On the Hypertrophy of the Interstitial Cells in the Testicle of the Guinea-Pig under different Experimental Conditions.

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[Plates 1 and 2.]

I.

Quantitative problems are, no doubt, of the greatest theoretical and practical interest in the study of the internal secretion of the sexual glands. We are explaining different physiological and clinical situations by changes in the quantity of internal secretion of the sexual glands or of other glands of internal secretion connected with the former. It suffices to mention normal puberty, menstruation and gravidity, eunuchoïdism and pubertas præcox. Quantitative problems were already discussed when the first steps were made in the study of the question of the site of the internally secretory function of the sexual glands (1). Bouin and Ancel (2) tried to cause by different experimental means a compensatory hypertrophy of the interstitial cells of the testicle; they extirpated the testicle in rabbits on one side and ligatured the vas deferens on the other side; they found a proliferation of the interstitial cells, whereas the number of the cells of Sertoli remained unchanged. Further, they extirpated the normal testicle of pigs with unilaterally retained testicle (3). They found in these experiments that the weight of the retained testicle was about twice as much as when the normal testicle was present. They found also in these cases a marked hypertrophy of the interstitial cells. Sand (4) confirmed these statements in his experiments on rabbits and guinea-pigs using his method of experimental cryptorchism. From all these experiments one could conclude that the hypertrophy of the interstitial cells takes place as a compensatory hypertrophy of the elements acting as an organ of internal secretion.

But there are some objections one can make against this conception. Ribbert (5) has shown that after unilateral castration the remaining testicle is greater than normally, and that there is a marked hypertrophy of the seminiferous part of the testicle. From experiments performed in our laboratory, we can state (6) that the hypertrophy of the remaining testicle is so marked that it weighs twice or thrice as much as a normal testicle of an

animal of the same age. Sand showed that the remaining testicle can undergo the same hypertrophy even when the vas deferens is ligatured; this is due to the fact that, even after the vas deferens is ligatured, the spermatogenesis can proceed in a normal way, and the degeneration of the tubules begins only when the spermatogenesis is more or less completed. So one may object that, when the testicular mass is diminished, there is hypertrophy not only of the interstitial cells but also of the seminiferous part. This is why I said, two years ago (7), that the situation seemed to be more complicated than Bouin and Ancel supposed, basing their conclusions on ingenious experiments, performed about twenty years previously, when nobody could foresee the extraordinary development which the study of the internal secretion of the sexual glands has undergone in recent years.

We again took up the question of the hypertrophy of the interstitial cells in the testicle in connection with another problem of internal secretion of the sexual glands. We studied the question as to how the development of the sexual characters of mammals depends upon the quantity of secretion of the sexual glands present in the body. For this purpose we used a method consisting of making unilateral castration and of cutting away more or less from the second testicle ("partial castration") (8). We made a great many of these same experiments on guinea-pigs, and we were able to state—in accordance with Pézard (8A)—that even very small particles, representing 1 or even less than 1 per cent. of the normal testicular weight, are sufficient for a normal masculinisation of an animal (9). In some cases we observed that the development of the sexual characters was slower than normally; but we have experimental evidence that this phenomenon of retardation was caused, not by a simple quantitative deficiency in internal secretion, but by a slower development of the sectioned testicle (10).

In all these experiments we had theoretically to confront a very important complication, *i.e.*, the possibility of a compensatory hypertrophy of the elements, to which we ascribe the function of internal secretion in the testicle. And indeed in some cases where the particles were especially small, we found an extraordinary development of the interstitial tissue. The size and the number of the interstitial cells may sometimes be enormous. In such a small segment of the upper pole of the testicle, nourished by the arteria spermatica interna, the number of interstitial cells may no doubt attain, or even greatly surpass, the number of interstitial cells in two normal testicles together, although such a particle represents, as mentioned, not more but even less than 1 per cent. of the weight of two normal testicles.

I never saw such an enormous hypertrophy of interstitial cells as in these small particles in upper partial castration; only one such case of enormous

hypertrophy of interstitial cells is to be found in the literature, in a paper recently published by Poll (11), who described the degenerating testicles of hybrids of birds. Anyone looking at a microscopical preparation of some of our cases, and comparing it with a normal testicle (Plate 1, fig. 1), would agree that the hypertrophy of the interstitial cells is here excessive—as in some tumours described in the pathology of the testicle in man.

In view of this hypertrophy one might object that even in our smallest particles there may have been a production of the sex specific secretion no smaller than the normal. It is true we have no definite evidence that the interstitial cells are the organ of internal secretion in the sexual glands; but there are so many facts showing that the interstitial cells have something to do with the internal secretion of these glands, that it is impossible to avoid this objection when we discuss the quantitative problems in the internal secretion of the sexual glands on the basis of experiments with partial castration. Whether or not the interstitial cells are really the organ of internal secretion in the testicle, the partial castration was, at any rate in some cases, counteracted by an hypertrophy of this organ.

We have some experimental evidence that this hypertrophy of interstitial cells is not a compensatory one, *i.e.*, that this hypertrophy is caused, not by an exaggerated function of these cells for the body as a whole, but by local conditions.

II.

The experimental evidence we have that the hypertrophy of the interstitial tissue is not a compensatory one, is of four different orders.

A. Going through all the cases where small particles of testicular substance were sufficient for a masculinisation, in different degrees, of guinea-pigs, we saw all transitions between a normal number of interstitial cells and a highly augmented number of the latter. But there seemed to be no constant relation between the number of interstitial cells and the degree of development of the sexual characters. We will give in another paper a full description of all our experiments with partial castration, considering them from the point of view of the problem of the site of the function of internal secretion in the testicle. Here only the following facts are of importance for us :—

(1) That there seems to be no constant relation between the number of interstitial cells and the degree of masculinisation, although there does not exist a case where masculinisation took place without fully developed interstitial cells being present in the testicular fragment.

(2) That a normal masculinisation is possible even when the number of

interstitial cells in a small testicular fragment is not very much augmented, so that the number of the interstitial cells is highly diminished in comparison with those in a normal testicle.

B. In the experiments with partial castration on guinea-pigs, mentioned above, we used in reality two different methods. In some of these experiments we left in the body, as previously said, a small segment of the upper pole of one testicle. In other experiments of this series we left a segment of the under pole of the testicle above the cauda epididymidis. In the latter we never saw the enormous hypertrophy of interstitial cells observed in some cases of "upper" partial castration, although in "under" partial castration a marked increase in the number of interstitial cells occurs. But the "under" testicular fragment degenerates, in general, so far as to become sclerotic, whereas the upper fragment can resist longer against sclerosation (11A). We explain this dissimilitude by a difference in the blood supply in the two methods. In the "under" partial castration the testicular fragment is supplied with blood by the arteria deferentialis, the artery of the vas deferens, which gives off branches from the under part of the testicle. These branches, as is known in human anatomy, have an anastomosis with the branches of the arteria spermatica interna supplying the upper half of the testicle. The art. def. is a small one in comparison with the art. sp. i., and we supposed that in our experiments the blood supply of an upper fragment was better than the blood supply of an under fragment. We found the plexus pampiniformis unchanged, so that it is very probable that a small upper testicular fragment received the same quantity of blood from the art. sperm. int. as the whole testicle. We think it right to conclude from these observations that the good blood supply explains, in a sufficient manner, the great development or the hypertrophy of the interstitial tissue in upper testicular fragments as related above.

C. Experimental evidence that the latter conclusion is true, and that the hypertrophy of the interstitial cells in the upper testicular fragment is caused by local conditions, is shown by the following observations. On six guinea-pigs of different ages the one testicle was cut into two fragments, both of which were left in the body; the upper one supplied by the art. sp. i., the under one supplied by the art. defer. (On the other testicle we made—for other experimental purposes—incisions going through about half or more of the testicle, but not touching the ductus epididymidis.) All these animals showed during four months of observation normal somatic sexual characters. A *résumé* of the six experiments we performed is given in the following Table:—

	No. of Protocol.	Duration of experiment.	Weight of animal.		Condition of upper fragment.	Condition of under fragment.
			At beginning.	At end.		
		days.	grm.	grm.		
I	*69*	123	230	430	Upper fragment grown together with under fragment. Tubules in full spermatogenesis; spermatozoa found in caput epididym. In the neighbourhood of the under fragment, the tubules are in the stage of desquamation, or in the juvenile stage. *Well developed interstitial cells in normal or slightly increased quantity.*	Some tubules with spermatozoa (?) and tubules with one stratum only. Fragment undergoing sclerosation. A few well-developed interstitial cells.
II	*70*	126	230	525	All seminiferous tubules in juvenile stage. *Interstitial cells in slightly increased quantity.*	No remains of fragment found.
III	*64*	128	280	580	Full spermatogenesis; spermatozoa. Some tubules with one stratum only. *Interstitial cells in markedly increased quantity.* Connective tissue grows inwards from level of incision.	Sclerosation.
IV	*76*	114	400	580	Seminiferous tubules with one stratum only. *Enormous hypertrophy of interstitial cells.*	Sclerosation. A few seminiferous tubules found, and a few well-developed interstitial cells.
V	*68*	123	290	510	Both fragments grown together. Sclerosation. only few interstitial cells (?)	No testicular tissue recognised;
VI	*75*	114	505	650	Sclerosation. Degenerating seminiferous tubules	in upper and in under fragment.

As we see from the weight of the animals used for these experiments, they were all at an age when the spermatogenesis in guinea-pigs has attained a very high degree, or when the production of spermatozoa begins; some of them were adult animals.

The result of these experiments is that in all the six cases there was, four months after the operation, a very marked degeneration of the under fragment of the operated testicle. This degeneration concerned both the seminiferous and the interstitial part of the testicular fragment, the latter being transformed more or less completely into connective tissue. Having observed a great number of testicles under different experimental conditions, I should like to mention here that there seem to be different forms of degeneration which the testicle may undergo; but I have not enough insight into this field of pathological anatomy to judge on this question.

Unlike the under fragment, the upper fragment was, in four cases, still resisting degeneration and sclerosation. No. 69 showed in the upper fragment, four months after the operation, tubules with spermatozoa which were even present in the caput epididymidis. Other tubules in this fragment were in the state of desquamation or in the juvenile stage. In No. 70 all tubules are in the juvenile stage, corresponding to that of an animal about three weeks old. In agreement with Benda (11B), we mentioned in another paper that it is in reality not justifiable to speak about a "degeneration" of the seminiferous tubules occurring after ligature or section of the vas deferens, transplantation, radiation, and so on. There is in reality only a process which leads up to a juvenile stage, a process which occurs in an indefinitely smaller measure also in the normal testicle. To understand that there is no other change than a return of seminiferous tubules *en masse* to a juvenile stage, it suffices to compare a preparation of No. 70 with one of a normal animal about three weeks old, as given in Plate 1, fig. 2. I do not think that this "backward development," to use a notion of Eugen Schulz (11C) is the only possible way of reaction of the seminiferous tubules in different experimental conditions, and I do not think that a seminiferous tubule which has returned to a juvenile stage will always have the same destiny or life-history as a juvenile tubule in a normal testicle; on the contrary, in our upper fragments and in other experimental cases we several times observed complete degeneration of such tubules. Evidently, the same experimental condition which may lead to backward development may also lead to complete degeneration of these juvenile tubules.

In No. 64 the upper fragment showed about the same condition as No. 69. Some tubules showed spermatozoa, others were in the juvenile stage. Beginning

from the level of the incision, connective tissue grows inwards in the testicular tissue and a few weeks afterwards this fragment would surely have been in the same condition of sclerosation as an under fragment. We found this condition in No. 68, where no testicular tissue was to be recognised in the mass of connective tissue. A degeneration of the upper fragment took place in No. 75, but in a somewhat different way. Another case, No. 76, is of the greatest interest for us. It is the fourth of the experiments where the upper fragment was still present and in good condition as compared with the under fragment already wholly degenerated. In this case (Plate 2, fig. 3) the seminiferous tubules have only one stratum of cells; I am not able to say whether there are here only cells of Sertoli or some spermatogonia also; the first is the more probable. The interstitial tissue was in a state of hypertrophy, like that in some cases of "upper" castration. This one case, where we have an enormous number of interstitial cells in an upper fragment, although the second testicle is present in the body, is sufficient to decide the question whether the hypertrophy of interstitial tissue in some cases of upper partial castration is a compensatory one or not; this hypertrophy is not a compensatory one, but one caused by local conditions.

D. Further experimental evidence is given by the following observations: Instead of sectioning the testicle near the upper pole, as in the foregoing experiments, we sectioned the testicle near the under pole and cut away a very small fragment of the under pole, together with the cauda epididymidis. We made this operation on both sides. In principle, this is the same operation as that performed unilaterally in the experiments reported in C, but with the difference that, instead of having a *small* fragment supplied by the art. sp. i. on *one* side, the animals of this series had *big* fragments on *every* side supplied by the art. sp. i. If an hypertrophy of the interstitial cells occurs under these experimental conditions also, it cannot be compensatory, because the quantity of testicular mass is not diminished by the operation.

We made three identical experiments, a *résumé* of which is given in the Table on page 139.

As we see, there was in two cases a very marked hypertrophy of the interstitial tissue, especially in No. 72, illustrated by fig. 4. The hypertrophy is not so striking as in some small upper fragments. But one must take into consideration that, on examining a great number of testicles even under identical experimental conditions, as already mentioned, all transitions in the quantity of interstitial cells exist; even the two testicles of the same animal treated in the same manner may show very striking differences as concerns the interstitial tissue and the seminiferous tubules. On examining

	No. of Protocol.	Duration of experiment.	Weight of the animal		Condition of testicle.
			At beginning.	At end.	
		days.	grm.	grm.	
I	73	54	470	495*	Both testicles grown together. All seminiferous tubules with one stratum only. *Interstitial cells in a markedly increased quantity.* Sclerosation at the level where the incision was made.
II	72	109	516	670	Right testicle: All seminiferous tubules in the juvenile stage. Sclerosation beginning at the level of the incision. *Very markedly increased quantity of interstitial cells.* Left testicle: Tubules in full spermatogenesis with spermatozoa, tubules in the stage of desquamation and with one stratum only. Sclerosation as on the right. *Increased quantity of interstitial cells.*
III	60	125	130	420	Both testicles grown together. A great number of seminiferous tubules in full spermatogenesis; others in the stage of desquamation and with one stratum only; some tubules in the juvenile stage. *Quantity of interstitial cells not increased.*

* The animal died of illness, and was weighed the last time nine days before its death.

the two testicles of a normal animal, one will also sometimes observe very striking differences in weight and in the development of the seminiferous and interstitial apparatus. Great differences are also to be found between two animals of the same litter. This is why it is often impossible to have real control animals in experiments where conclusions should be based on weight or age relations.

On looking at fig. 4, one will see that there is a striking resemblance between the condition of this testicle and the testicle of a young animal (fig. 2). The condition of the seminiferous tubules, the condition of the interstitial cells, and the distribution of the latter embedded in a granular or homogeneous mass, all these remind one in a very striking manner of the testicle of a guinea-pig of about 2 or 3 weeks of age, when the testicle of this species has entered on its rapid development to spermatogenesis and puberty. This juvenile stage of the testicle, which one can observe under experimental conditions, is all the more interesting in that our animal No. 72 was at the time of the operation fully grown, weighing already more than 500 grm. There can be no more striking instance of the

fact that a "backward development" of the testicle is possible under experimental conditions.

III.

After all, there can be no doubt that the hypertrophy of the interstitial cells, as observed under different experimental conditions, has nothing to do with the function of the testicle for the organism as a whole, but that this hypertrophy is caused only by local conditions in the testicle itself.

Kyrle (12), who studied the hypertrophy of the interstitial cells under different experimental conditions, has suggested that this hypertrophy has something to do with a regeneration process which the seminiferous tubules are undergoing. This point of view is in connection with another conception of the function of the interstitial cells, namely, that these cells represent a trophic organ for the seminiferous tubules. The latter conception plays a great *rôle* in the attacks, made in the last few months by different German authors (13), upon the theory of Bouin and Ancel (which was supported and further developed by Tandler, Steinach, Sand and myself) that the interstitial cells are an organ of internal secretion.

This is not the place to discuss the question whether our conception of the internally secretory function of the interstitial cells is right or not. Indeed this conception will not remain unaltered and it is impossible now to say how much of this theory will permanently stand in view of the further development of scientific research in our special field. And, it may be, the results of the experiments made in our laboratory and reported by myself in this communication will be interpreted by some as a withdrawal in some measure from the position I held and tried to strengthen in my book on the "puberty gland." But on the other hand I think that there are not yet sufficient data to attribute definitely to the interstitial cells a special local function in relation to the seminiferous tubules, although the possibility of such a function cannot be denied, even though the interstitial cells should play a *rôle* as an organ of internal secretion. It is necessary for us to emphasise this point since Stieve in a recent publication (13) has seriously misrepresented our views.

Summary.

In experiments with partial castration one may observe in small testicular fragments enormous hypertrophy of the interstitial tissue, the number and the size of the interstitial cells being very markedly augmented. This hypertrophy is not a compensatory one, as is shown by the following experimental evidence:—

A. Hypertrophy of the interstitial cells is not present in all cases of partial castration and very small testicular fragments with a not very much

augmented relative number, and consequently with a highly diminished absolute number, of interstitial cells may suffice for a normal masculinisation.

B. On comparing testicular fragments supplied by blood in different ways (a fragment from the upper and a fragment from the under pole of the testicle) one finds that the fragment which seems to be better supplied by blood shows more pronounced tendency to hypertrophy of interstitial cells.

C. Enormous hypertrophy of interstitial cells may take place in an upper testicular fragment even when the other testicle is present in the body.

D. Marked hypertrophy of interstitial cells may take place when we transform both testicles into upper "fragments," by sectioning the testicle near the under pole and by so cutting away only a very small quantity of testicular mass.

In view of all these experiments it seems clear that hypertrophy of the interstitial cells, as observed in different experimental conditions, has nothing to do with the internally secretory function of the testicle in its relation to the organism as a whole. This hypertrophy is caused by local conditions, and is not brought about in response to general compensatory requirements.

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DESCRIPTION OF PLATES.

(The figures are drawn by Miss L. Lehbert, Dorpat.)

PLATE 1.

Fig. 1.—Two sections through the testicle of a normal animal aged about 4½ months (Prot. No. 27). On the left: interstitial cells in form of a triangle between tubules in full spermatogenesis; on the right: interstitial cells embedded in a granular mass in the neighbourhood of blood-vessels.

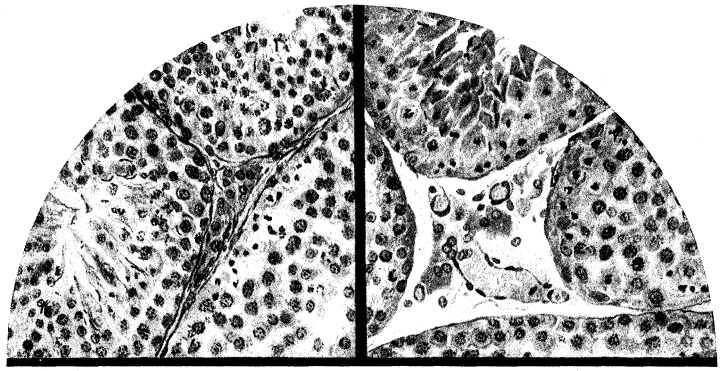
Fig. 2.—Two sections through the testicle of a normal animal about three weeks old (Prot. No. 32). The interstitial cells are rich in protoplasm, the nucleus is large. In the left half the interstitial cells are embedded in the granular mass.

PLATE 2.

Fig. 3.—Section through the upper testicular fragment of an animal subjected to the "complex" testicular section (Prot. No. 76). The hypertrophy of the interstitial cells is enormous. The seminiferous tubules with only one stratum of cells (cells of Sertoli).

Fig. 4.—Section through the testicle of an animal subjected to the operation described in D (p. 138). (Prot. No. 72). The seminiferous tubules are in the juvenile stage, although the animal was fully grown when operated on. The operation was performed about four months previously. The number of interstitial cells is very markedly increased.

All the testicles or testicular fragments were fixed in the solution of Helly (solution of Müller with 5 per cent. formol) and stained by eosin and hæmatoxylin. Only fig. 4 is made from a fragment fixed in Flemming and stained by Heidenhain's iron-alum hæmatoxylin. All the preparations were made by Dr. Wagner.



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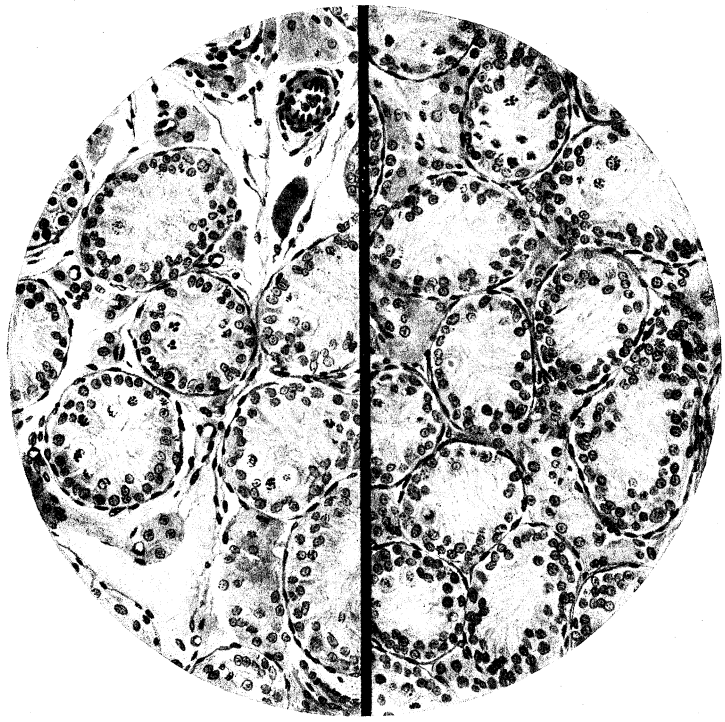

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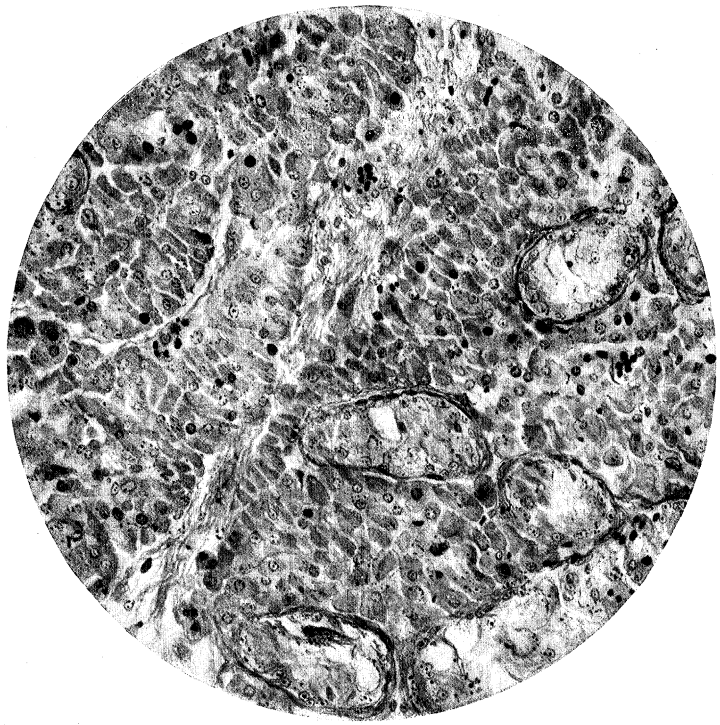

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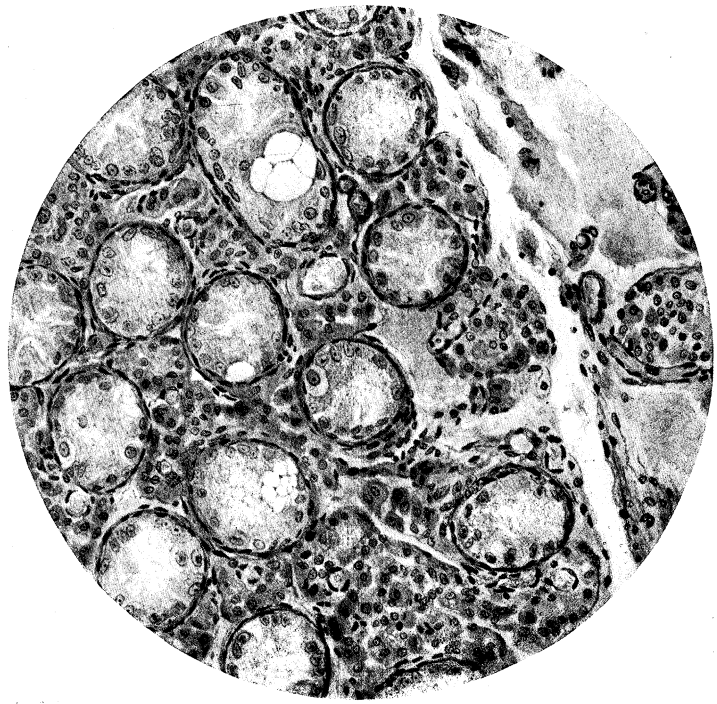

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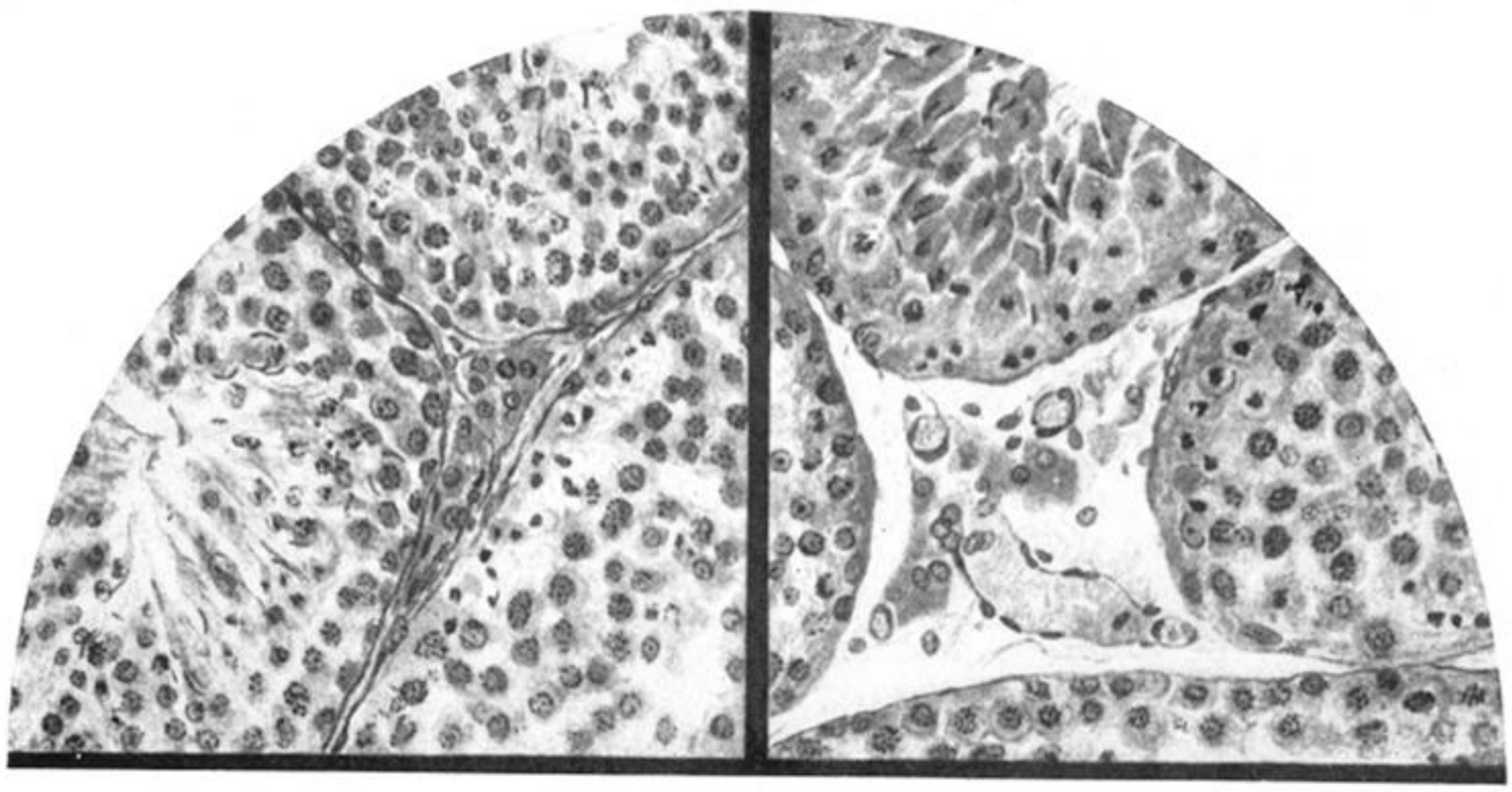

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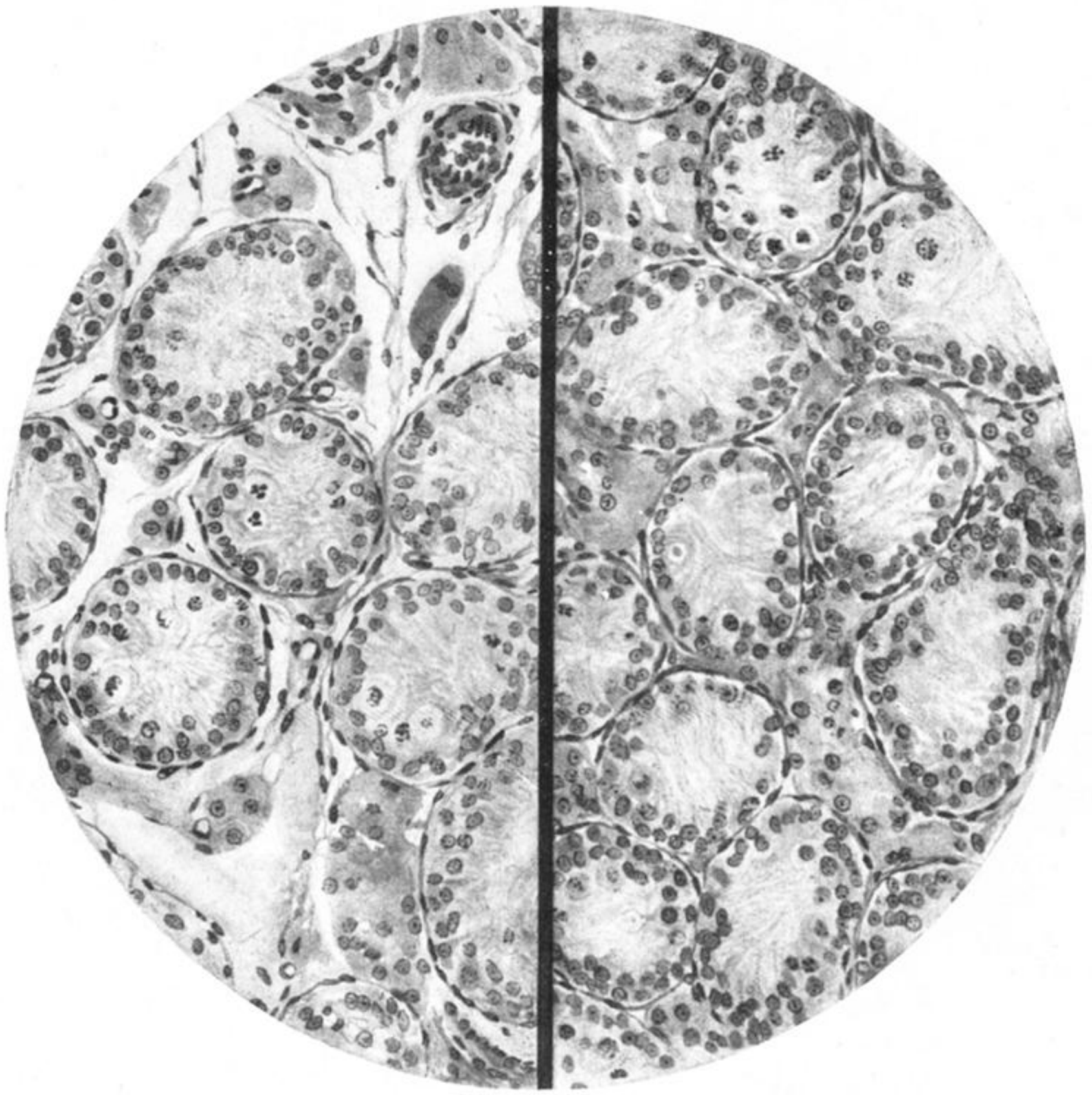

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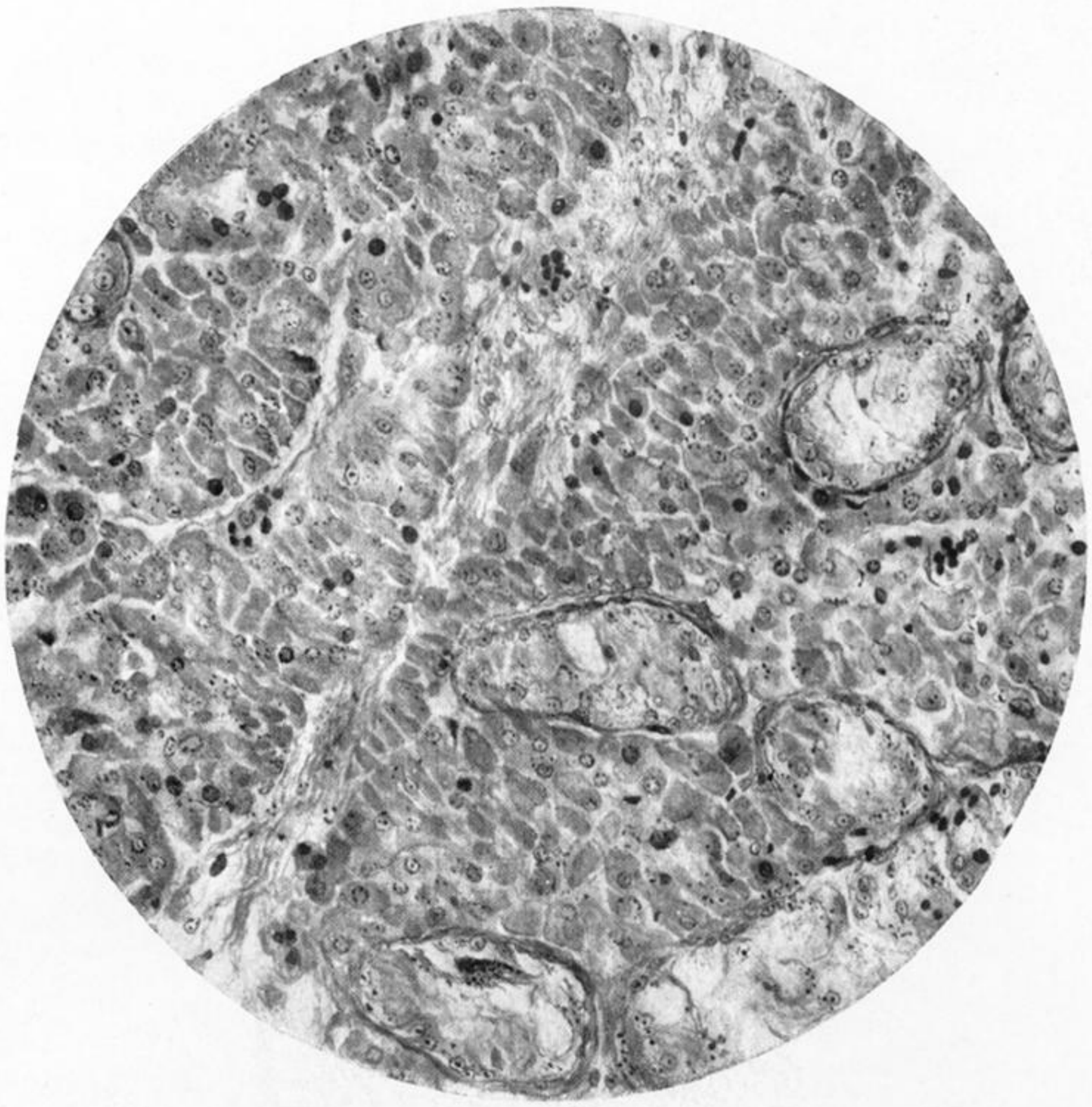

Fig. 3.

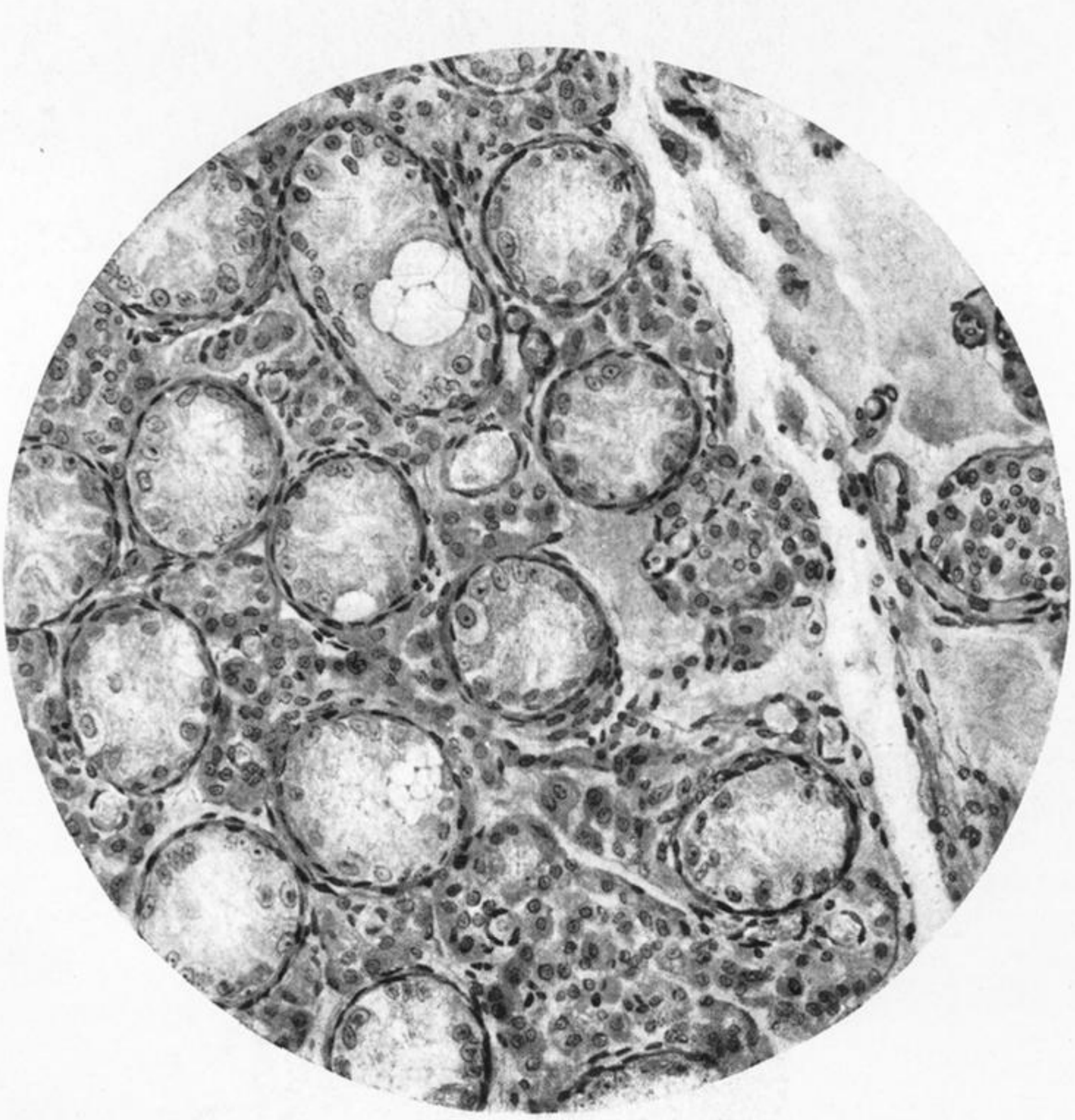

Fig. 4.