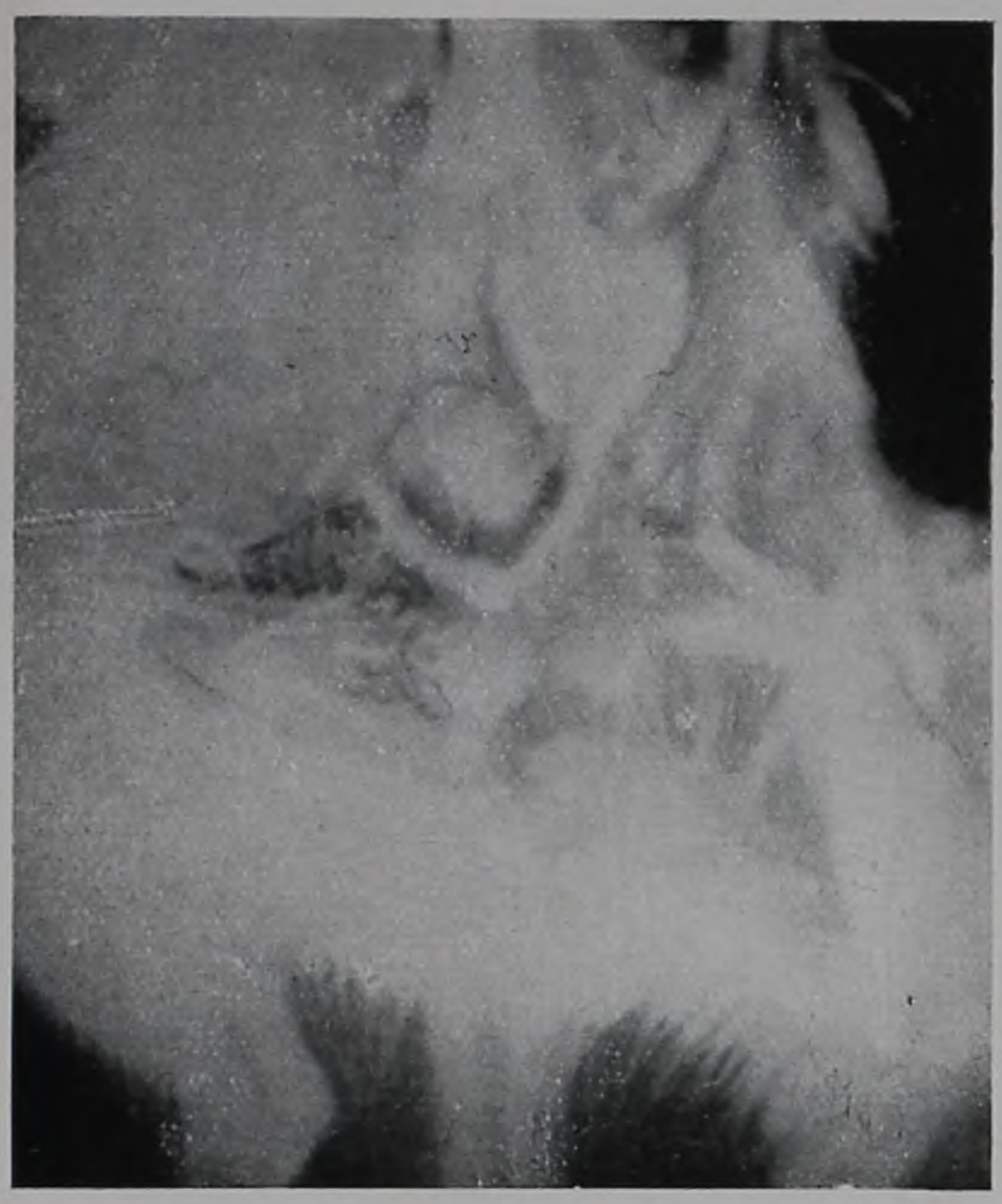
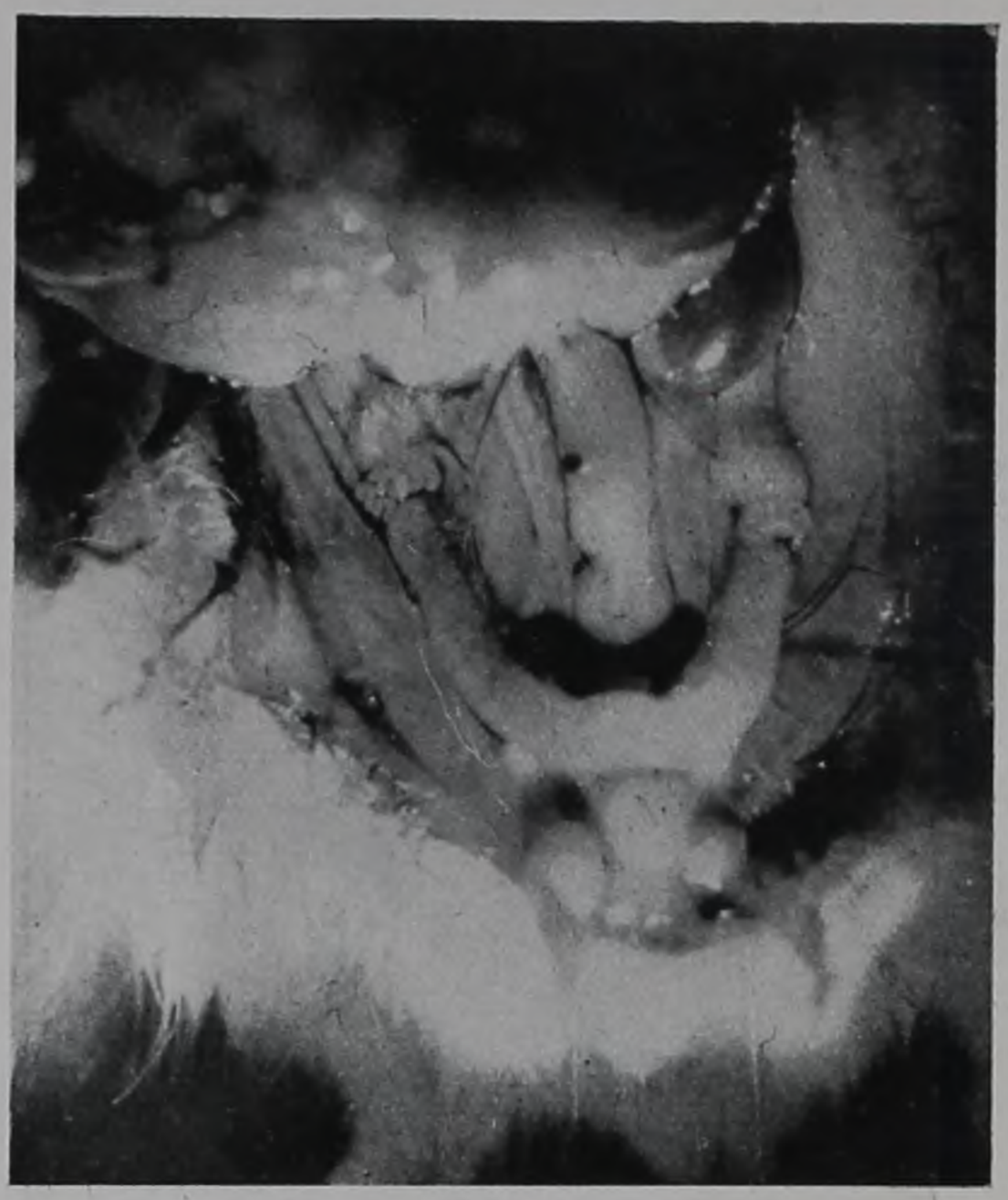


Figure 1.



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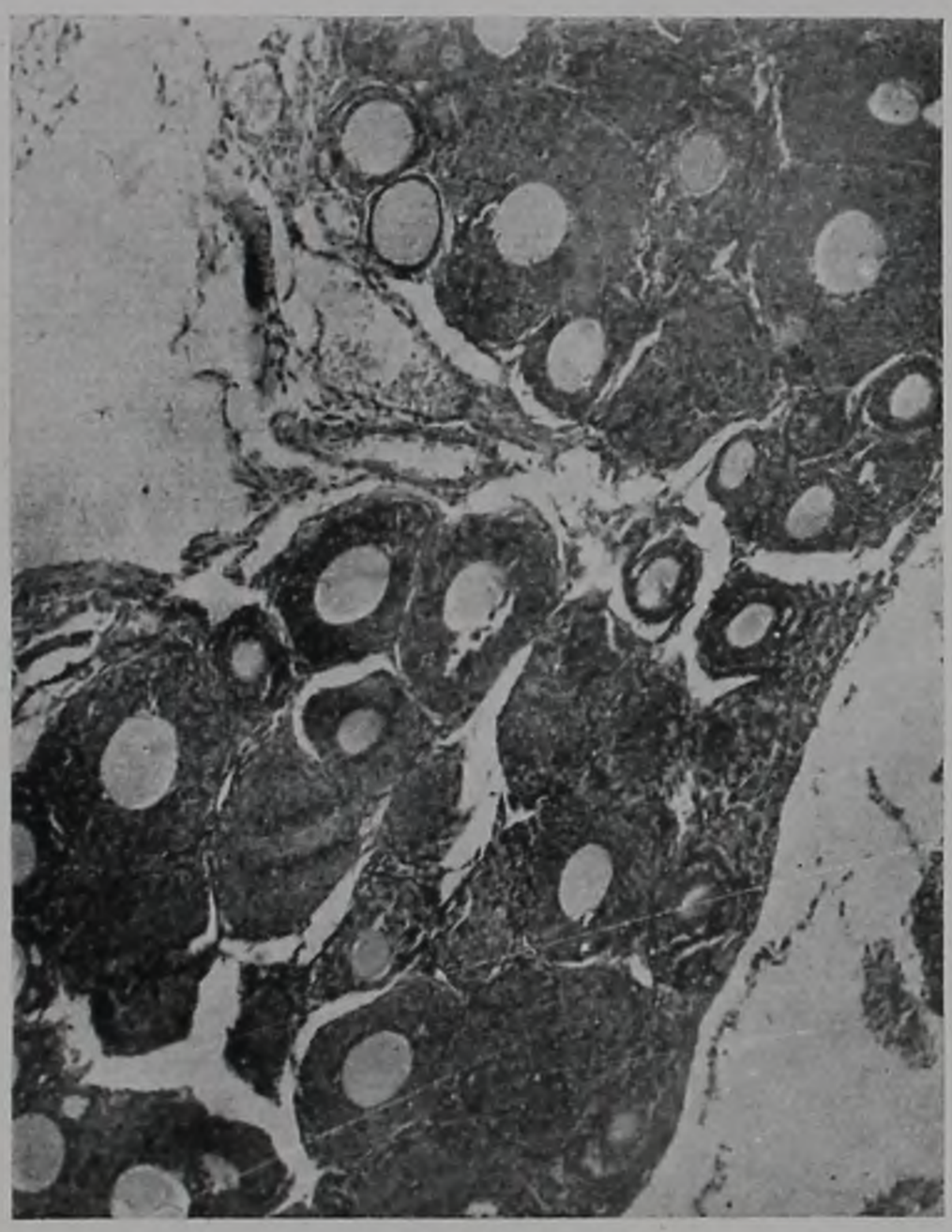

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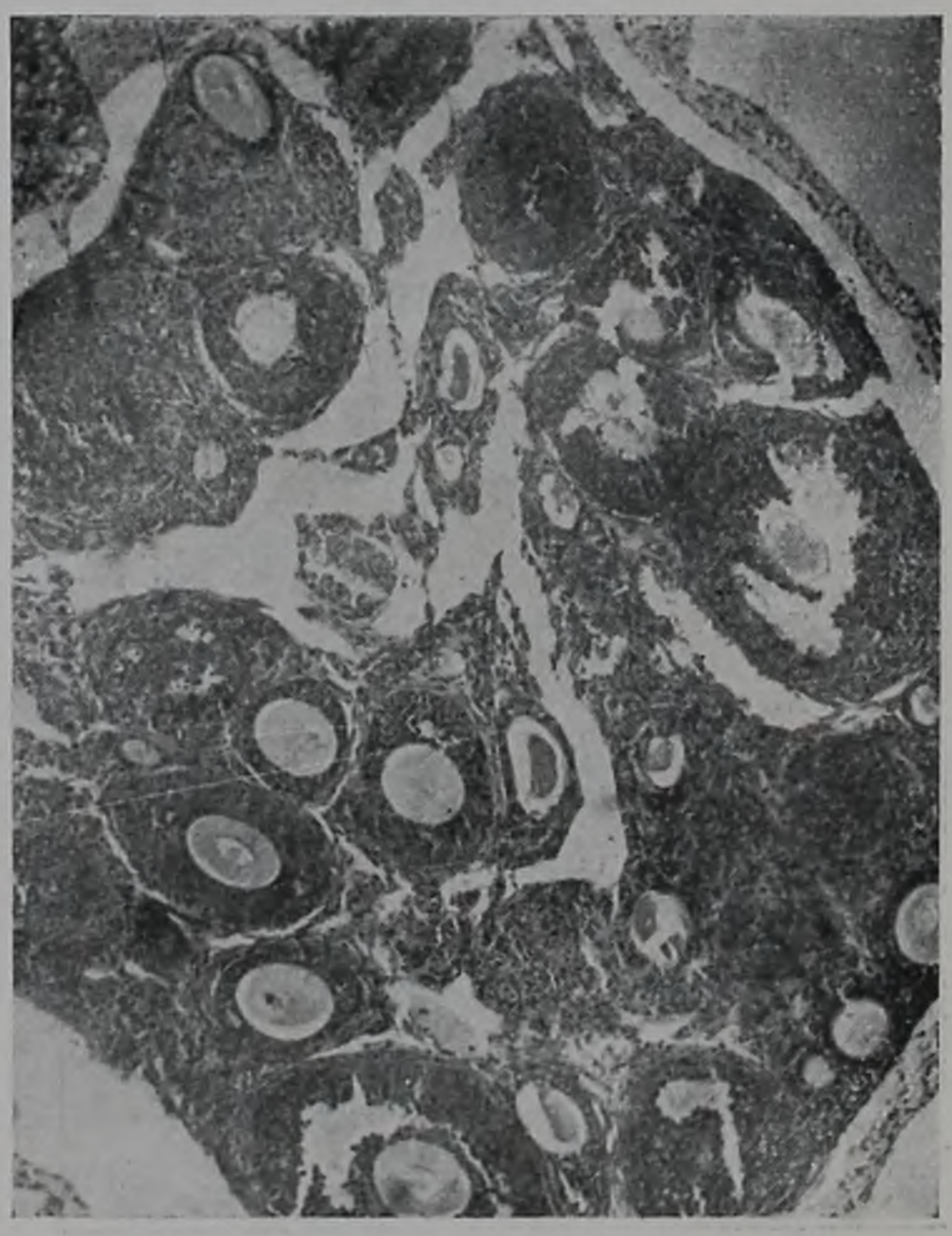

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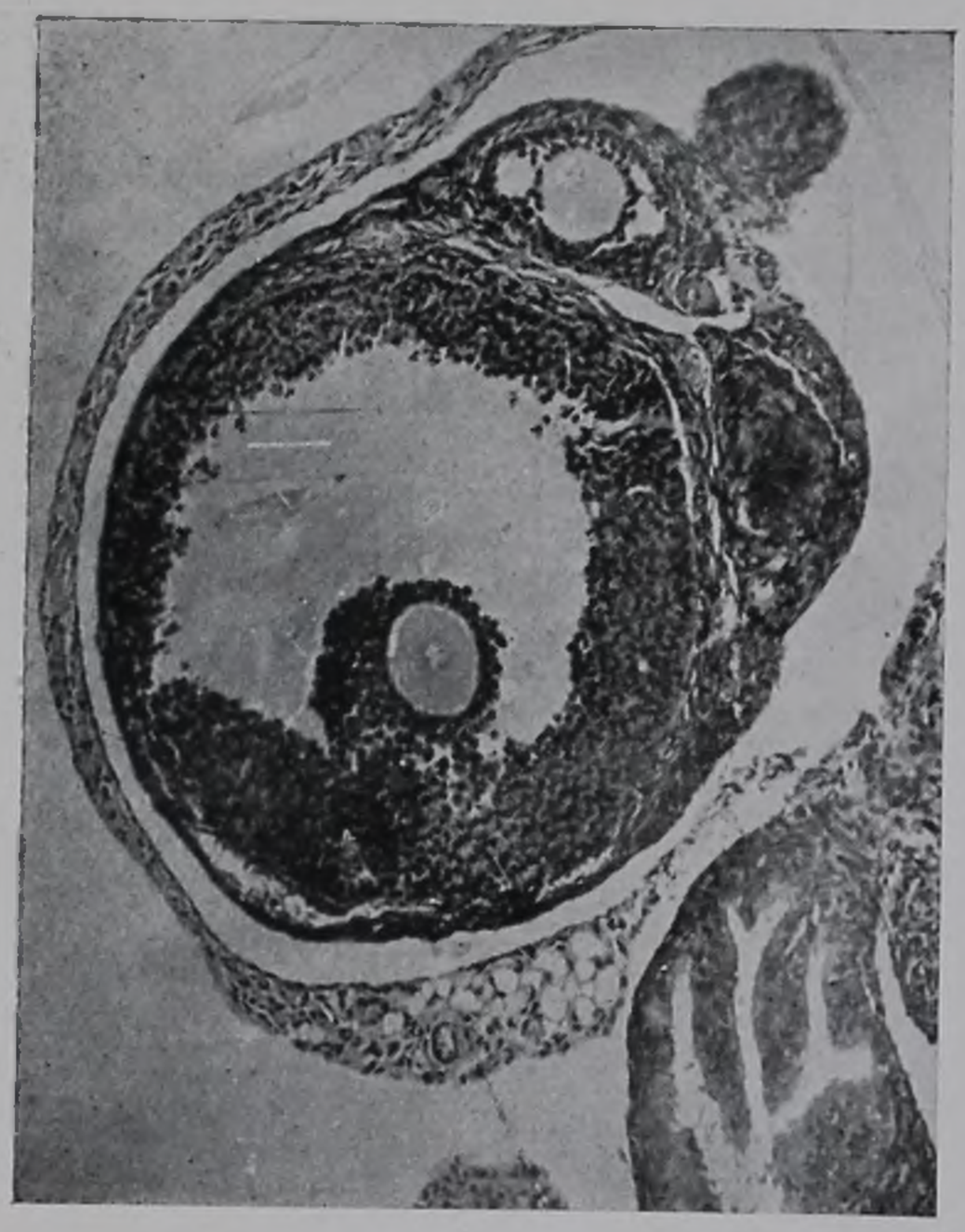

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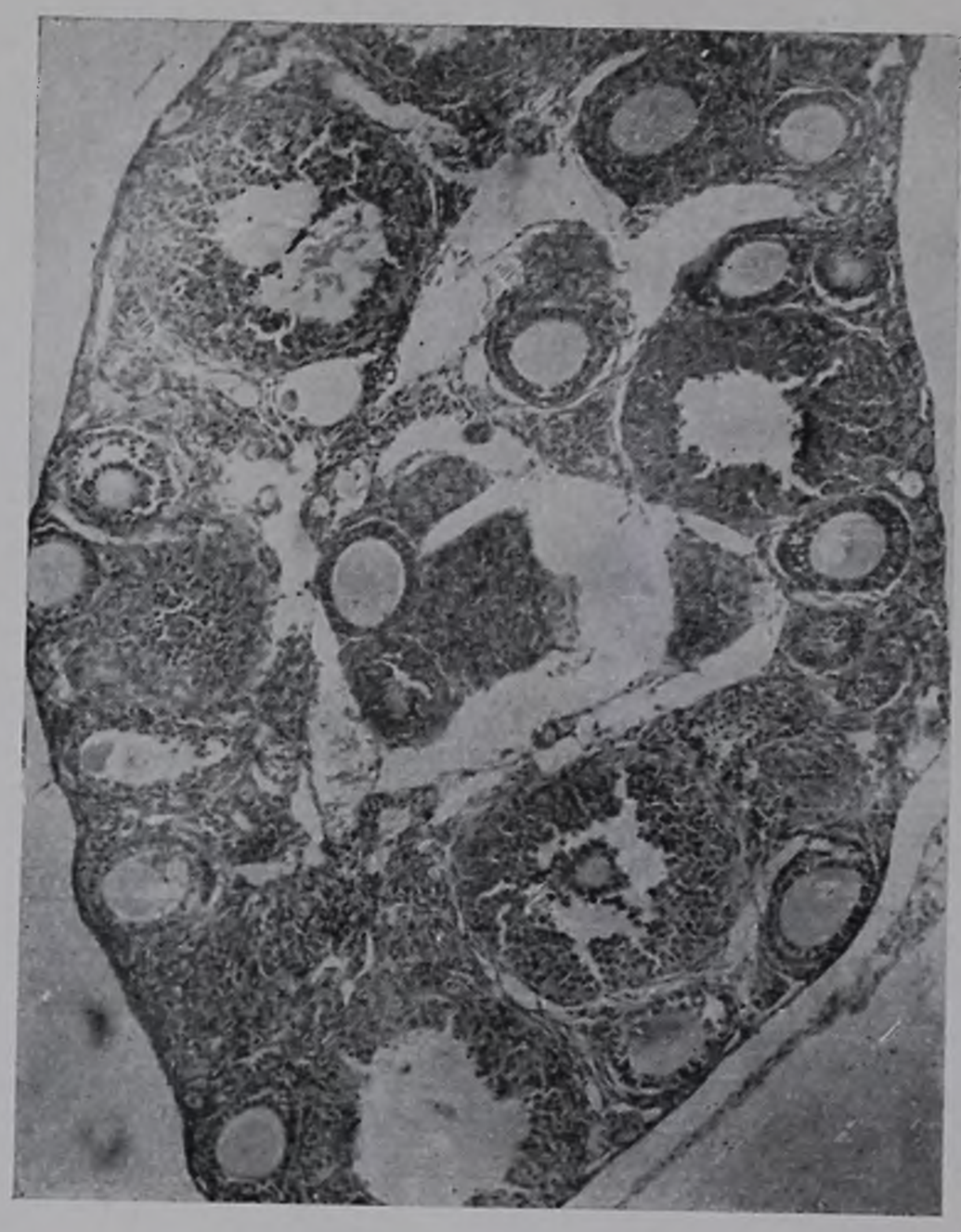

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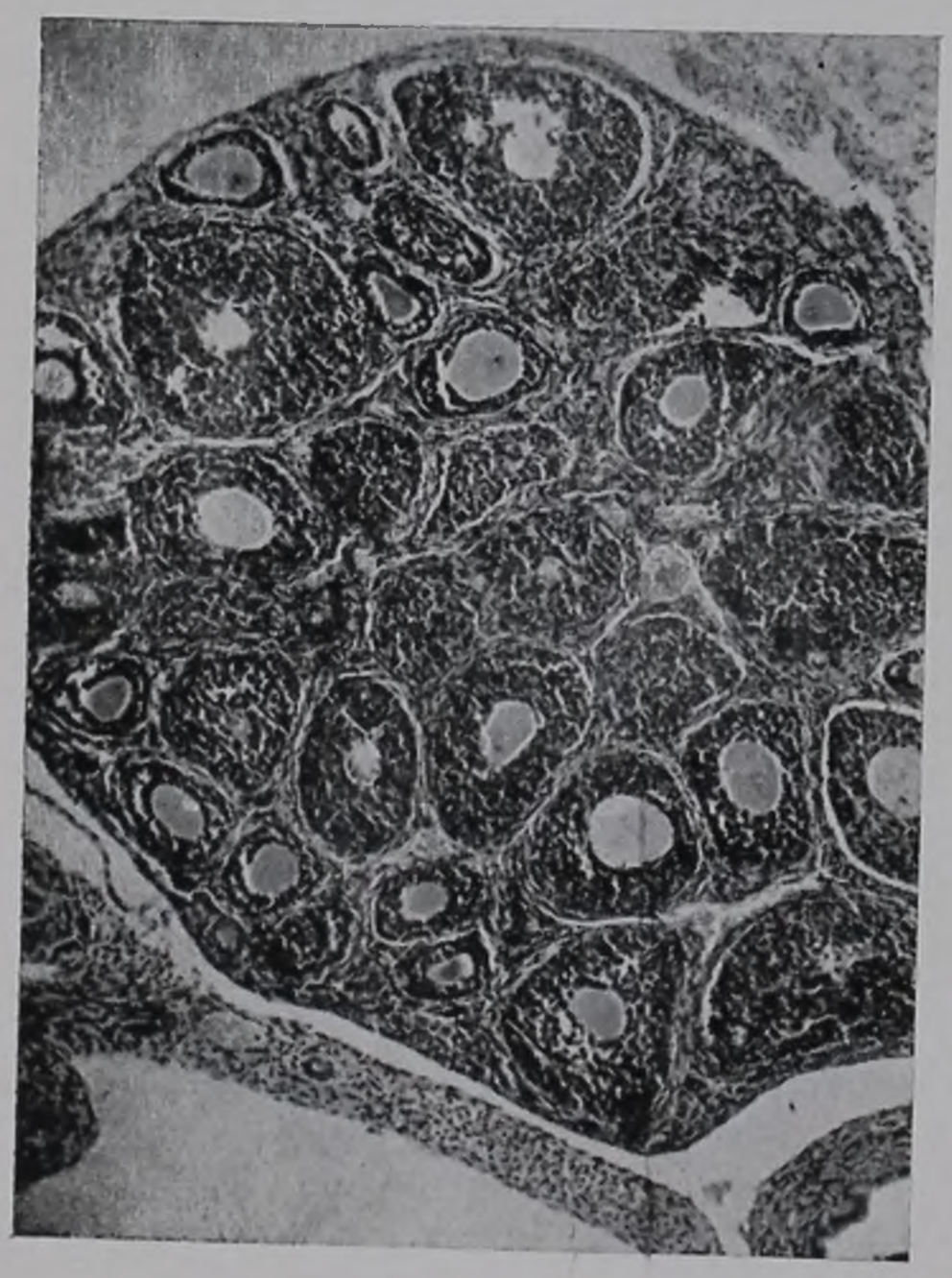

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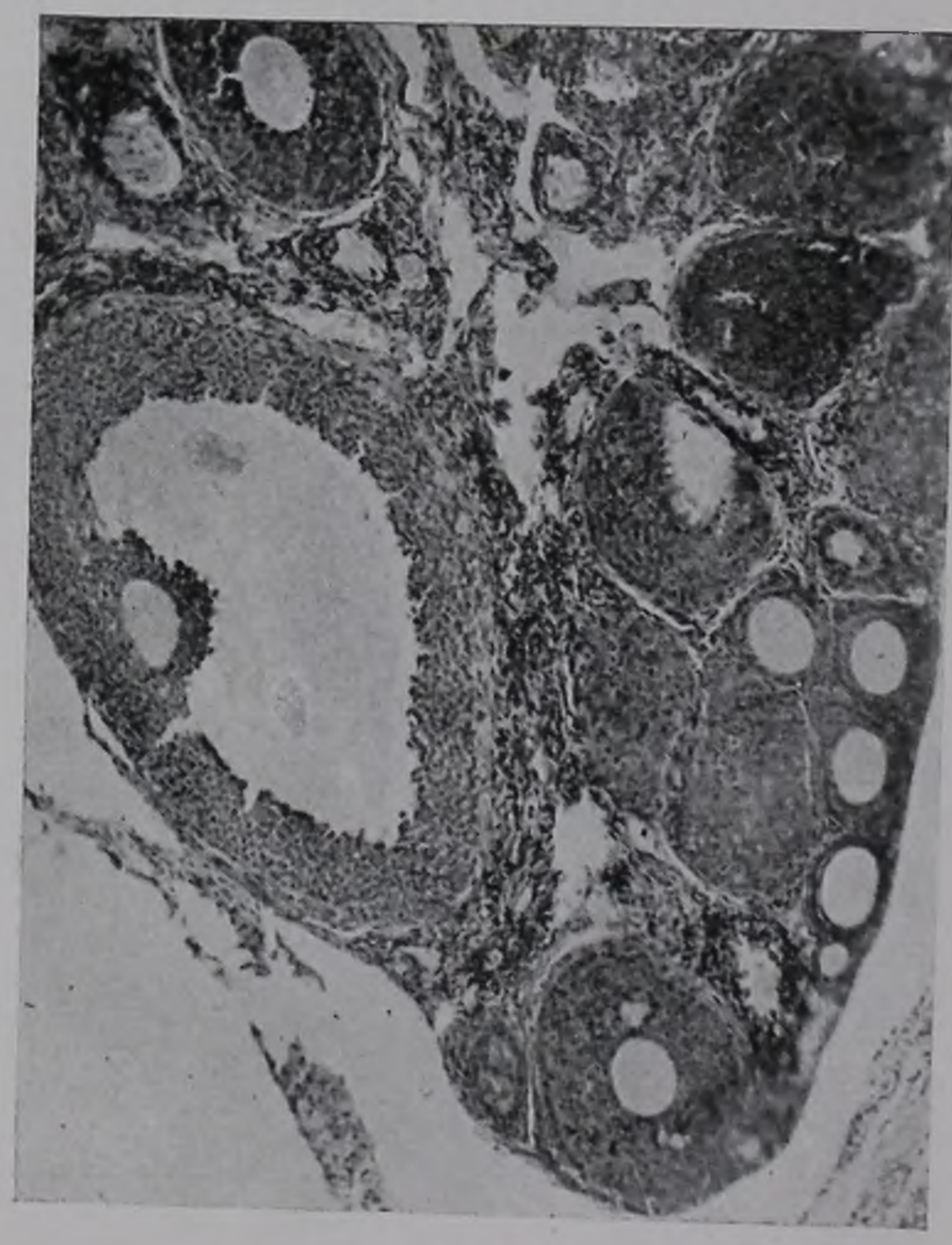

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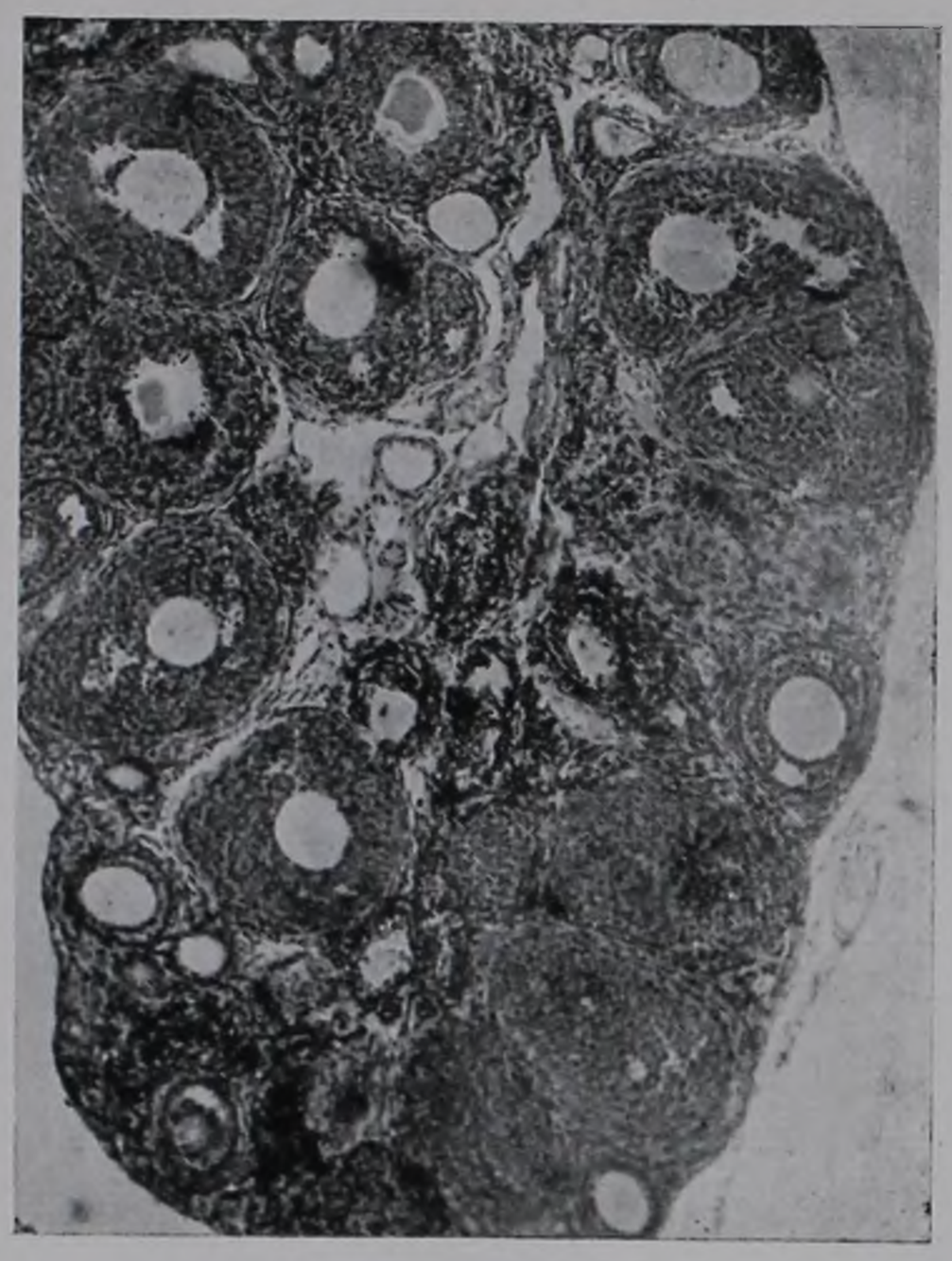

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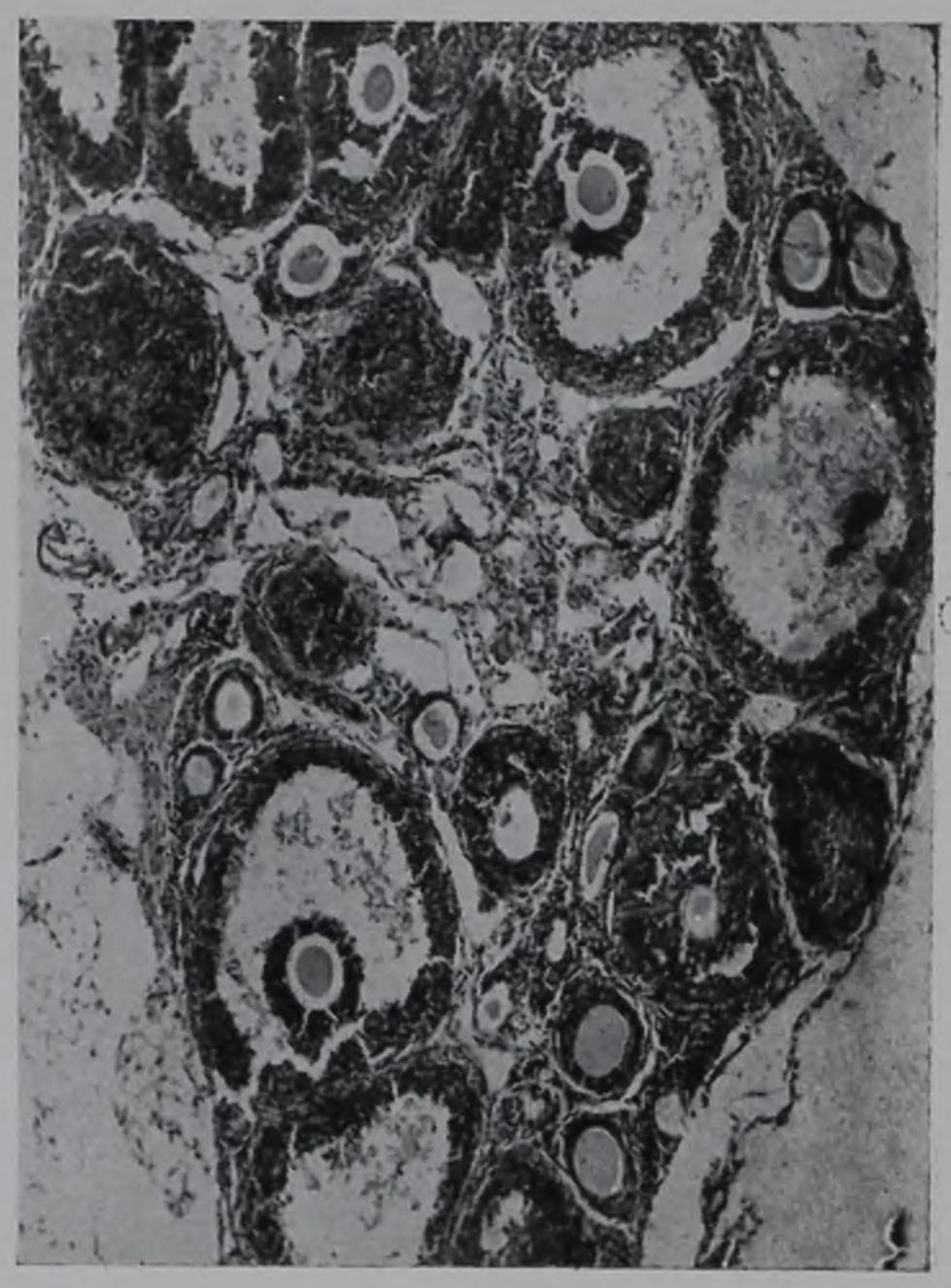

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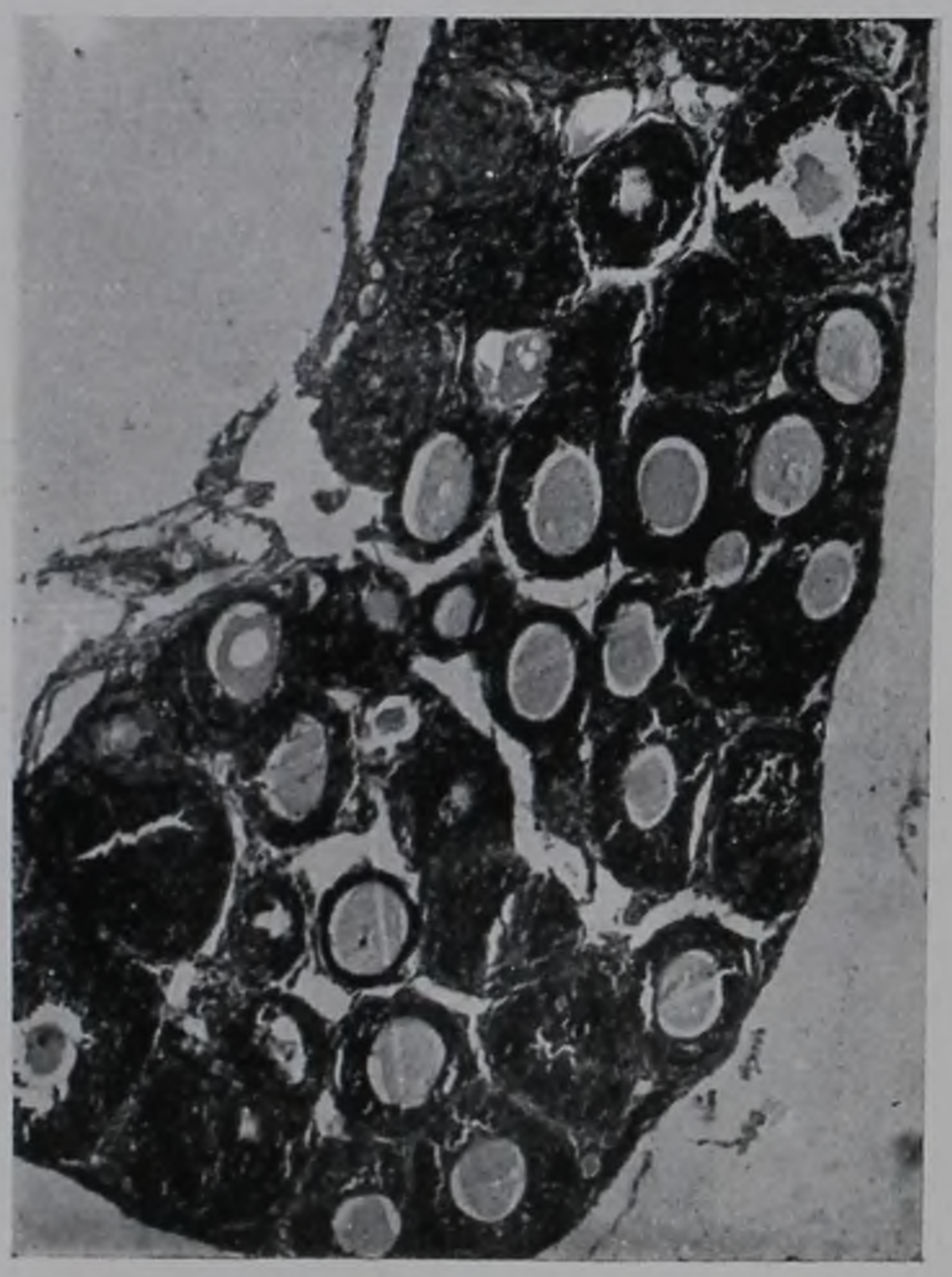

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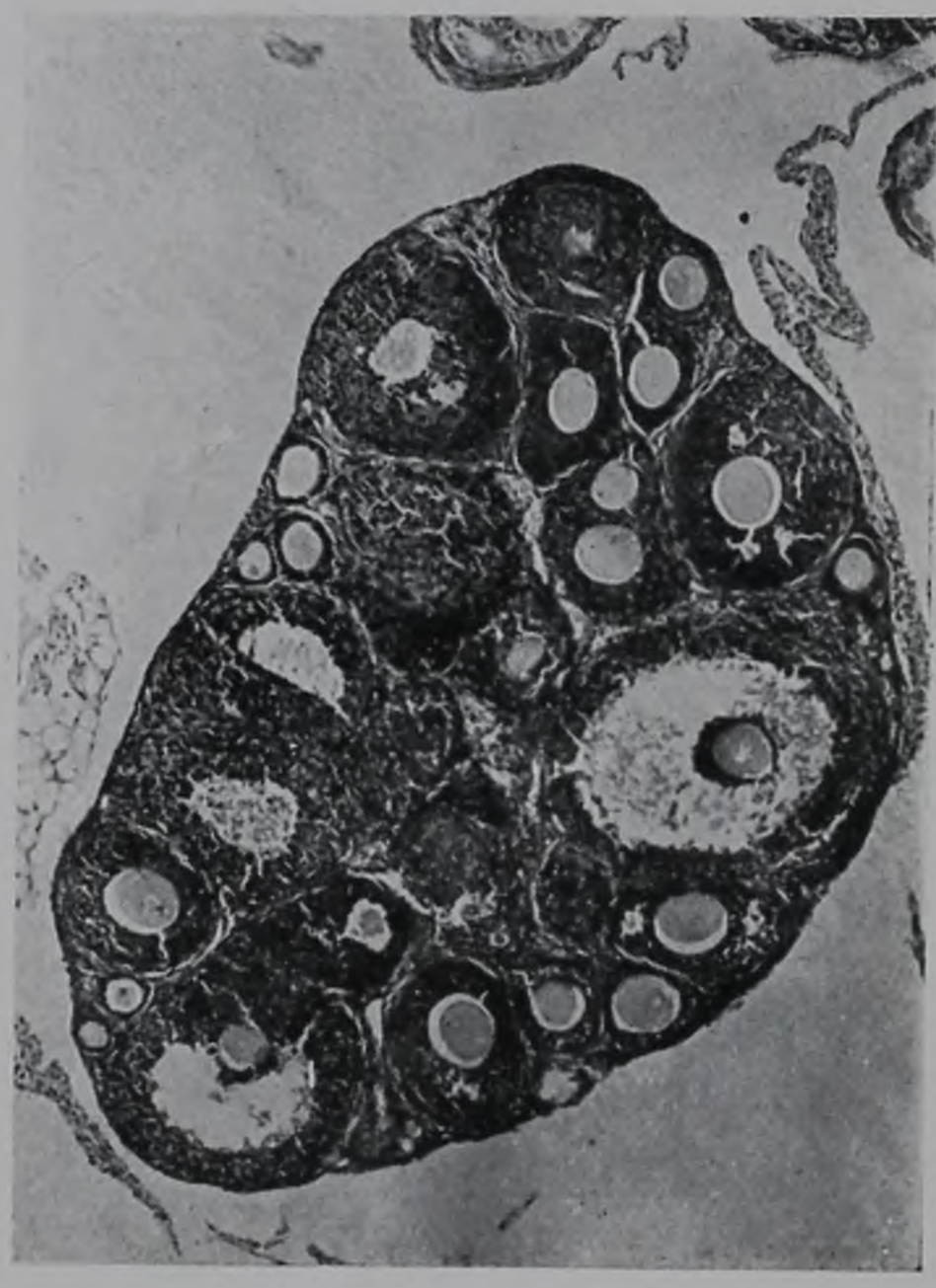

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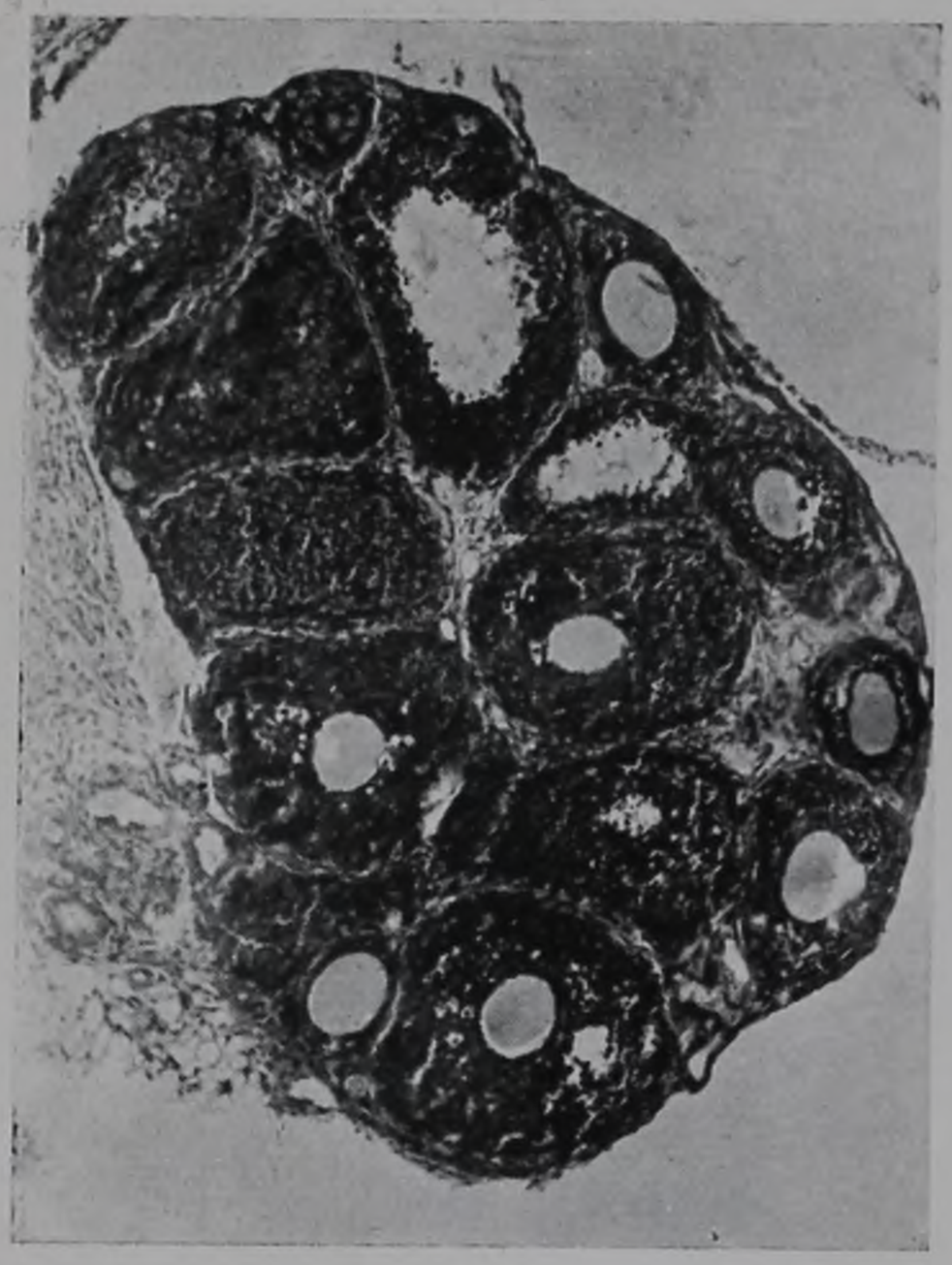

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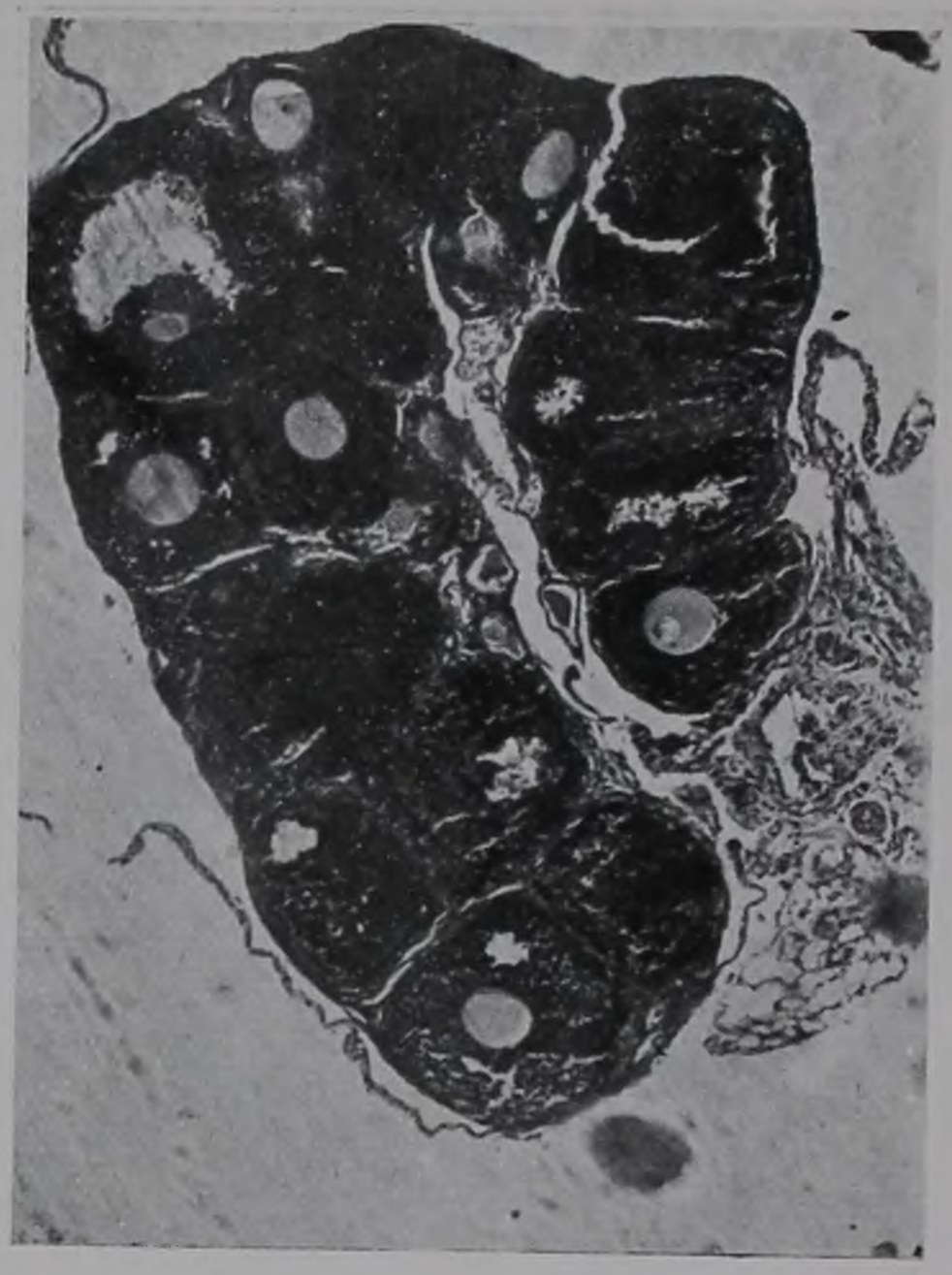

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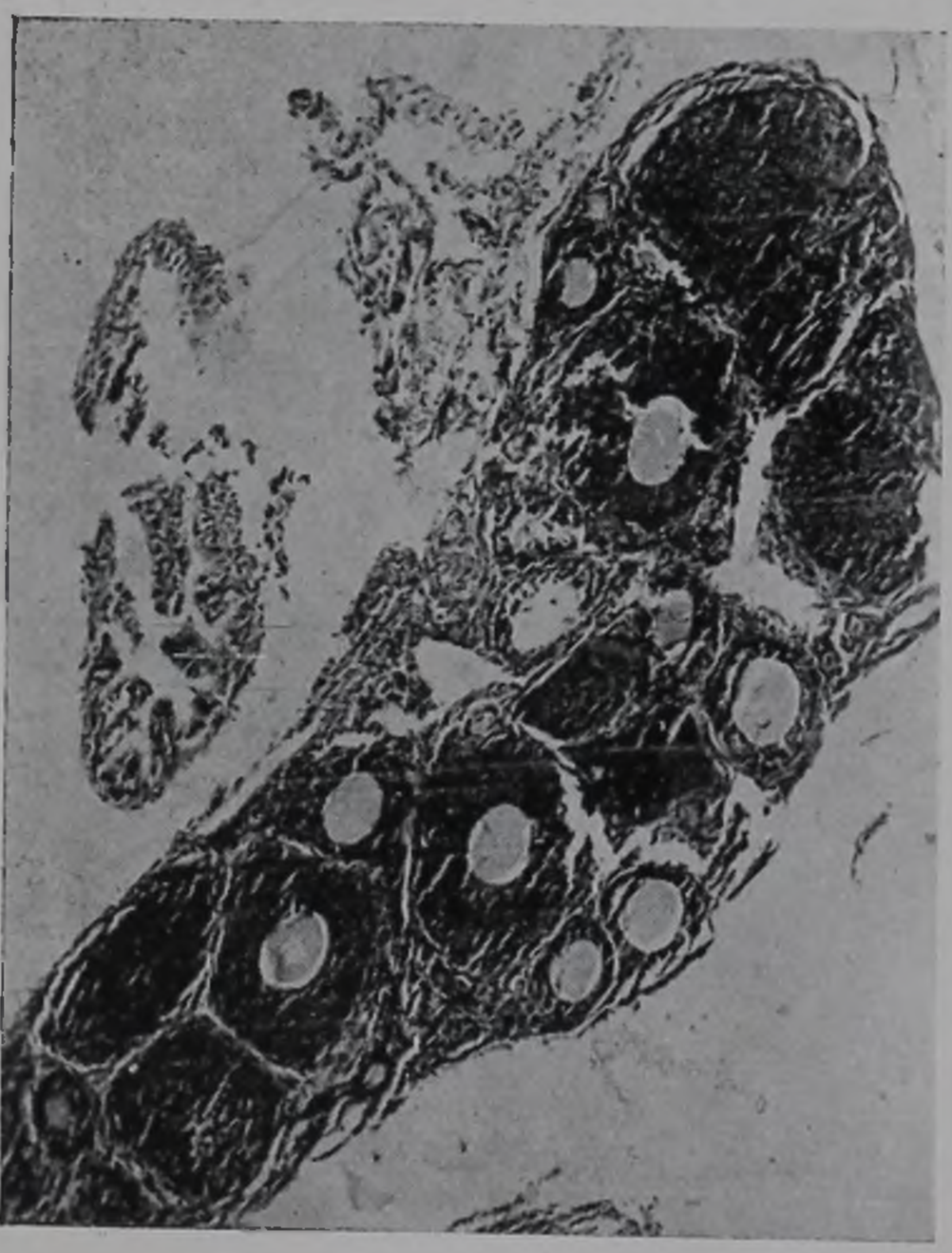

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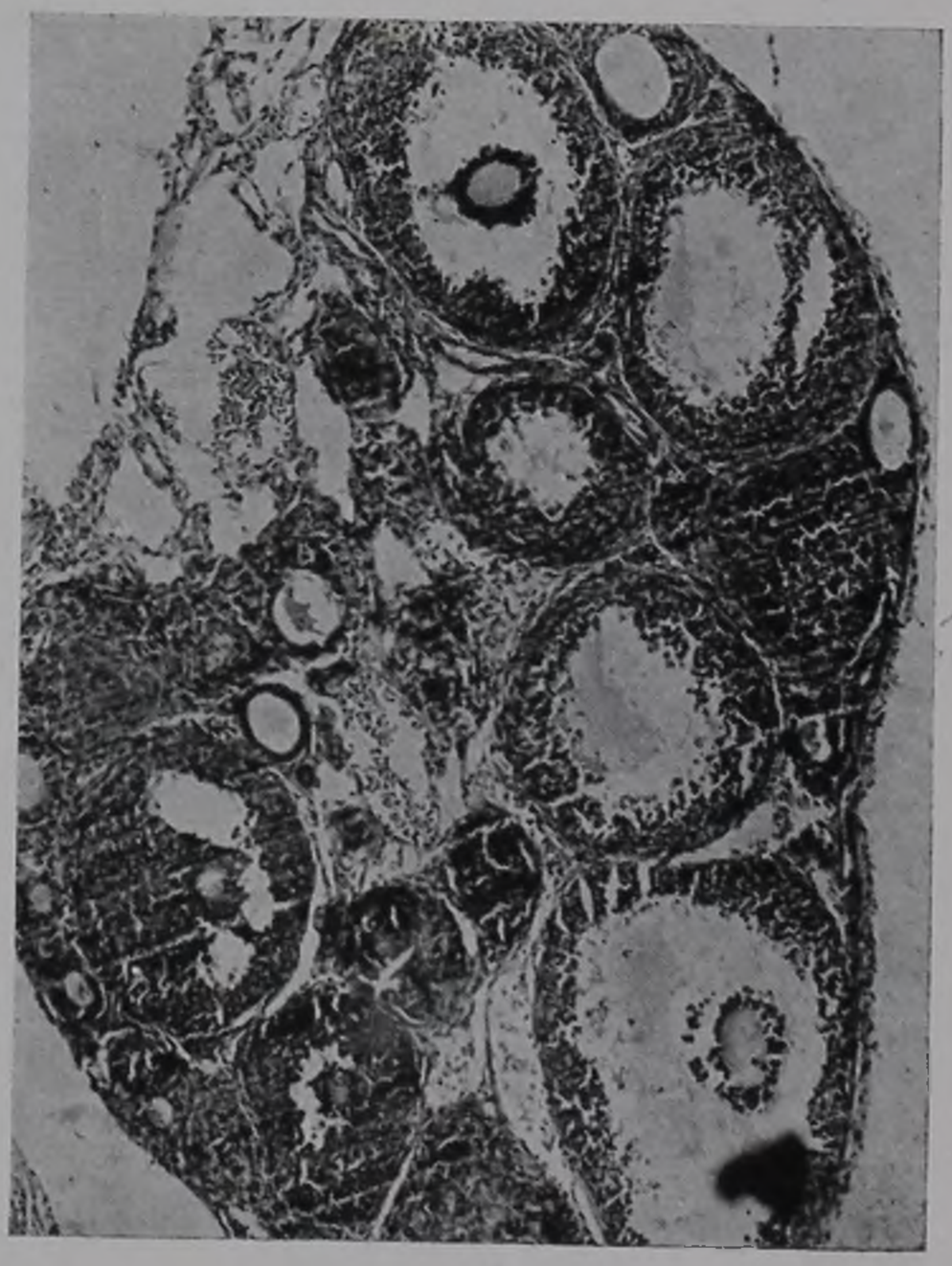

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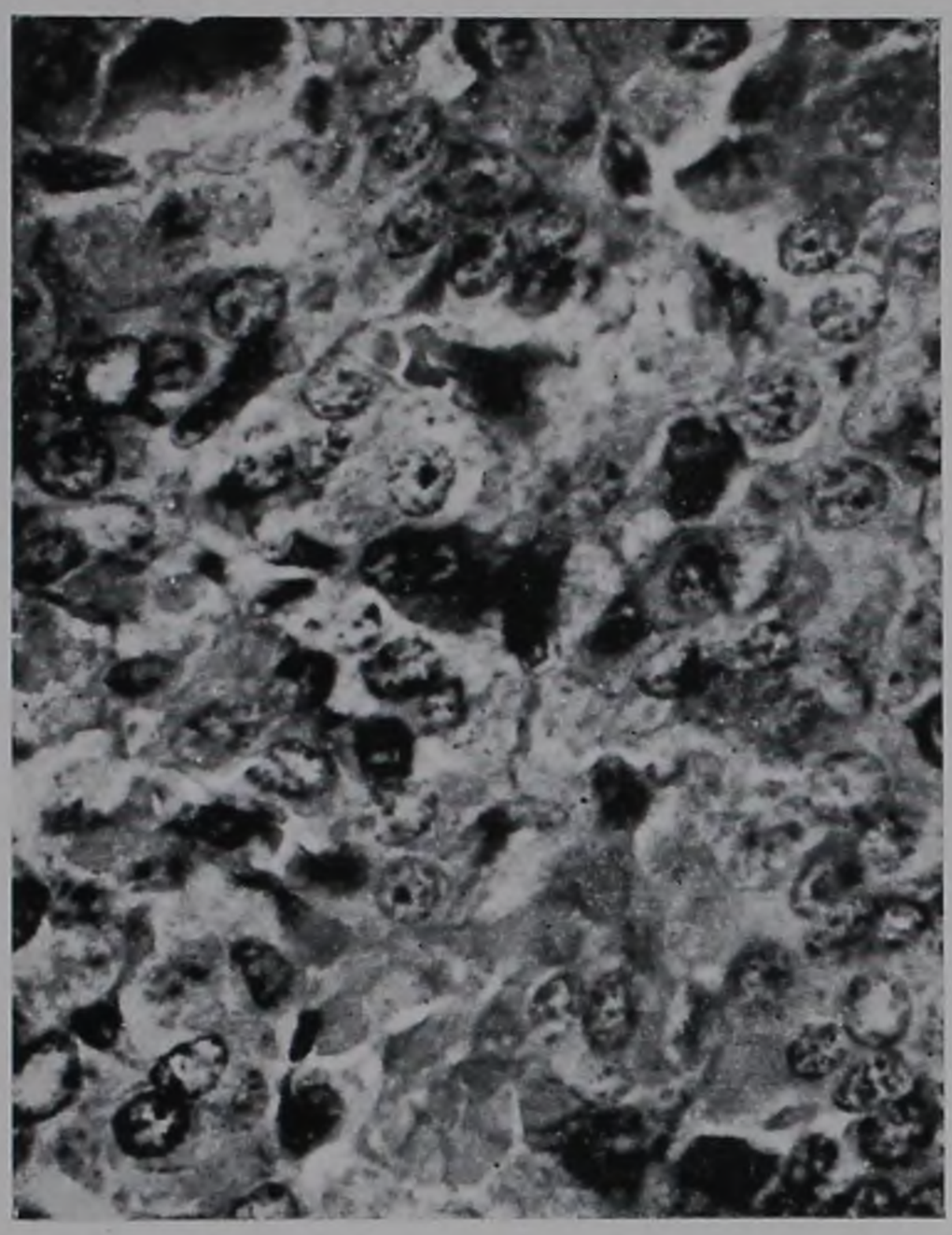

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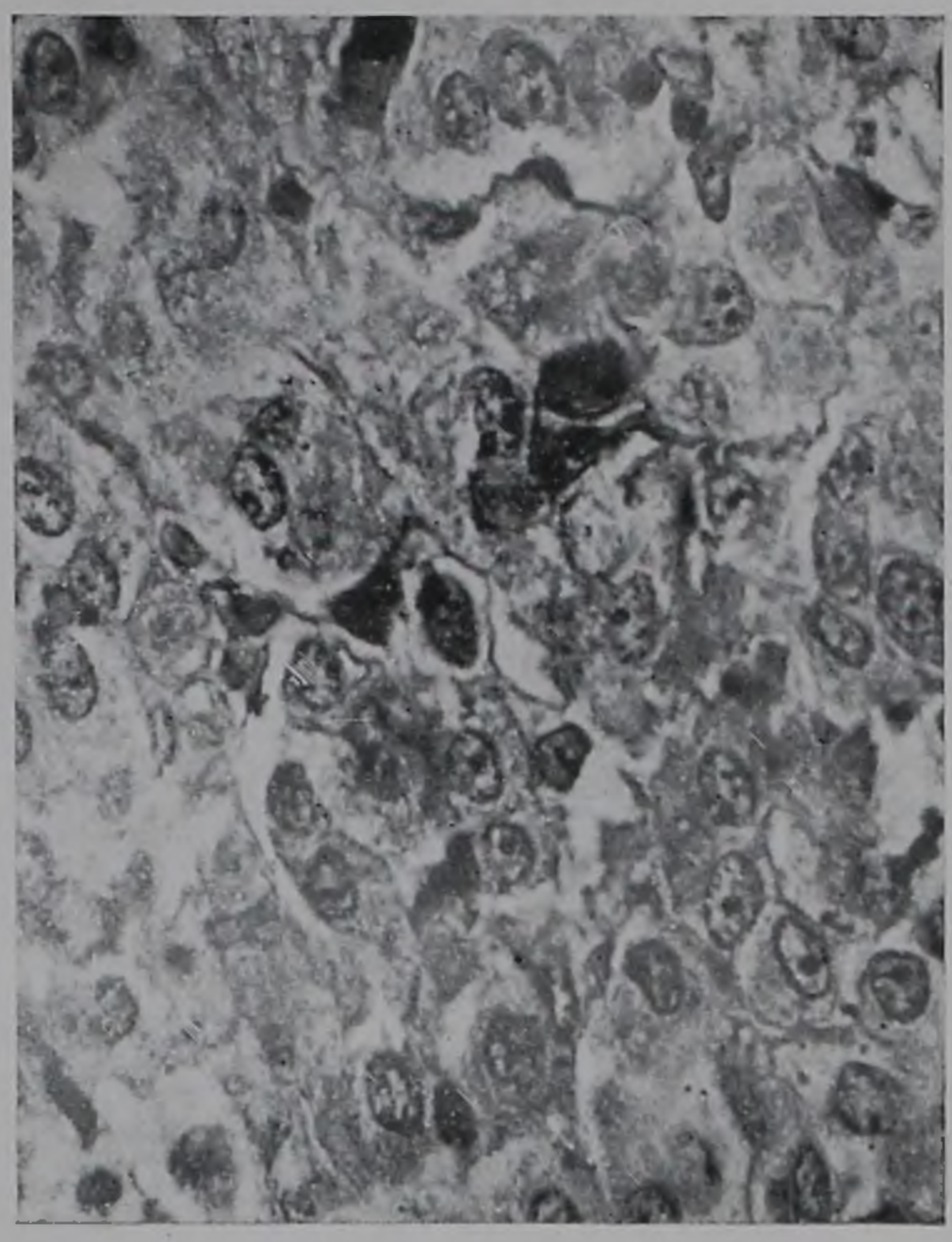

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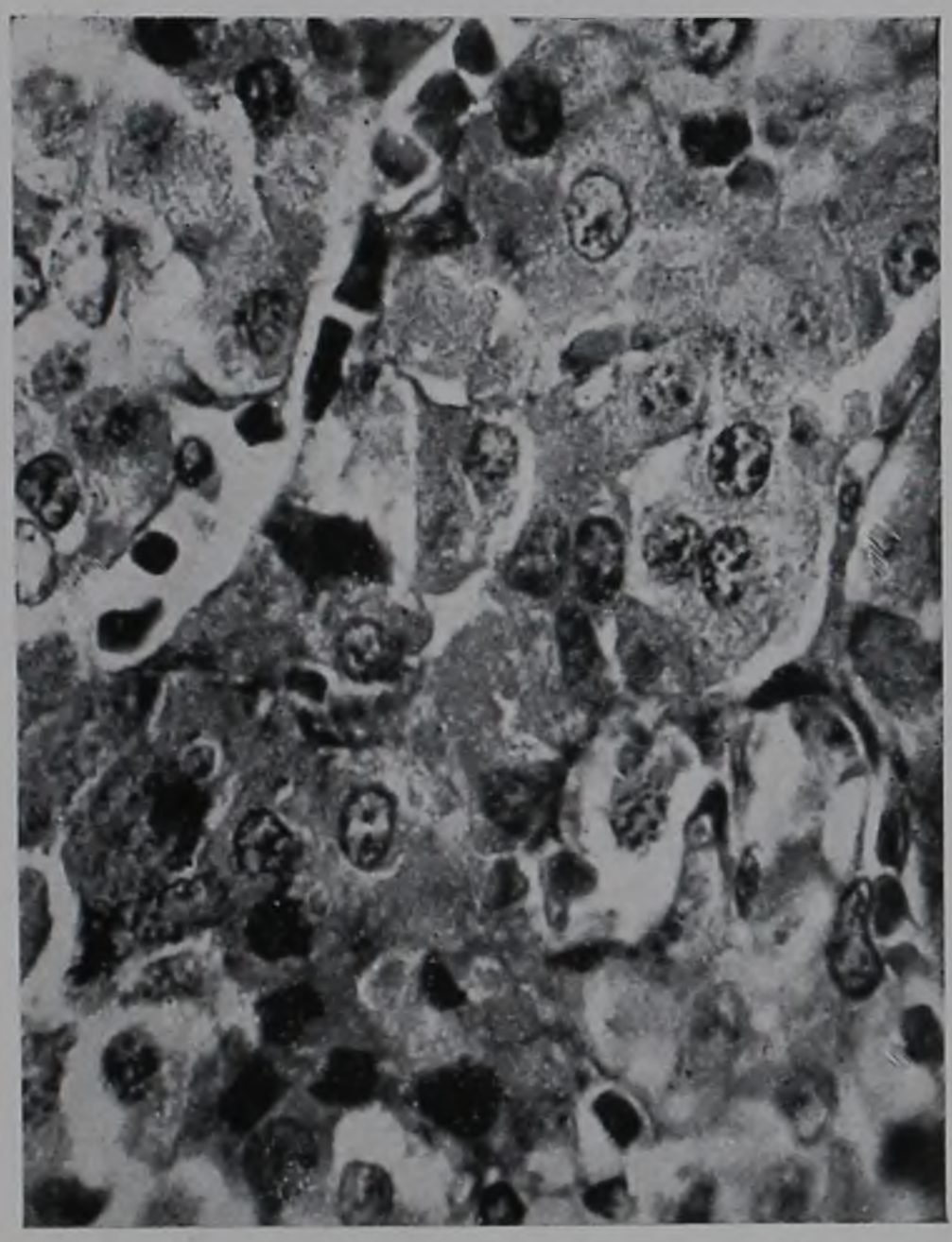

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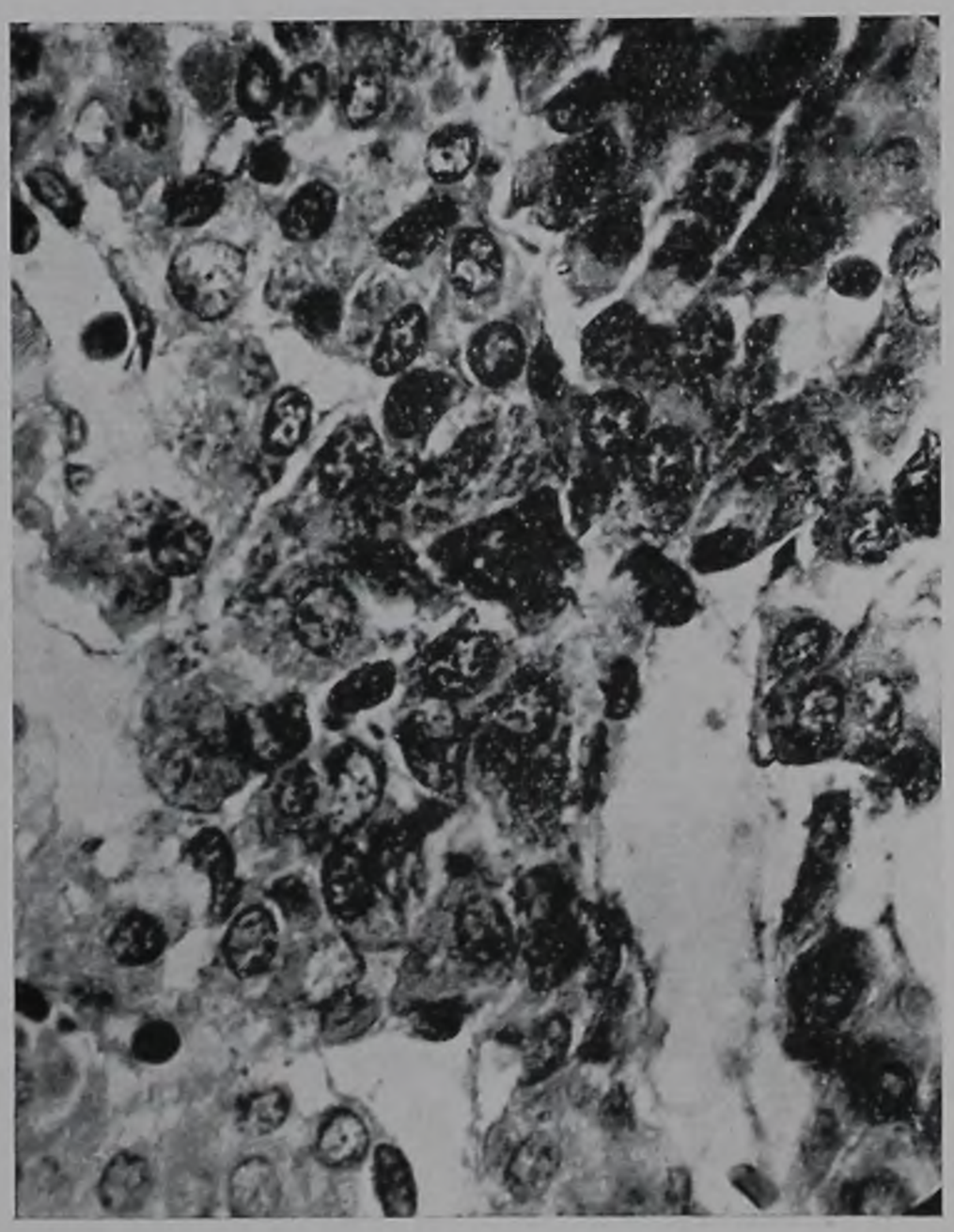

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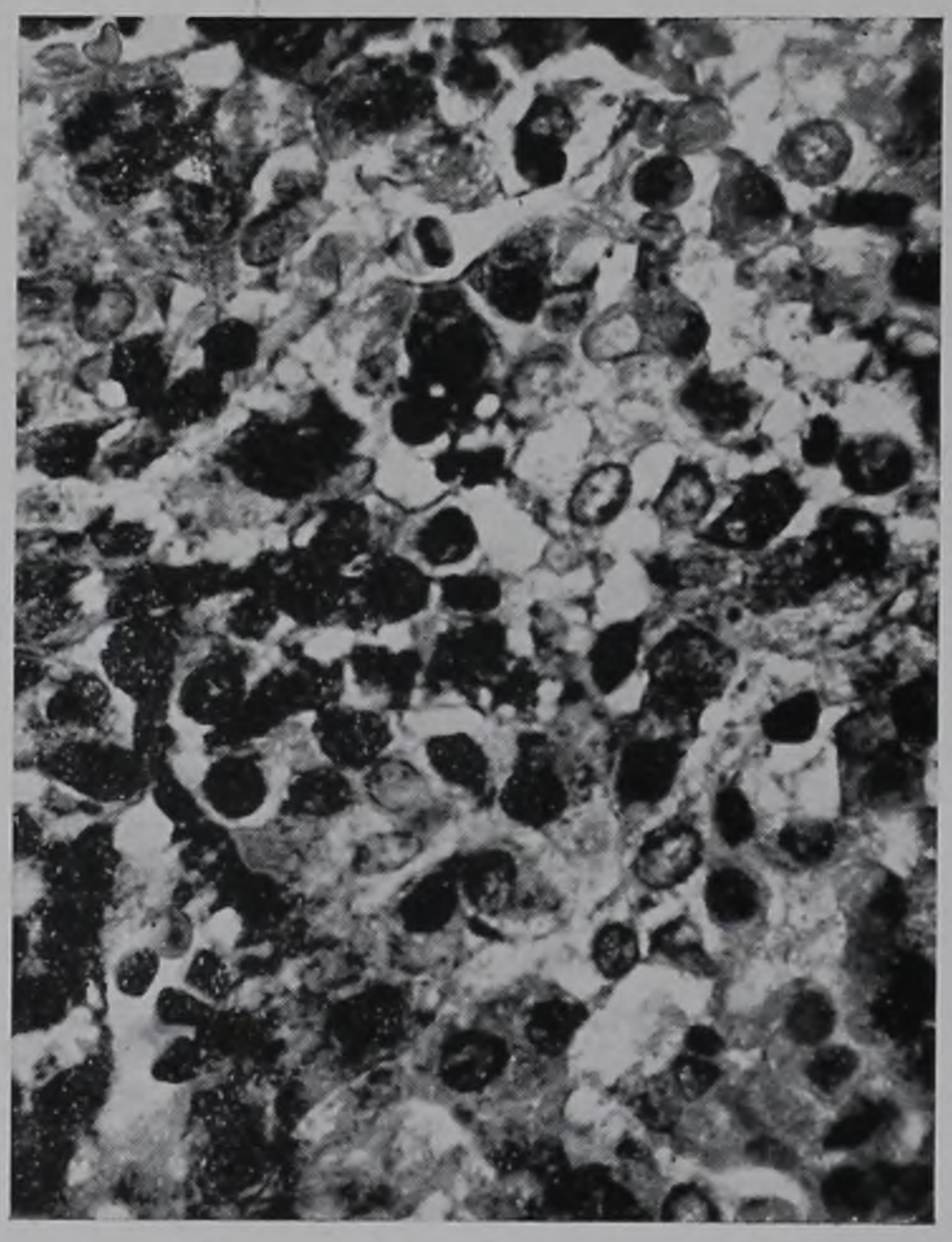

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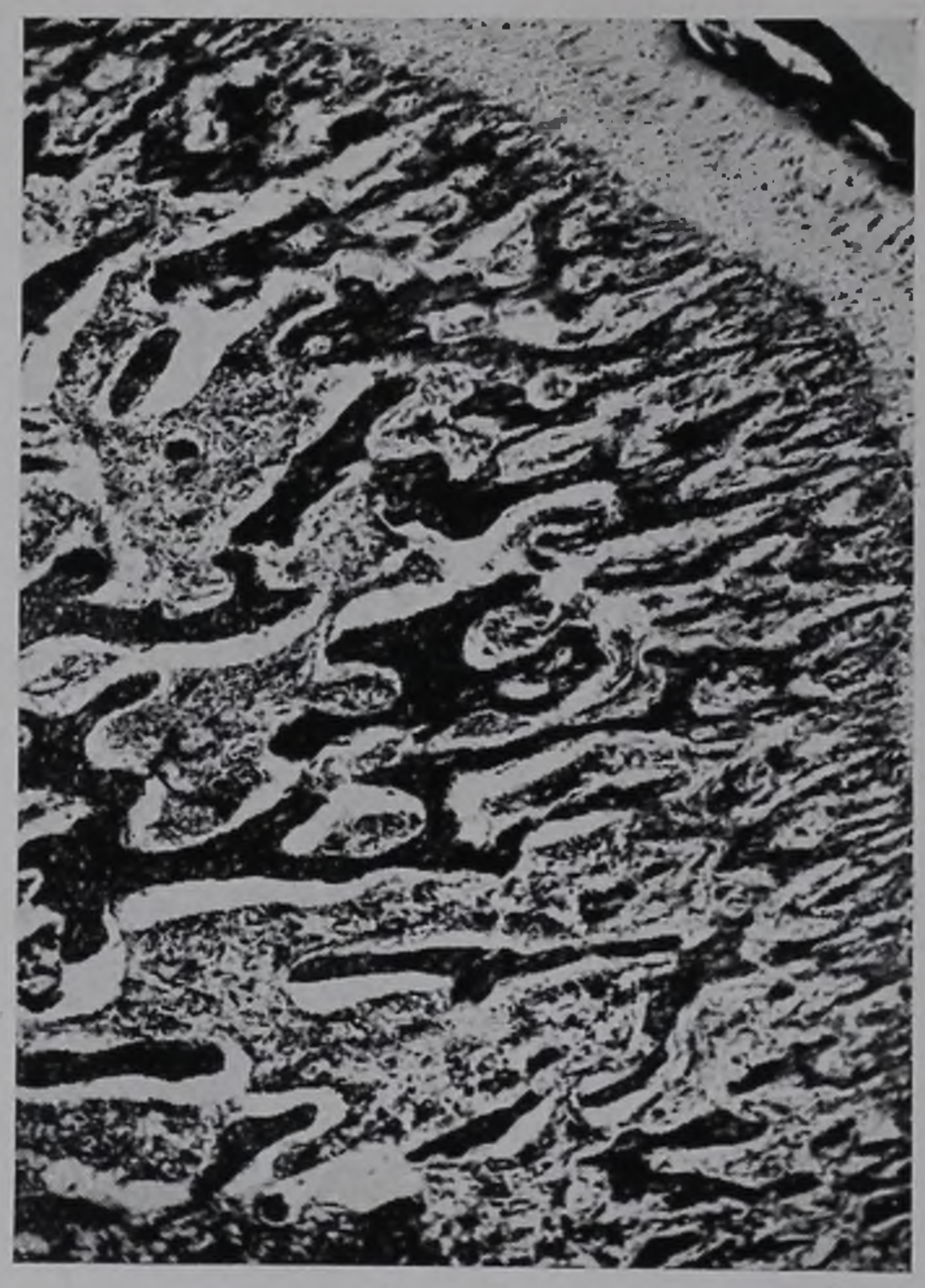

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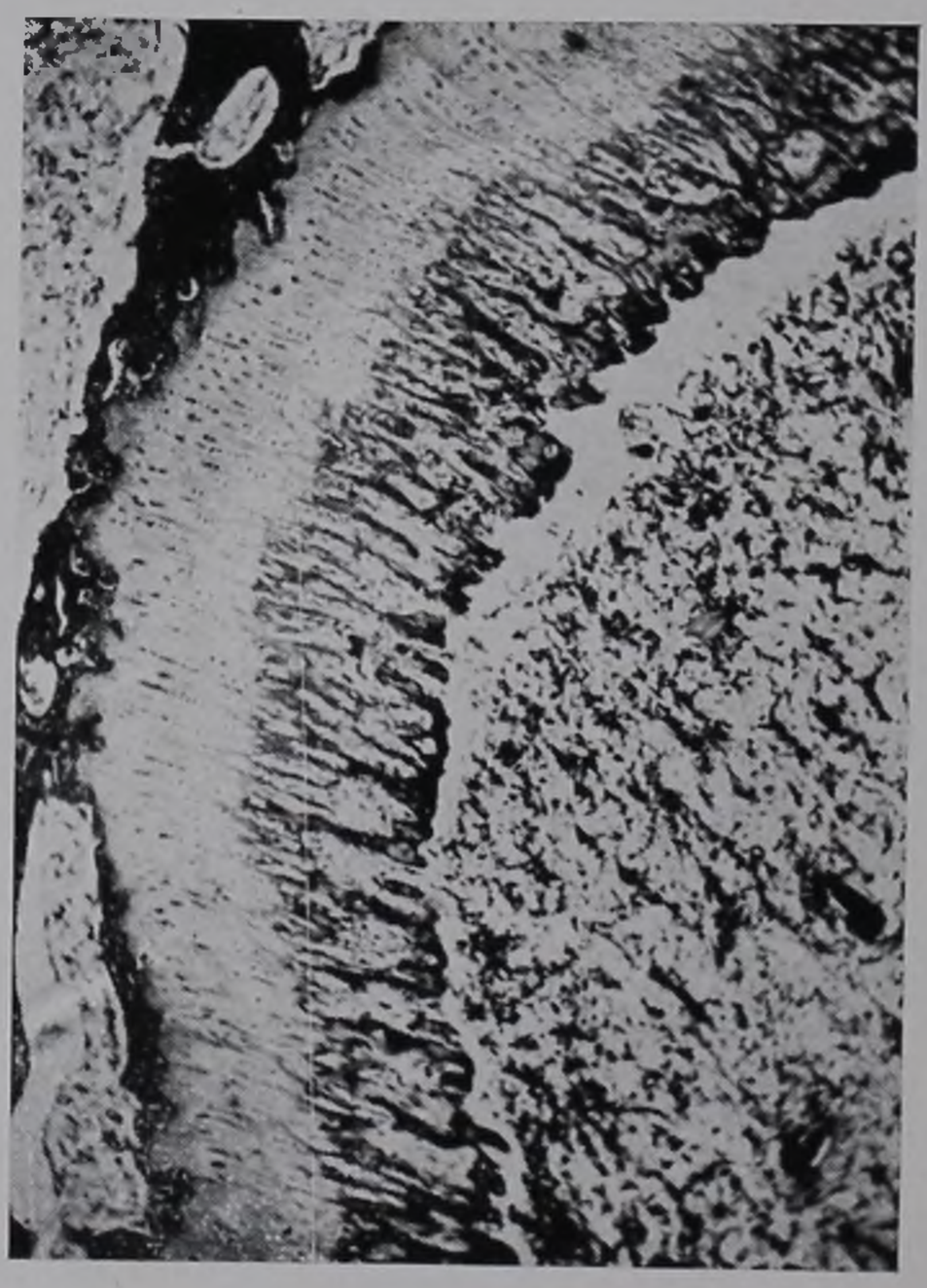

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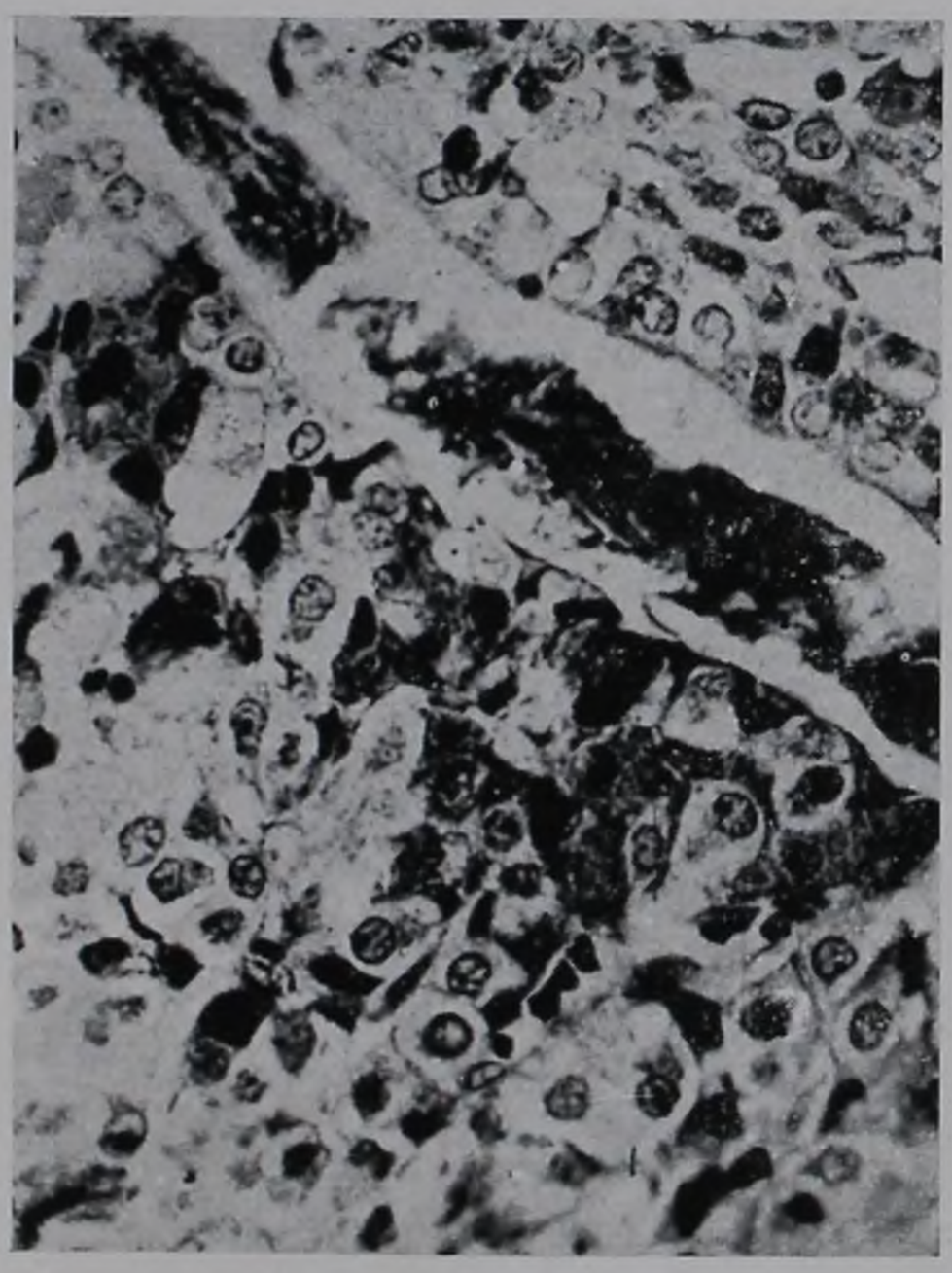

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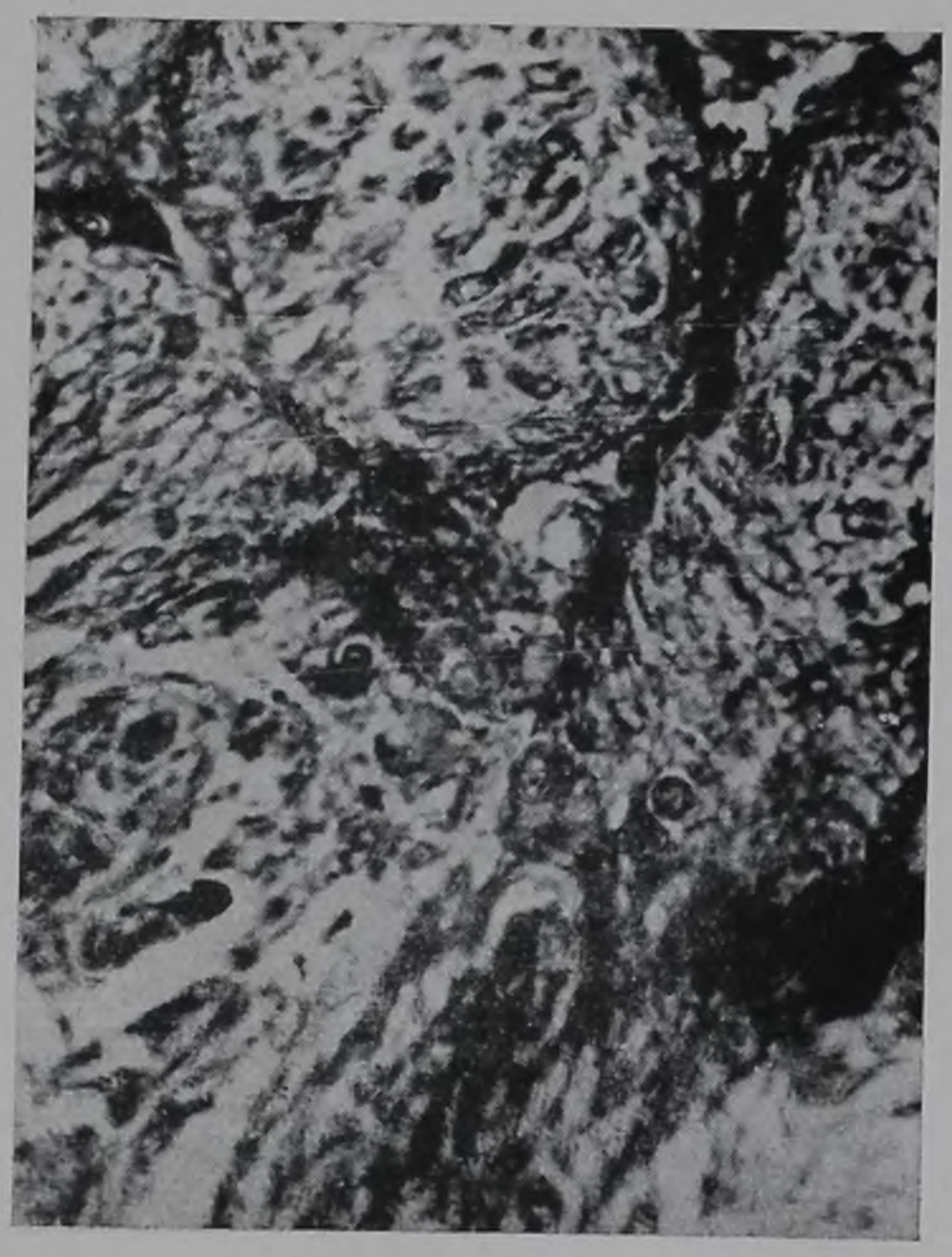

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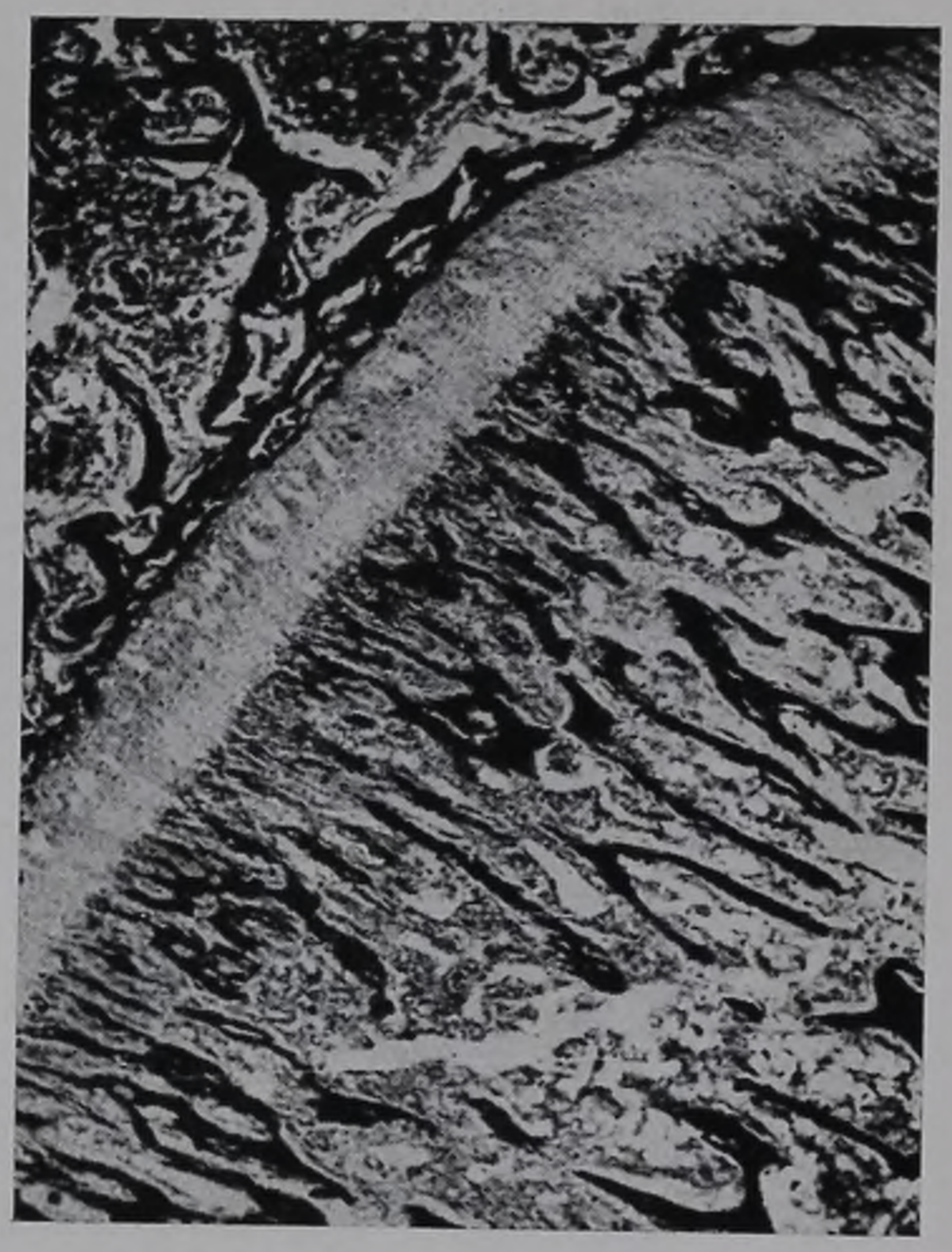

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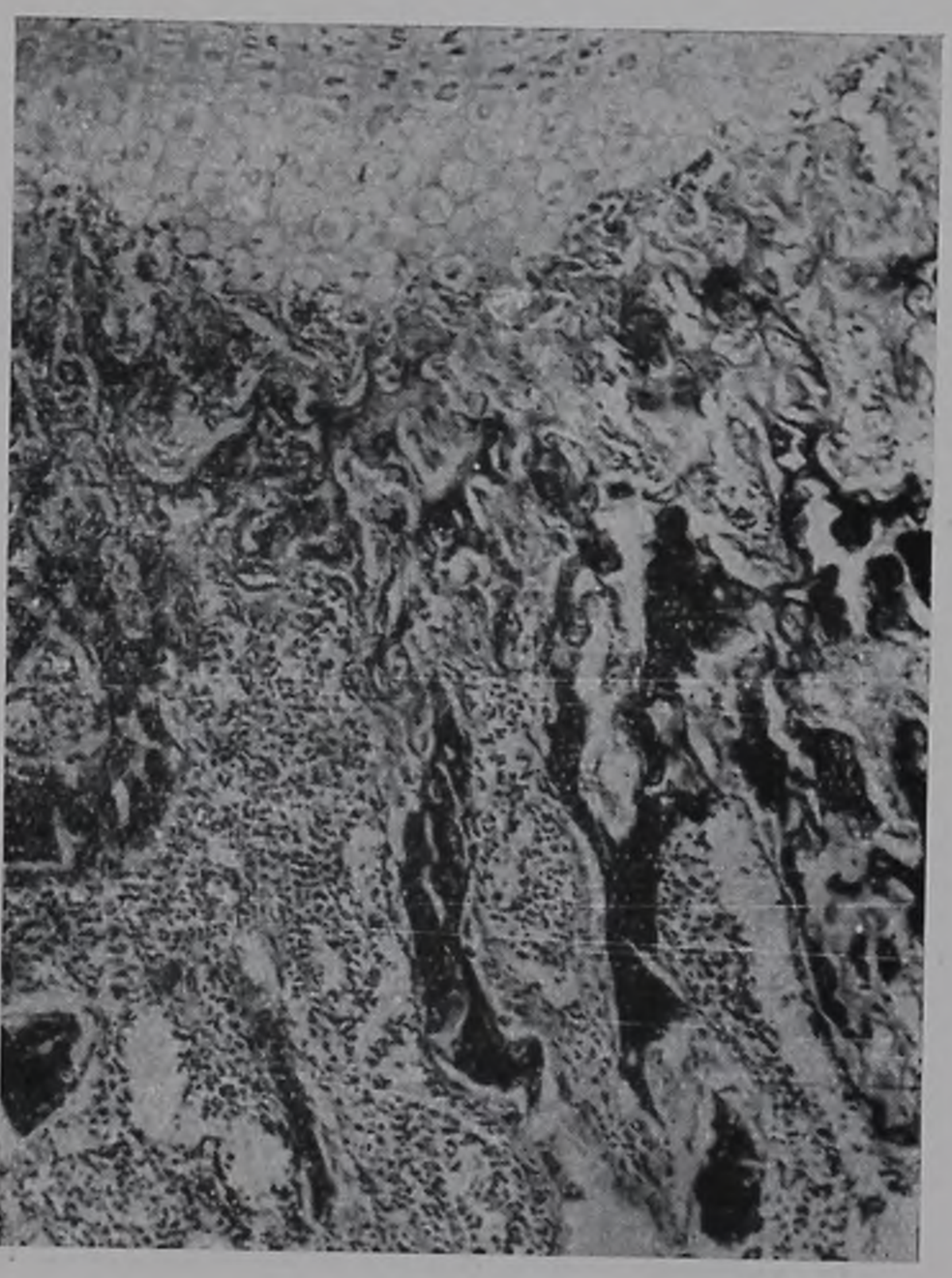

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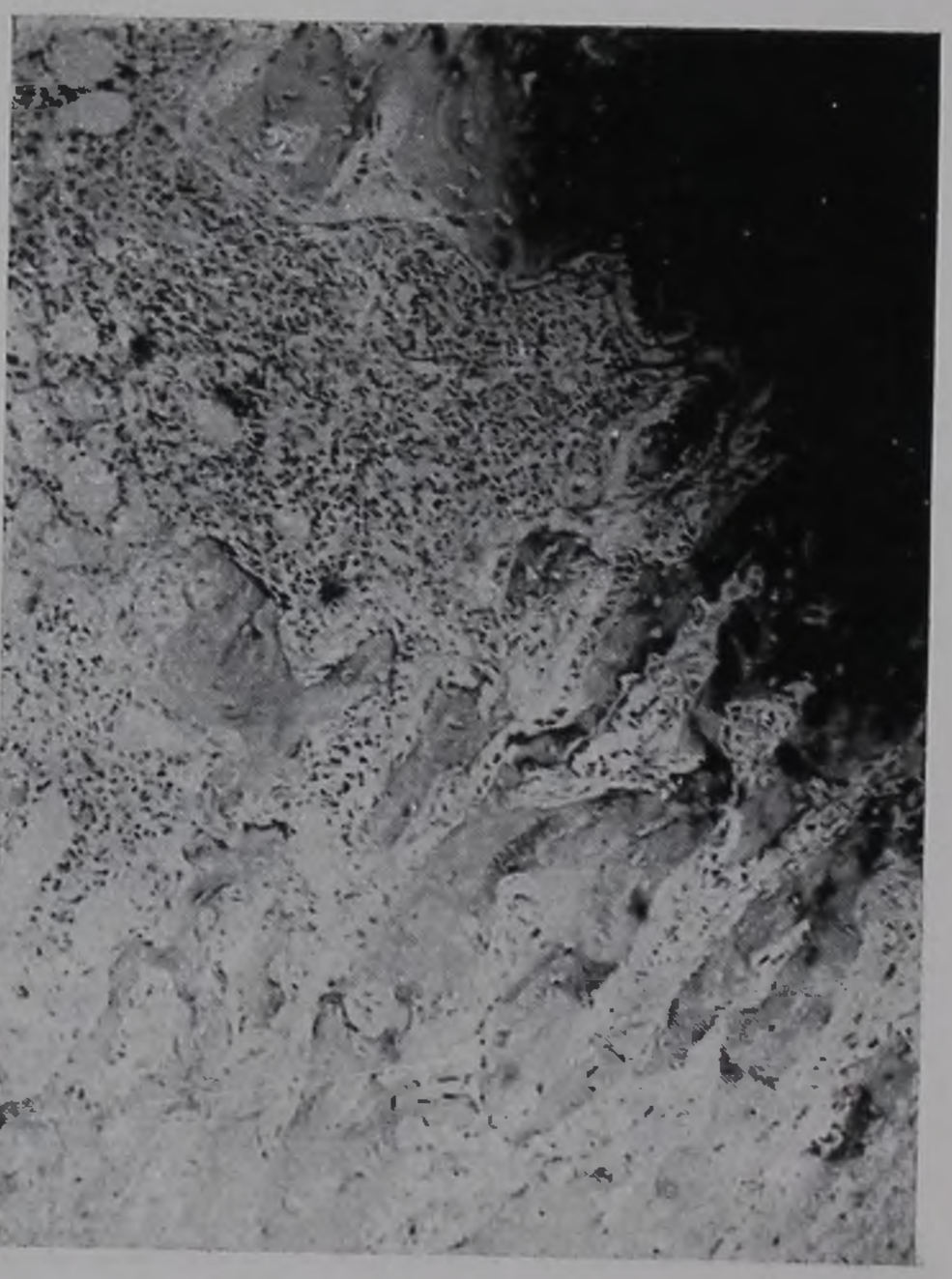

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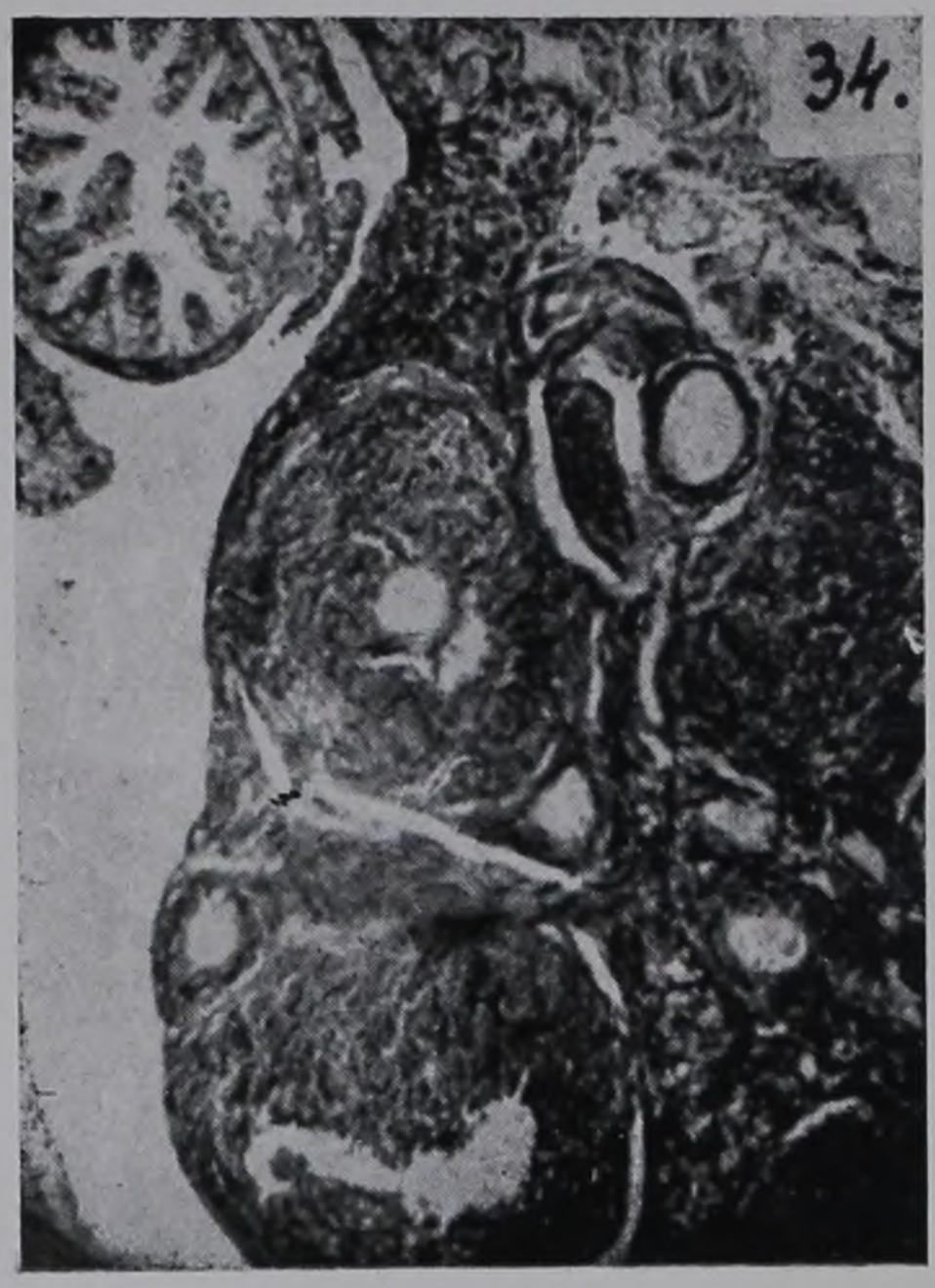

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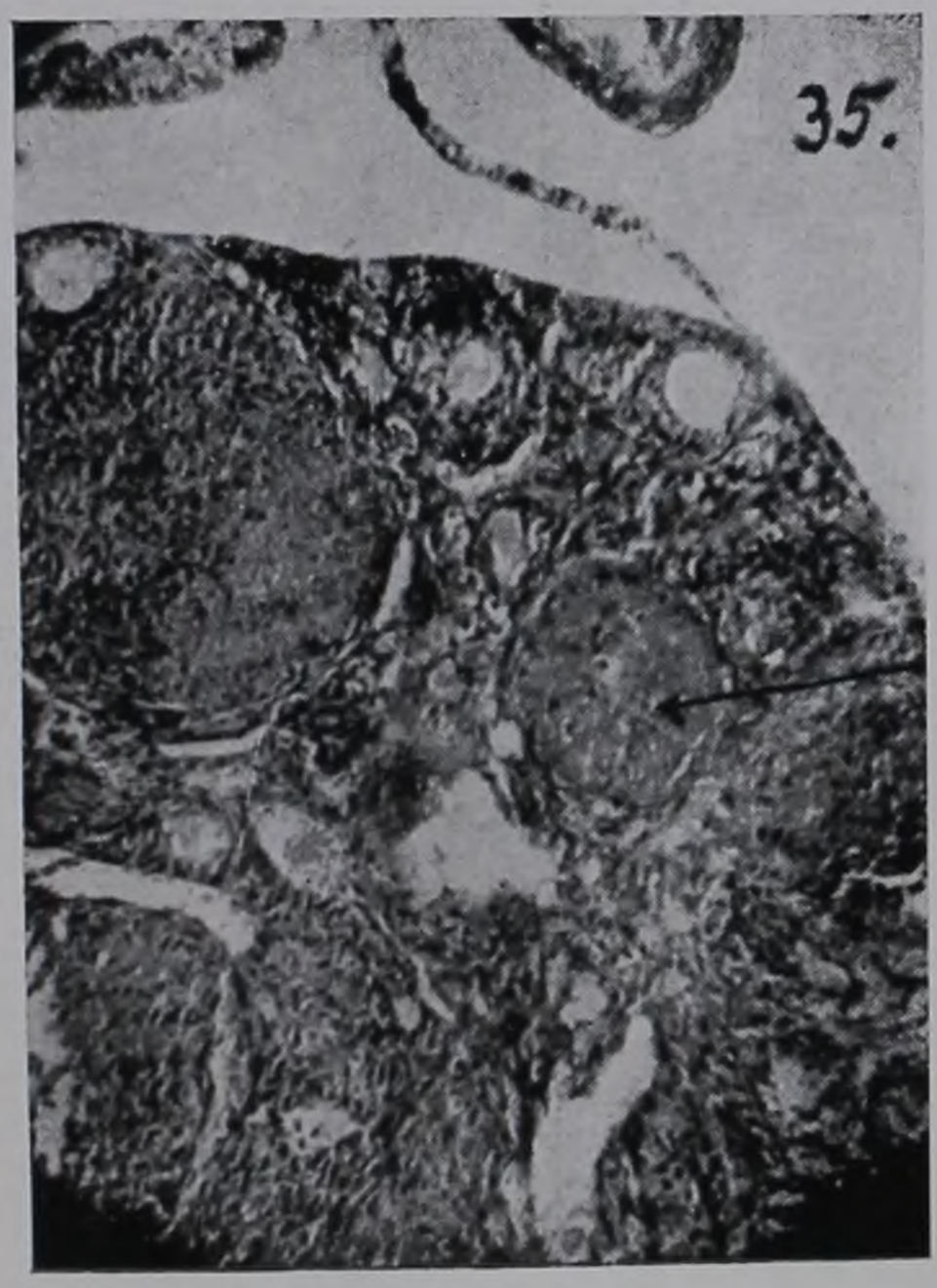

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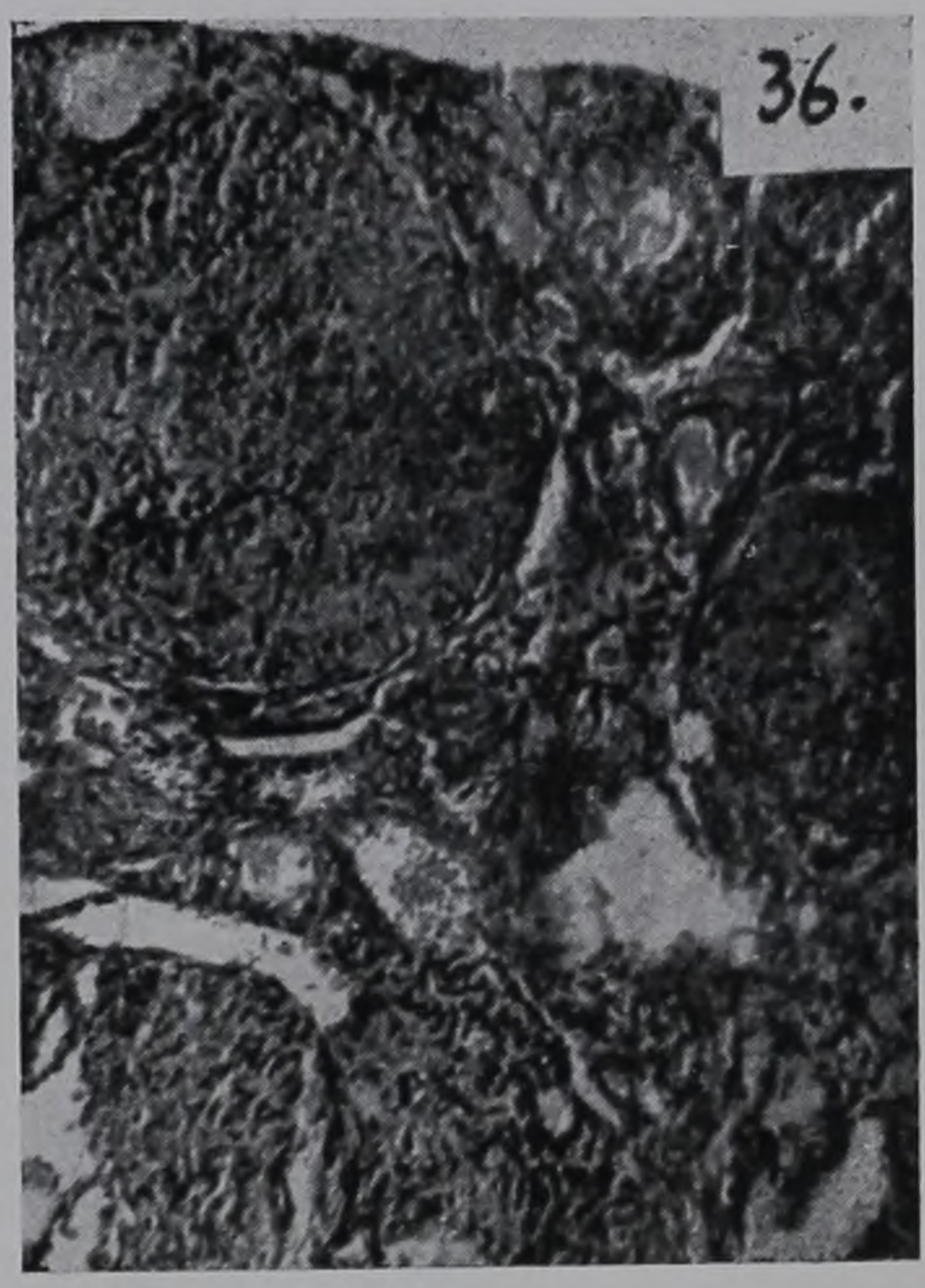

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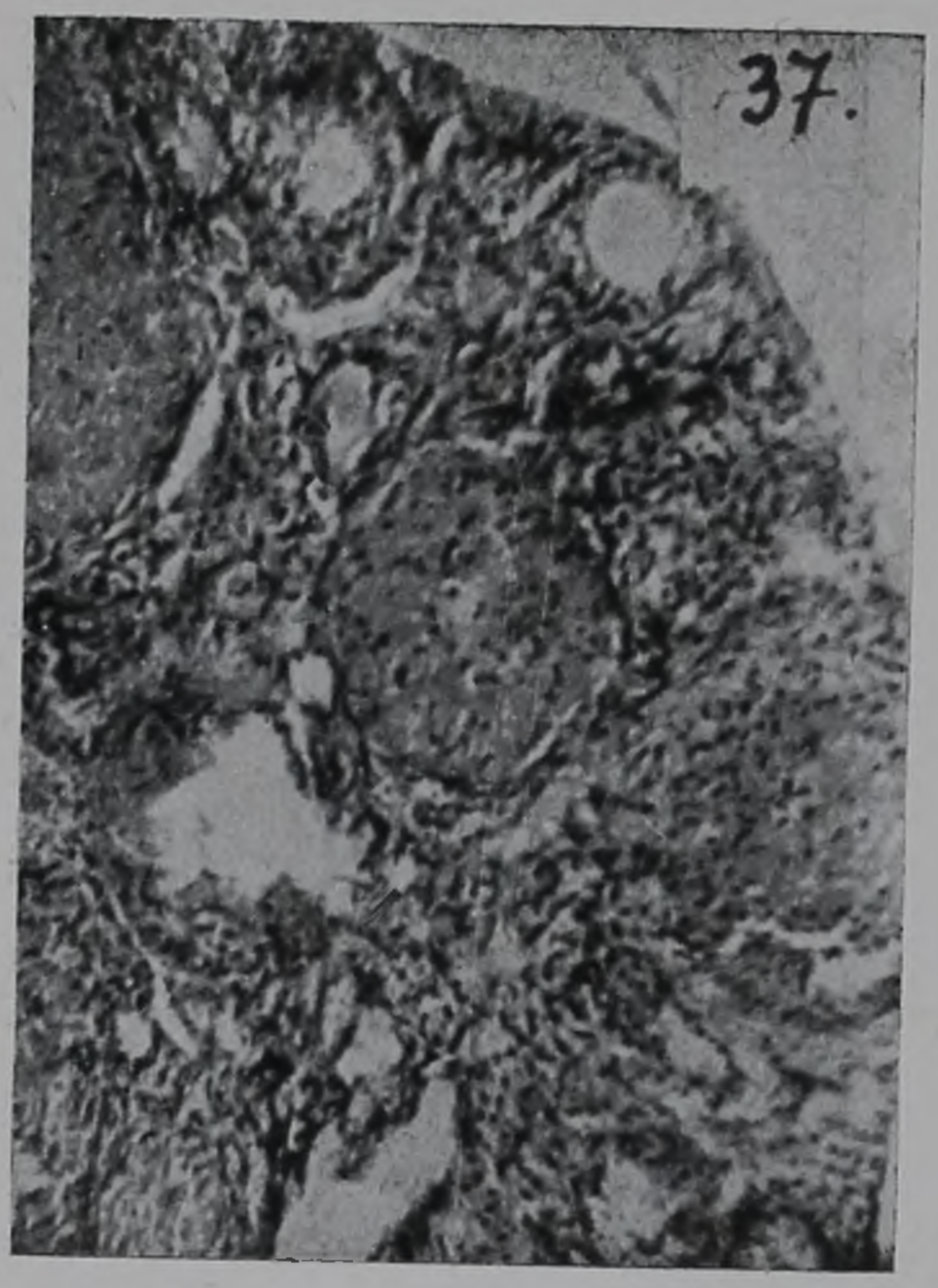

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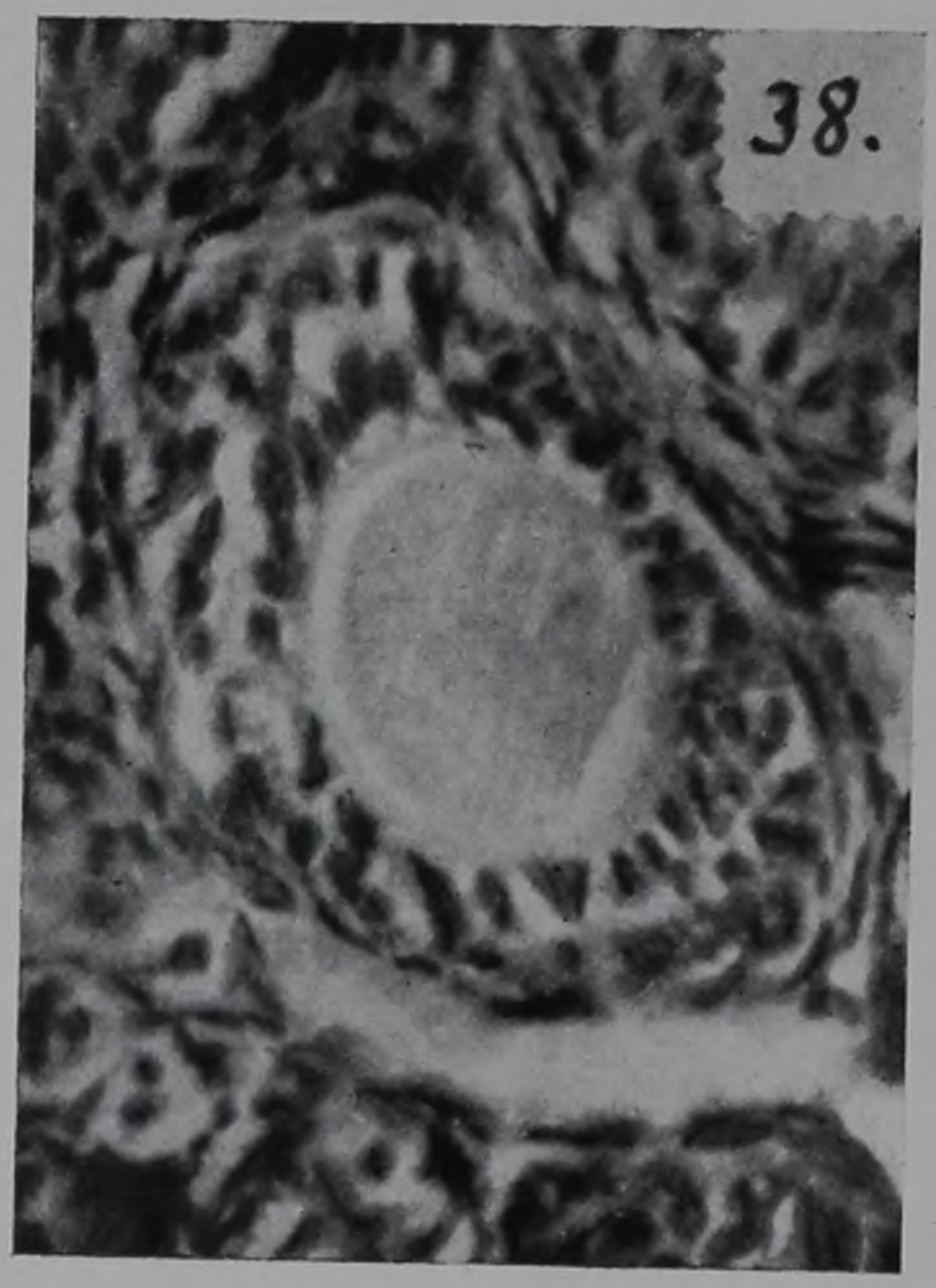

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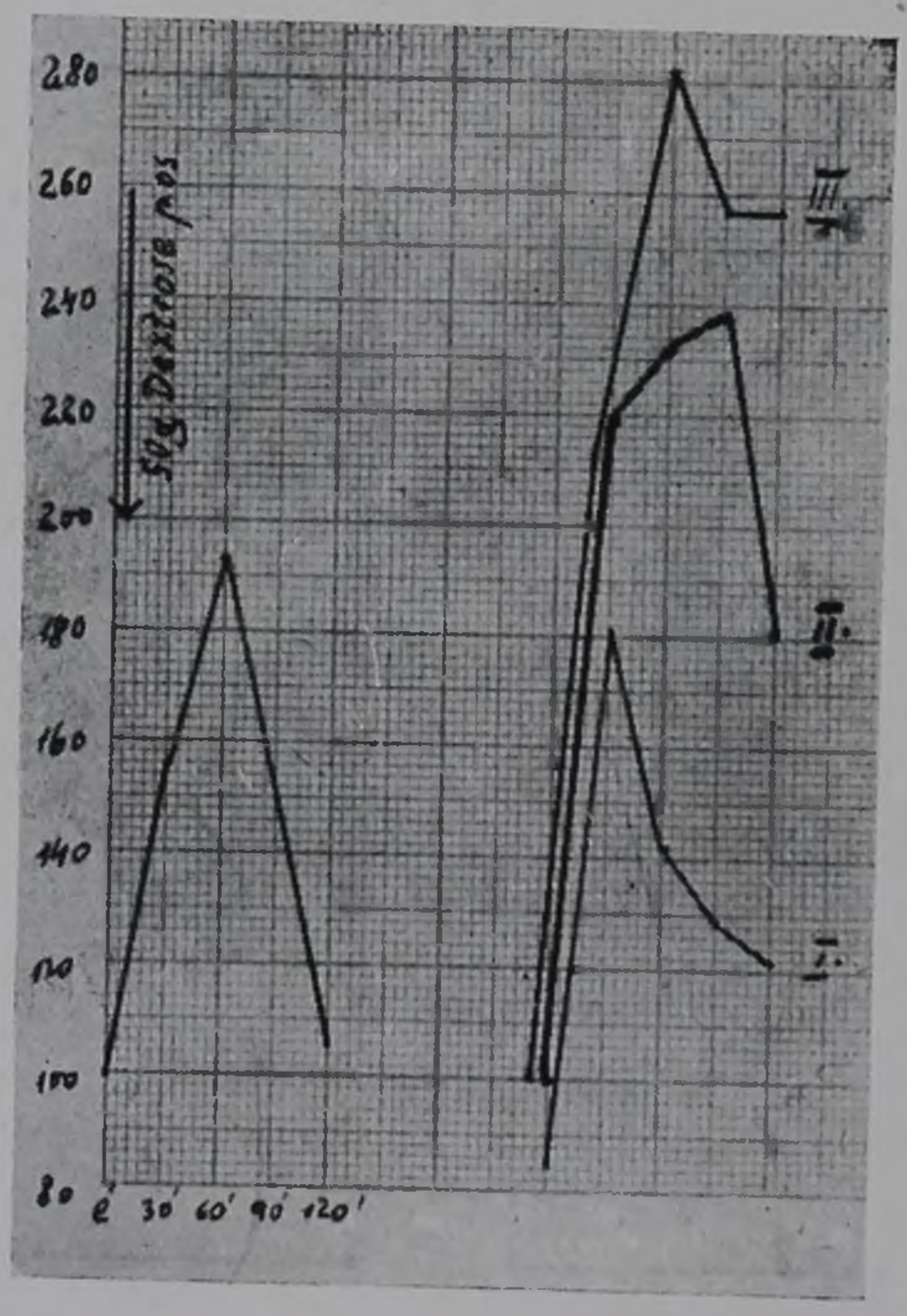

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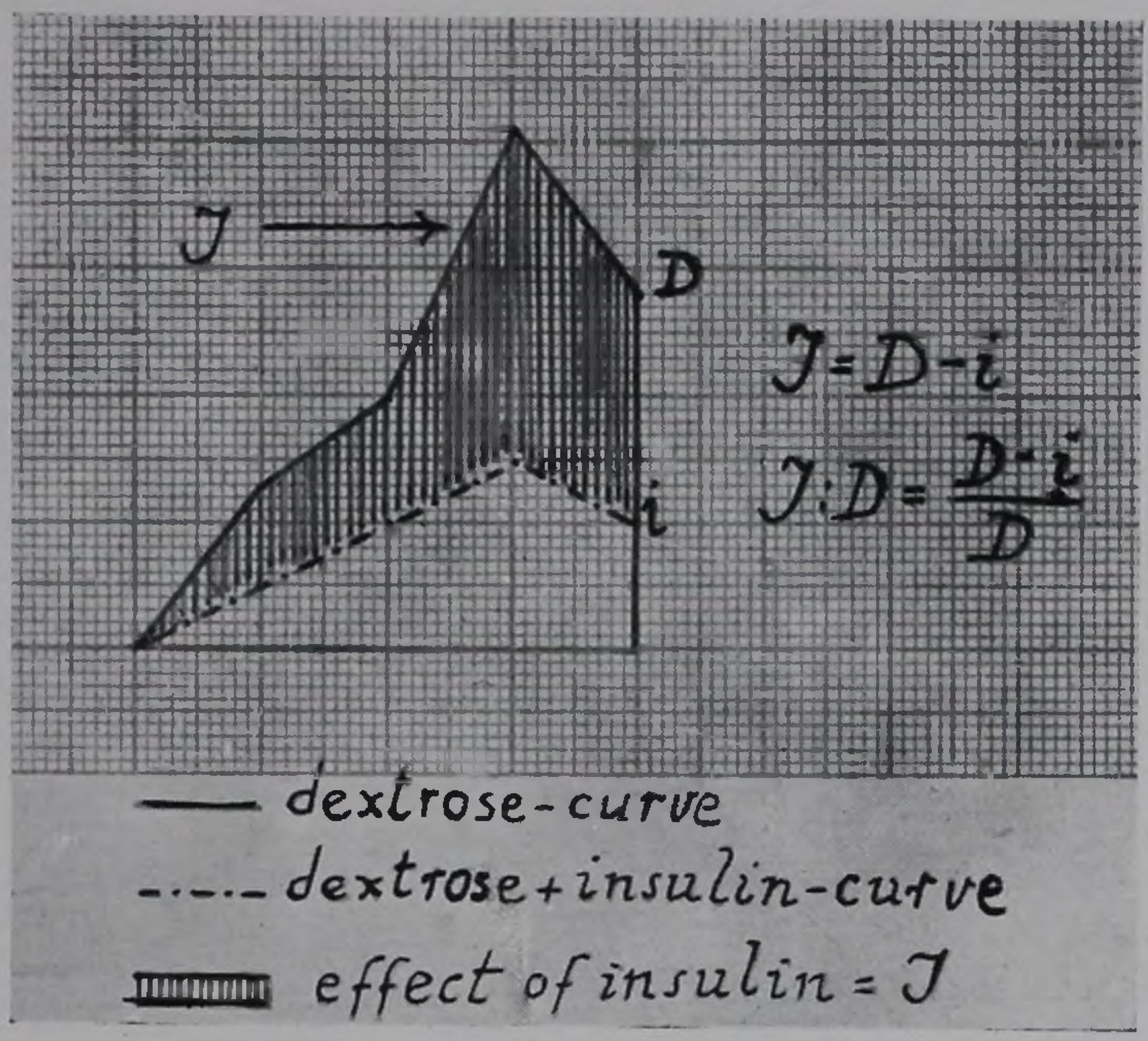

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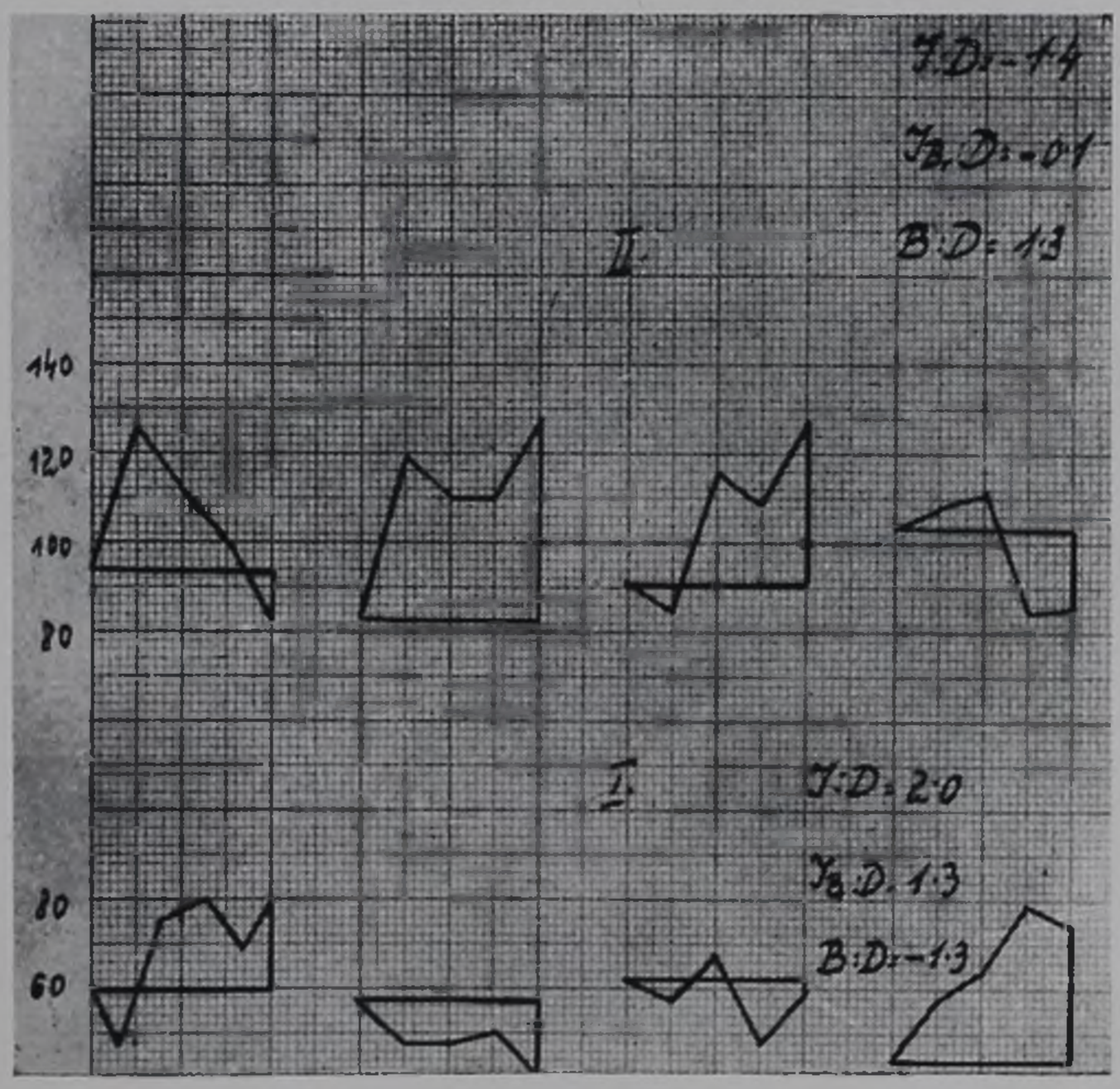

Figure 41.

have shown that our approach to get nearer the solution of the problem was not quite incorrect.

The effect of aneurin undoubtedly represent a pharmacological effect. The dose required is 50—100 times the normal one employed in cases of deficiency.

It is believed that the experiments reported speak in favour of the assumption that in carbohydrate metabolism the attacking point of vitamin B_1 is the hypophysis-hypothalamus system.

Some researchers concerned with the hypophysis discover more and more hormones of the anterior lobe the number of which has gradually exceded twenty. Others have tried to prove that some of the newly discovered hormones were identical with others already known. The experiments now reviewed show comparatively modest results. They have not led to the discovery of new hormones, they imply with some probability that there are not so many, and they have provided some interesting new data on the question of the stimulation, or the inhibition, of the anterior lobe of the hypophysis. Part of the experimental results have been confirmed by foreign researchers, but the rest awaits still further investigation or confirmation. It would be a deep satisfaction if the interest of investigators in these borderline fields could be raised by our modest efforts and we shall be particularly happy if further work inspired by them can justify our results.

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Literature on the correlation between ketosis and the anterior lobe of the pituitary can be found in my paper entitled „Neues Verfahren zur Stimulation der gonadotropen Funktion der Hypophyse", Schweiz. Med. Wschr. 1942. 20. 541.

As for the correlation between vitamin B_1 and the anterior pituitary lobe my work entitled „Zusammenhang zwischen Vitamin B_1 und Hypophysenvorderlappen", Schweiz. Med. Wschr. 1943. 2. 41 should be referred to. My paper on the effect of Vitamin B_1 on the hypophysis of mature female rabbits was published in the Orvosok Lapja, 1945. No. 3., another (written with Elisabeth Winkler) „The effect of Vitamin B_1 on the hypophysis and ossification of female rabbits and rats in pubescence", in the Orvosok Lapja, 1946. No. 10.

Literature on Vitamin B_1 and carbohydrate metabolism is to be found in the folloving works: *Julesz, Gábor, Megyesi:* Magyar Belorvosi Archivum I. 2. 1948. *Magyar, Imre:* Zeitschr. f. d. ges. exp. Med. 104. Bd. 4. H. 1938. — *R, Tislowitz:* Klin. Wschr. 16. Jg. Nr. 7. 226. 1937, I, — *Aszódi* and *Mosonyi:* Klin. Wschr. 16. Jg. No. 35. 1217. 1937. II. — *Stepp, Künau, Schröder:* 1944. Die Vitamine und ihre klinische Anwendung. — Les Vitamines B. Leur rôle dans le métabolisme hydrocarboné. Leur emploi dans le traitement du diabète: Jean Daniel *Gringoire.* 1933. — Der Vitamin B_1-Stoffwechsel des Menschen und der Tiere unter normalen und pathologischen Zuständen: *Lajos Purjesz* 1941.

AUS DER II. MEDIZINISCHEN KLINIK (DIREKTOR: PROF. E. HAYNAL),
UND DEM BAKTERIOLOGISCHEN INSTITUTE (DIREKTOR:
PROF. F. FARAGÓ) DER PÁZMÁNY PÉTER UNIVERSITÄT IN BUDAPEST.

UNTERSUCHUNGEN ÜBER DIE IMMUNITÄT GEGEN PERTUSSIS.

VON DR. ANTON SZÖKE, ASSISTENT DER KLINIK.

Auf Grund der Untersuchung der letzten Jahre gilt heute die Aufassung, dass das Bact. pertussis eigentlich dreierlei Antigene enthält: das Bacterium-Antigen (Endotoxin), das Agglutinogen und das Pertussistoxin. Vom Standpunkte der Antigenwirkung sind die drei Komponenten nicht gleich wirksam. Gegen das Bakterienantigen konnte bereits früher durch die intravenőse Immunisierung von Kaninchen ein Gegenstoff erzeugt werden (*Evans* und *Maitland* 1937.) Die Erzeugung des Gegenstoffes gegen das Bakterienagglutinogen schreitet die seit langem bekannten Wege. Die Erzeugung des Antitoxins gegen das Pertussistoxin gelang jedoch bis zum heutigen Tage nicht. Man kann bei den Versuchstieren die wohlumschriebenen pathologischen Wirkungen des Pertussistoxins durch das gewöhnliche Immunserum nicht beeinflussen. So gelang es auch nicht die in der Kaninchenhaut durch intrakutane Einspritzung lebender Keime oder spezifischer Stoffe entstehende nekrotisierende Wirkung zu neutralisieren sowie die im Verlaufe der experimentell hervorgerufenen Mäusepertussis entstandenen Folgen der trachealen Infektion durch die prophylaktische Einspritzung von Immunserum zu verhindern (Faragó 1941/42., Evans, 1944.). Doch ist die Frage der Immunität gegen das Bakterientoxin nicht nur aus theoretischen, sondern auch aus praktischen Gründen von entscheidender Bedeutung, weil nach neueren Untersuchungen die toxische Komponente im Verlaufe der Erkrankung eine wenigstens genau so wichtige Roile spielt, wie die bakterielle Komponente (*Sprunt* und *Martin* 1943., *Faragó* und *Makara* 1942.)

Es gibt keine Unterschiede in der Antigenwirkung verschiedener Pertussisstämme. Die Toxizität der einzelnen Stämme kann aber beträchtliche Unterschiede anfweisen. Wir trachteten deshalb von unseren Stämmen die am meisten toxischen zu Versuchszwecken zu wählen. Zur Bestimmung des Grades der Toxizität bedienten wir uns der *Gundel-Schlüter*'schen Reaktion (1933), bei der von der dicken Bakteriensuspension 0.1 ccm in die depilierte Kaninchenhaut intrakutan eingespritzt werden. Von den im Bordet Gengou'schen blutigen, sowie dem im Bakteriologischen Institut eingeführten semisynthetischen Nährboden gezüchteten Stämmen wies der Stamm S_1 die grösste Toxizität auf und dieser wurde deshalb zu unseren Versuchszwecken benützt. Es ist noch zu erwähnen, dass die Toxi-

zität des im semi-synthetischen Nährboden gezüchteten Stammes nur dann abnimmt, wenn die Qualität des Nährbodens nicht ganz einwandfrei ist.

Im Verlaufe der Untersuchungen über die Toxizität verschiedener Stämme nach intrakutaner Einspritzung tauchte die Beobachtung auf, die wir hier kurz mitteilen wollen. Der Umstand, dass im Institut zu Versuchszwecken Kaninchen nicht in genügender Menge vorhanden waren, zwang uns dazu, die bereits eingeimpft gewesesen Kaninchen wiederholt zu impfen. Unseres Erachtens war dies gestattet, umsomehr, als auf Grund früherer, im Institute durchgeführter Versuche gezeigt wurde, dass beim Kaninchen, gegen die toxische, nekrotisierende Wirkung des Bac. pertussis keine Immunität entsteht. (Es muss aber betont werden, dass bei den obenerwähnten Versuchen die Kaninchen immer i. v. immunisiert wurden). Wir beobachteten, dass die zweite intrakutane Serie einen Monat nach den ersten intrakutanen Impfungen nur minimale nekrotisierende Wirkungen hervorrief. Durch diese Beobachtung fühlten wir uns veranlasst nachzuprüfen, ob nicht durch intrakutane Darreichung von Keimen bei Kaninchen ebenfalls ein antitoxisch wirkendes Serum erzeugt werden könnte. Zu diesem Zwecke verabreichten wir einem Kaninchen 4 und einem 5 in zweiwöchentlichen Abständen erfolgende Dosen von je 4 Milliarden Keimen intrakutan. Die Impfungsreaktion wurde bei jeder Einspritzung kleiner, so dass nach der 3—4. Injektion anstatt der Nekrese nur mehr eine geringe Verhärtung an der Einstichstelle auftrat. Diese Beobachtung zeigt also, dass das Kaninchen durch eine verhältnismässig geringe Keimzahl (16—20 Milliarden) intrakutan immunisiert werden kann.

Parallel mit der intrakutanen Impfung immunisierten wir Kaninchen auch intravenös. Dabei bekamen die Kaninchen in 4 je 10 Tage dauernden Schüben je drei Impfungen in einem Schub, wodurch jedes Kaninchen in 12 Impfungen zusammen 120 Milliarden Pertussiskeime erhielt. Zum Vergleiche der antitoxischen Immunität wurde auch hyperimmunes Menschenserum benutzt, das nach einem besonderen Verfahren durch Faragó erzeugt wurde und über dessen therapeutische Wirksamkeit sich dieser auch klinisch überzeugen konnte.

Die antitoxische Wirksamkeit des Serums wurde zweifach kontrolliert: Erstens vermischten wir lebende Pertussisbazillen (4 Milliarden Keime) mit verschiedenen Mengen (0.1 und 0.3 ccm) von Serum von Kaninchen, die vorher intravenös, bzw. intrakutan immunisiert wurden, sowie mit hyperimmunem Menschenserum, liessen es bei Zimmertemperatur im dunklen Raume drei Stunden lang stehen und impften dann die Mischung Kaninchen in die enthaarte Hautstelle intrakutan ein. Zweitens wurde kontrolliert ob die Wirkung des durch die Waschung von Pertussiskeimen gewonnenen toxischen Extraktes durch das Immunserum neutralisiert werden kann. Durch die früheren Untersuchungen von Faragó (1942) wurde nämlich gezeigt, dass das durch intravenöse Immunisierung gewonnene Kaninchenserum die toxische Wirkung des durch die

Waschung von Pertussisbazillen hergestellten Extraktes nicht zu neutralisieren vermag. In unterstehender Tabelle sind die Ergebnisse unserer Versuche zusammengefasst dargestellt:

Grösse der Hautnekrose	Antigen	i. v. immunisiertes Kaninchenserum 0·1 ccm.	0·3 ccm.	intrakutan immunisiertes Kaninchenserum 0·1 ccm.	0·3 ccm.	hyperimmunes Menschenserum 0·1 ccm.	0·3 ccm.	Kontrollversuch: Normales Kaninchenserum 0·1 ccm.	0·3 ccm.
	4 Milliarden lebende Pertussiskeime	10×13	11×12	8×6	4×3	5×4	4×2	12×14	14×15
	Extrakt aus 4 Milliarden Keimen	11×10	10×8	6×5	4×4	0	0	14×15	14×14

Die neutralisierende Wirkung verschiedener, intrakutan eingespritzter Pertussissera.

(Grösse der Hautnekrose in mm ausgedrückt).

Aus der Tabelle geht hervor, dass die intrakutane Immunisierung ein wirksames Mittel zur Herstellung antitoxischen Immunserums ist. Durch die Verabreichung wesentlich geringerer Keimmengen wurde bei den Kaninchen eine Antitoxinerzeugung in Gang gesetzt, die in der nekrosewidrigen Wirkung des Serums ihren Ausdruck findet. Die intravenöse Verabreichungsart erwies sich dagegen, früheren Erfahrungen entsprechend, für die Erzeugung eines antitoxisch wirkenden Immunserums nicht als geeignet. Aus der Tabelle ist gleichzeitig zu ersehen, dass das hyperimmune Menschenserum die toxische Wirkung des Pertussisbazillus beinahe vollkommen, die Wirkung des Extraktes voll zu neutralisieren vermochte.

ZUSAMMENFASSUNG.

Im Verlaufe unserer Untersuchungen gelang es uns im Gegensatz zu den bis zum heutigen Tage erfolglos gebliebenen Immunisierungsversuchen ein gegen das Keuchhusten wirksames antitoxisches Kaninchenserum zu erzeugen. Im Gegensatz zur früher üblichen intravenösen Verabfolgung erwies sich zu diesem Zwecke das intrakutane Immunisierungsverfahren für geeigneter, da das durch diese Art erzeugte Serum die Wirkung der lebenden Keime und des durch deren Waschung gewonnenen Extraktes in gleicher Weise fast vollständig zu immunisieren vermag. Durch unsere Untersuchungen konnte die Frage, warum die Diskrepanz zwischen der intravenösen und der intrakutanen Verabreichug besteht, vorläufig noch nicht geklärt werden. Wahrscheinlich kommen die intravenös gegebenen Keuchhustenbazillen mit den die Immunitätsstoffe erzeugenden Einrichtungen des tierischen Organismus

durch eine andere, weniger wirksame Weise in Berührung, als die intrakutan verabreichten Keime, die am Orte der Impfung verbleibend, einen länger anhaltenden Antigenreiz bieten, als die intravenös verabreichten. Im Laufe userer Untersuchungen konnten wir uns auch von der ziemlich hochgradigen Antitoxinwirkung des hyperimmunisierten Menschenserums überzeugen.

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