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## On the "Islets of Langerhans" in the Pancreas

H. H. Dale

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III. *On the "Islets of Langerhans" in the Pancreas.\**By H. H. DALE, M.A., B.C., *George Henry Lewes Student.*

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## [PLATES 2 AND 3.]

## CONTENTS.

	Page
i. Introductory . . . . .	25
ii. Historical . . . . .	26
iii. Histological Methods . . . . .	29
iv. The Resting Pancreas of the Mammal . . . . .	30
v. Effect of Secretory Activity on the Mammalian Pancreas . . . . .	33
vi. Effect of Prolonged Fasting on the Mammalian Pancreas . . . . .	34
vii. The Resting Pancreas of the Toad. . . . .	35
viii. Effect of Prolonged Fasting on the Toad's Pancreas . . . . .	38
ix. Effect of Secretory Activity on the Toad's Pancreas . . . . .	38
x. Effect of Occluding the Pancreatic Duct in the Mammal . . . . .	40
xi. Summary and Discussion . . . . .	42

i. *Introductory.*

THE observations here described are the outcome of an investigation of the histological changes produced in the pancreas by the activity called forth by "secretin" (BAYLISS and STARLING (1)). In all former investigations of these changes the secretion of the gland has been provoked by the natural stimulus of digestion or by the administration of pilocarpine. The former is at best a slow stimulus, and the histological variations at different periods of normal digestion slight; the latter was found in practice much inferior to secretin as a pancreatic stimulant, judging by the volume of juice secreted. It seemed worth while, therefore, to repeat the former histological observations with the use of secretin,

\* The expenses of this research have been in part defrayed by a grant from the Government Grant Committee of the Royal Society.

which, besides being a far more powerful stimulant than pilocarpine, has the advantage that its action is an intensification of the normal physiological stimulus. It may be said at once that, in regard to the cytological details of the changes involved in the actual secretion—the discharge of the zymogen granules and the growth from the base of the cell of the chromatophilous substance—nothing new was observed. The changes were in many cases more complete, but of the same kind as those described as a result of the action of pilocarpine. My attention, however, was early drawn to very obvious differences produced in the structures called "islets of Langerhans," "intertubular cell-clumps," &c. These changes form the main subject of this paper, and a short historical account seems called for of the views which have been held as to the nature and function of these structures, which will be here referred to as "islets of Langerhans" or "islets."

## ii. *Historical.*

LANGERHANS (2), in 1869, first described, in the pancreas of the rabbit, certain roundish areas of tissue, varying in diameter between 0·1 and 0·24 millim., regularly distributed among the ordinary alveoli, but staining a deep yellow with MÜLLER'S fluid, consisting of small cells, polygonal in outline, with homogeneous cell-substance and round nuclei without nucleoli. He saw no indication of their function, but suggested a possible connection with the nervous apparatus of the gland. SAVIOTTI (3), in the same year, confirmed the observation, describing the cells of the areas in question as being exactly like those composing the ductules. In some cases a continuity with the ductule-epithelium could be made out. This was contradicted by v. EBNER, (4) who, injecting the pancreatic duct, found none of the injection mass in the areas described by LANGERHANS, and could discover in them no trace of lumen. RENAUT (5) described the pancreas as a lympho-glandular organ, the alveolar tissue being intersected by trabeculæ of lymphoid tissue. The nodes of this system of trabeculæ were the areas described by LANGERHANS, which were called by RENAUT "points folliculaires." KÜHNE and LEA (6), in 1882, described, in the pancreas of the living rabbit, glomeruli with wide tortuous capillaries, which were even more plainly seen in injected specimens. In hardened specimens these glomeruli were found to correspond with areas which they named "intertubular cell-clumps" (*Zellenhäufen*). Their description of the cells composing them differs in no important respect from that of LANGERHANS and other observers, but they described, in addition, a capsule of connective tissue, surrounding each clump and cutting it off completely from the neighbouring alveoli. They found them also in the dog, cat, man, and of particularly large size in the monkey (*Macacus cynomolgus*). They suggested that the clumps were of the nature of small lymph glands. PODWYSOZZKI (7) described them as being very similar in appearance to lymph-follicles, but was of opinion that they were not really lymphatic tissue, and suggested the name "pseudofollicles."

GIBBES (8) found the islets in the dog, cat, guinea-pig and ape, and discussed their nature and function, but without reaching a definite conclusion.\*

Up to this point all observers had regarded the islets as permanent structures, quite distinct from the secreting acini. In 1886 LEWASCHEW (9) first pointed out the great variation in their abundance in different specimens of pancreas from dogs and cats. He saw no capsule, but in many cases an apparent continuity between an islet and adjacent alveoli. In many alveoli he found one or more cells resembling those of the islets, and others intermediate between these and normal alveolar cells. All intermediate stages could be found between a group of normal alveoli and a fully formed islet. The islets were more abundant in the pancreas of a fully fed or a pilocarpinised animal, than in that of an animal starved for a few days. His injection results were somewhat uncertain, but in some cases he found lumina, like those of alveoli, in the islets. LEWASCHEW, then, regarded the islets as groups of alveolar cells, altered by fatigue, and supposed that they were reconverted into alveoli during rest.

This view of the islets, as consisting of alveolar cells altered by fatigue, has been held also by DOGIEL (10), PISCHINGER (11) MANKOWSKY (12), TSCHASSOWNIKOW (13) and STATKEWITSCH (14). STATKEWITSCH found that a similar change was caused by prolonged starvation. Of the others, PISCHINGER and MANKOWSKY agree with LEWASCHEW as to the reconstitution of alveoli from the islets during rest; TSCHASSOWNIKOW and DOGIEL regard the islets as consisting of cells in process of degeneration, terminating in absorption.

The embryological investigations of LAGUESSE (15) led him to conclusions of a somewhat similar nature. Already, in 1887, BIZZOZERO and VASALE (16) had pointed out that the islets were abundant in guinea-pig embryos—a fact which, at first sight, seems to tell strongly against LEWASCHEW's view, and has, indeed, been held to do so (OPPEL (17)). LAGUESSE, who first gave to these structures the name of "islets (*îlots*) of Langerhans," described the histogeny of the pancreas in the sheep and the trout. In the sheep embryo the primitive buds, from the anastomosing tubules which form the pancreatic rudiment, are described as being of the nature of islets (*îlots primaires*). These later become converted into secretory acini, which are again transformed into islets. These, after growth by vigorous cell-division, are yet again converted into a larger number of acini. LAGUESSE regards this process, both as a method of growth, and as representing an alternation between externally and internally secreting (exocrine and endocrine) conditions of pancreatic tissue. He considers that the process may continue to some extent throughout life, and thus explains LEWASCHEW's results.

In this connection should be mentioned also the observation of OGATA (18), who,

\* The view attributed to him by many German writers, that the islets are embryonic remnants, was not advocated by GIBBES, who, indeed, mentions it, but only to dismiss it as "scarcely probable" in view of their wide distribution.

in the frog's pancreas, discharged by pilocarpine, found patches of tissue in which the cells had quite lost their normal alveolar arrangement, and the similar appearance observed by STEINHAUS (19) and VER ECKE (20) in Amphibia, and by ELLENBERGER and HOFMEISTER (21) in the horse. None of these observers suggests any connection between such areas and the islets of Langerhans.

It should also be noted that KOLOSSOW (22), who describes bridges of tissue between adjacent gland cells, finds that this connection exists between the cells of the islets and the adjoining secretory cells. He concludes that the islets are composed of glandular elements.

Many observers have maintained, against LEWASCHEW and his supporters, the earlier view which regards the islets as a tissue completely distinct in origin and function from the secreting acini. HARRIS and GOW (23) described the islets in many animals under the name of "secondary cell-groups," and regarded them as a special part of the secretory mechanism, possibly concerned in forming one of the pancreatic enzymes. They found that the cells of the islets became smaller during activity. MOURET (24) and PUGNAT (25) return to the conception of the islets as consisting of lymphoid tissue. JAROTSKY (26) attributes LEWASCHEW's results to the use of a fixative so imperfect as alcohol. Using corrosive sublimate, he never found intermediate forms at any stage of activity. He suggested that the islets furnish a prezymogen, carried in the lymph-stream to the alveoli, and was the first to point out the greater richness in zymogen granules of the alveoli immediately round an islet. This latter was most marked in starving animals and in those fed on fat, least so in those fed on carbohydrate diet. DIAMARE (27) denies the variation of islets with activity, and the presence of intermediate forms. He found, that the small bodies described in the abdominal cavity of fishes by STANNIUS (28) resembled islets in structure. He regards the islets as special epithelial structures, probably furnishing an internal secretion. A similar view is held by MASSARI (29) and by v. EBNER (30). GIANELLI and GIACOMINI (31), in the islets of reptiles, describe an alveolar structure and the presence of ducts. The alveoli of the islets are sometimes continuous with those of the general secreting tissue. They do not, however, adopt LEWASCHEW's theory, but, like HARRIS and GOW, regard the islets as furnishing a special constituent of the secretion. The view of the islets as furnishing the internal secretion of the pancreas has stimulated the investigation of these structures in cases of diabetes mellitus, and a considerable volume of conflicting evidence has been obtained. OPIE (32), who first pointed out the preponderance of islets in the splenic end, as compared with the rest of the pancreas, in mammals, examined the pancreas in a number of cases of diabetes. In a certain number he found the islets affected by hyaline degeneration, or by inflammatory change, which in some cases was selective, leaving the alveoli intact. Similar results were obtained by WEICHELBAUM and STANGL (33), HERZOG (34), SSOBOLEW (35), and GENTÉS (36), though each found a certain proportion of cases with intact islets. Many other observers have failed



to find evidence connecting diabetes with disease of the islets (KASAHARA (37), WRIGHT and JOSLIN (38), V. HANSEMAUN (39), DIECKHOFF (40), M. B. SCHMIDT (41), GUTMANN (42)). In glycosuria, produced by injection of adrenalin (HERTER (43)) or of leucomaines (LÉPINE (44)), no histological changes in the pancreas were detected.

Of cases of pancreatic disease, without diabetes, the various observers named record a certain number in which the islets were unaffected, but others in which the islets were diseased, scarce, or even indistinguishable.

The evidence as to the relation of the islets to carbohydrate metabolism is, therefore, at best inconclusive.

In the same connection observations have been made, as to the effect, on the structure of the pancreas, of the various procedures by which it has been found possible to destroy its function of external secretion, without affecting the carbohydrate metabolism—ligature of the duct, injecting the duct with oil, or removing the gland from its normal connections and forming a subcutaneous graft. MOURET (45) found that all the pancreatic tissue disappeared, leaving only certain irregular groups of cells, which he does not seem to have regarded as islets. MANKOWSKY (12) found that both the alveoli and the islets disappeared, leaving nothing but ducts and connective tissue. SCHULTZE (46), SSOBOLEW (32), LAGUESSE (47) found that the alveoli rapidly disappear, leaving only ducts and islets embedded in connective tissue. According to LAGUESSE, many islets are new-formed by budding from the ducts. Ultimately these also undergo sclerosis and disappear.

There are thus three principal theories of the nature and functions of the islets of Langerhans, each supported by a certain amount of evidence: (1) That they are the result of a transformation, temporary or permanent, of the ordinary secreting tissue of the pancreas; (2) that they are a special part of the secreting tissue, furnishing a particular constituent of the secretion; (3) that they are entirely independent structures, analogous to such ductless glands as the medulla of the adrenal bodies or the anterior lobe of the hypophysis cerebri, and furnish an internal secretion essential for normal carbohydrate metabolism. The theory put forward by LAGUESSE combines the first and third of these. The evidence here to be presented is entirely in favour of the first. Its relation to the others will be discussed later.

### iii. *Histological Methods.*

The animals used were the dog, cat, rabbit and toad. After trying a number of fixatives I found the most generally useful to be a mixture containing corrosive sublimate and formaldehyde. This was made fresh as required by mixing three or four volumes of a saturated watery solution of the sublimate with one volume of strong formal (about 40 per cent.). It, therefore, contained 8–10 per cent. formal-

dehyde. In some cases corrosive sublimate alone was used. The pancreas of dog or cat was cut into thin slices: the rabbit's pancreas, being spread out into a thin layer in the mesentery, needed no such section: the toad's pancreas was hardened entire. The tissue was left in the hardening fluid for 24 hours, washed for 24 hours in running water, passed through alcohols and xylol and embedded in paraffin in the usual way.

In the case of the mammals the sections were cut a thickness varying from 2–4  $\mu$ . The toad's pancreas being of looser construction, it was found better to cut it into 5  $\mu$  sections for study of the general topography of the gland.

In almost all cases the sections were stained with eosin and toluidene-blue. The stains were used in 1-per-cent. watery solutions. The sections, fixed to the slide and freed from paraffin by xylol, were brought through alcohols to dilute tincture of iodine, in which they were left till deeply yellow, to remove excess of corrosive sublimate. The iodine was washed out in 60-per-cent. alcohol, and the sections brought into distilled water. They were then stained with the eosin solution until distinctly overstained, 5–10 minutes, varying with different specimens. The excess of eosin was removed by 60-per-cent. alcohol, until the zymogen granules could be seen under a low power stained very distinctly deeper than the rest of the section. The eosin was then fixed with dilute acetic acid, the sections washed again in distilled water, and stained for  $\frac{1}{2}$ –2 minutes, according to the rapidity of staining, with toluidene-blue. After washing in water again, they were passed through alcohols to 95 per cent., in which they were left till sufficient toluidene-blue was removed to give clear differentiation; then through absolute alcohol into xylol; and finally mounted in Canada balsam.

#### iv. *The Resting Pancreas of the Mammal.*

It should be pointed out, in the first place, that the term "resting" is more convenient than accurate. In a mammal, such as the rabbit, the stomach and small intestine practically always contain food and the pancreas secretes continuously. In the dog the process is more intermittent, but the stomach and small intestine generally contain some remains of food even after 24 hours' fasting. In the cat, under such conditions, the stomach and duodenum are practically empty, and there is a genuine intermission. If it be borne in mind that by "rest" is meant the condition of the gland, taken from an animal unfed for about 24 hours, but otherwise normally nourished, and that the word is thus applied to a condition of activity probably very different in the three animals referred to, it will be convenient to use the term in contrasting such glands with those in which excessive activity has been provoked by artificial means. In a section of such a resting pancreas, stained, as above described with toluidene-blue and eosin, the base of each cell of a secreting alveolus and the chromatin of its nucleus stain deeply with toluidene-blue, the large nucleolus or

nucleoli, and the secretory granules which fill the inner zone of the cell stain bright red with eosin. Among the deeply stained alveoli the ductules and the islets of Langerhans stand out clearly, relatively pale. The epithelium of the ductules consists of pale cubical cells, with oval nuclei containing a faint network which stains purple with both dyes and a small eosinophil nucleolus. This ductule epithelium can frequently be seen continued into the lumen of an alveolus as the centro-acinary cells, which possess nuclei of exactly the same type. The islets of Langerhans consist of irregular polygonal cells which, for the most part, are stained like the ductule epithelium, both as regards cell-substance and nuclei. A few of the nuclei, however, are irregular, shrunken, and deeply stained. Occasionally, but rarely in the resting gland, an islet can be found which is apparently continuous with the epithelium of a ductule, and the similarity of the islet-cells to those of the epithelium is then very obvious.

The abundance and size of the islets varies in different glands, and in different parts of the same gland. As OPIE pointed out, the islets are always more abundant and larger at the splenic end than in the rest of the gland.

By comparison of a number of sections from different specimens of the three species examined, the sections being taken always from the splenic end of the pancreas, it is seen that the islets are most abundant in the pancreas of the rabbit, less so in that of the dog, and still less so in the cat; and it is worth noting that the same order applies to the relative continuity of the digestive process in these three animals.

It is possible, also, in the pancreas of the dog or cat, roughly to predict the abundance or scarcity of islets by the naked-eye appearance of the fresh gland. It will be found that a cat's or dog's pancreas, for example, which has the opaque white appearance of a fully loaded gland has few islets even at the splenic end, while one which, from some unknown difference in the process of nutrition, has the translucent grey appearance of the discharged, or partly discharged, gland will show a relatively greater abundance of islets. Fig. 1, Plate 2, shows part of a section across the splenic end of a dog's pancreas, which has about the average abundance of islets in that animal. Fig. 2, from a cat, is from a pancreas which contained an unusually large number of big islets, which, even at the splenic end, are usually rare in the cat.

The structure of the islets, as seen under a high power, is different in the rabbit from that seen in the cat and dog. The islets in the rabbit stand out less clearly from the surrounding alveolar tissue than those of the cat and dog. This is due to the fact that the islet-cells in the rabbit stain fairly well with toluidene-blue, and better with eosin. The result is a pinkish-purple tint of all the islet-cells. The same is true of the ductule epithelium of the rabbit. There is no real difficulty, however, under low or high power, in recognising the islets. They are seen to be usually of rounded, compact form, and, at first sight, sharply marked off from the surrounding secretory alveoli. With a high power many of the islets show a division of the cells, by intervening connective tissue and blood capillaries, into packets which



have a shape strikingly similar to that of the secretory alveoli. In such, an outer layer of larger cells can be distinguished from an inner layer of smaller cells, exactly like centro-acinary cells. The appearance, in fact, is exactly that of a group of alveoli in which the secreting cells have lost their characteristic basal basophil staining and zymogen granules, their nuclei having become more centrally placed, and assimilated to those of the centro-acinary cells, and in which the lumen has become obliterated by a falling together of the cells. In most of such islets it can also be seen that the sharp circumscription is only apparent, and is not due to a capsule, as described by KÜHNE and LEA. The divisions of the islet, of suggestive alveolar shape as described above, can often be found actually continuous, beyond the islet margin, with undoubted normal secreting alveoli, containing zymogen granules, and staining characteristically both in cell-substance and nucleus.

In many islets the appearance of alveolar structure and of continuity with the secretory tissue is not seen. In such islets the cell-substance is reduced, and the cells displaced, so that the nuclei are irregularly crowded together. The blood capillaries are also enlarged and tortuous, so that the islet has the form of a coarse network.

In the dog and cat the islets also vary not only in size and number, but in structure. In cases where the islets are large and abundant—such are commoner in the dog—it is easy to find islets showing traces of alveolar arrangement, some of the cells staining in parts like secretory cells, whilst continuity with the secretory alveoli surrounding the islet is often very clearly seen. Such an islet is shown in fig. 3. In this, a common type of such continuity is seen, the islet tissue being continued into the centro-acinary cells of an adjacent alveolus, of which the secreting epithelium seems, therefore, to form a cap over a prolongation of the islet. In the islet itself traces of alveolar structure are obvious even in the photograph, and in one place cells are seen which, though evidently part of the islet, stain like secretory cells, and contain zymogen granules. On the other hand, sections of islets are found in the cat and dog which have no apparent connection with surrounding alveoli, but consist of closely packed, polygonal cells, filling the meshes of a plexus of large blood capillaries. Between such a structure, however, and that described above, and illustrated in fig. 3, there can be found all conceivable intermediate stages.

About the continuity and connection between secretory and islet tissue there can, I think, be no doubt. The question remains, however, whether such a structure as that shown in fig. 3 represents a new formation of secreting tissue from an islet, or of an islet from secreting tissue. The impression gathered from a consideration of such a preparation by itself is strongly in favour of the latter view. The unequal distribution of the staining reaction, so that an isolated cell, or part of a cell, shows the basophile reaction, which fades away towards its edge; the presence of cells which have no basophile properties, but retain a few eosinophile granules, which appear to be undergoing solution; the faint trace of alveolar outline, like the shadow of a

structure which is being lost; none of these would be expected in a new formation of alveoli. In the same direction points the fact that the abundance of these intermediate forms is greatly increased by such influences as cause increase in the number and size of the islets. To the consideration of these we must now proceed.

v. *The Effect of Secretory Activity on the Mammalian Pancreas.*

For the study of this effect only the cat and dog were taken as types of mammalia. The effect on the rabbit was not observed, since, as shown by BAYLISS and STARLING, secretin makes only a small difference in the rate of its pancreatic secretion.

The dogs and cats were anæsthetised with morphia and A.C.E. mixture, a cannula tied in the pancreatic duct, so that the rate of secretion could be watched, and secretin solution injected into the jugular vein in doses of 5–10 c.c. at such intervals as to keep the gland constantly secreting for many hours, until it began to show signs of exhaustion. In many cases this condition was never attained. In one case, after 14 hours continuous secretion the gland responded to secretin as well as ever, and had the opaque naked-eye appearance of a loaded gland, from which, on section, it showed no recognisable histological difference.

The effect of different specimens of secretin, and the response to the same specimen of different animals, showed unaccountable variations. With a powerful secretin and a responsive pancreas, especially if the animal be bled towards the end of the experiment with each administration of secretin, it is often possible to reduce the gland to such a state of exhaustion, that further doses of secretin produce hardly any, or no secretion.

In making comparisons of exhausted with resting glands, in respect of the abundance of islets which they contain, certain obvious sources of error must be eliminated. It is clearly useless, for reasons stated above, to compare the splenic end of one with the free end of the other gland. One cannot, therefore, remove part of the gland at the beginning of the experiment and compare it with the rest of the gland subjected to secretin. On the other hand, by taking sections from the splenic end of the pancreas from two different animals, even when kept under conditions apparently identical, it is impossible to be certain of eliminating the differences due to individual variation. No difference, therefore, which can come within those limits of variation can be considered conclusive.

I have in all cases taken sections from the splenic end of the pancreas. By comparison of a number of specimens from resting glands with a number of specimens from glands in which activity has been provoked short of exhaustion, whether by pilocarpine or by secretin, one received a general impression of greater abundance of islets in the active glands.

Such are the differences, presumably, observed by LEWASCHEW and his followers, and denied by their opponents. This contradiction of evidence is not difficult to

understand. I have never found, under such conditions, such an abundance of islets as could not be matched by isolated specimens obtained from a resting pancreas. To carry conviction on the point, it would be necessary to cut serial sections through the pancreas, and count and measure the islets.

The case is different when the gland is thoroughly exhausted. The sections from the exhausted pancreas of a dog, shown in figs. 4 and 5, show islets in such abundance and of such a size as are never seen in any part of a resting gland.

All of these islets, moreover, show signs of active formation. The traces of alveolar structure in most of them are very distinct; their outline is irregular, on account of encroachment on the surrounding alveoli; and transitional forms are abundant, both on the margin and in the interior of the islets. In fig. 5 is seen practically the whole of a lobule which, for the most part, is converted into tissue indistinguishable from that of the islets, but still contains many incompletely altered alveoli which are visible in the photograph.

Comparing the cat's pancreas in fig. 6 with that in fig. 2, the contrast is not so striking. Fig. 2, however, shows an abundance of islets unusually large in the resting cat's pancreas, while the gland shown in fig. 6 was not reduced to complete exhaustion—a condition which I have never yet produced in the cat. It may still be claimed with confidence that fig. 6 shows such an abundance and progressive formation of islets as is never seen in the resting pancreas of the cat.

When the specimens are examined under high powers of the microscope the difference caused is even more evident than with the low magnification shown. It can then be seen that, apart from the large areas of definite islet tissue such as the low power shows, a large proportion of the remaining alveoli show partial change into what must now be called the islet condition, some of their cells having lost their normal staining properties and having become assimilated to the centro-acinary cells.

Another feature, which is, unfortunately, not shown in the figure, is the frequent apparent continuity of the islets with the epithelium of the smaller ductules. The origin of such an appearance is quite clear, if we imagine the area of gland adjacent to and drained by such a ductule undergoing this change, which we have seen to be of such a nature as to assimilate the secretory cells to the centro-acinary cells and the ductule epithelium.

Such preparations afford clear evidence that the conversion of secreting tissue of the pancreas into islets of Langerhans, of which we saw indications in the resting gland, is greatly accelerated and rendered much more extensive by excessive secretory activity.

#### vi. *Effect of prolonged fasting on the Mammalian Pancreas.*

It might, at first sight, be expected that fasting would have an effect opposite to that produced by secretory activity, and that the pancreas of a starved animal would

contain even fewer islets than the resting pancreas of a normally fed animal. The statement of STATKEWITSCH that starvation caused formation of islets from alveoli, was contradicted by JAROTSKY. The objections to making the matter the subject of deliberate experiment are obvious. An opportunity was afforded by the capture of a stray cat which, from its emaciated condition, had probably been without food for some time. The cat was killed, and the stomach and small intestine found to be empty, but fæcal matter was found in the cæcum and colon. The pancreas was small, greyish and translucent, having the appearance of an exhausted gland. Sections from the splenic end showed a pancreas of the discharged type, though a few zymogen granules were present. There was a great abundance of large islets, with clear evidence of progressive formation, as in the gland exhausted by secretin. Such a section, under a low power, is shown in fig. 7 (Plate 3). The abundance of islets is again such as is never seen in a resting pancreas of the cat.

The examination of this one specimen entirely corroborates the statement of STATKEWITSCH. The discussion of the meaning of this change, and of the difficulties involved in this conception of the origin of the islets, must be postponed till the effect of these same conditions on the Amphibian pancreas has been described.

#### vii. *The Resting Pancreas of the Toad.*

Previous observations on the islets of Langerhans in the Amphibian pancreas have not taken account of the effect of secretory activity. LEWASCHEW could not discover islets in cold-blooded animals, while v. EBNER and DIAMARE, who described them in the frog and in various Amphibia, regarded them as completely independent organs, altogether unaffected by the secretory activity of the rest of the gland. It seemed especially important, therefore, to extend to the Amphibian pancreas these observations of the effect of secretin and of fasting.

The toad was found to bear much better than the frog the effects of long captivity and of the injection of secretin. It was therefore taken as the Amphibian type.

The question first arises as to what can be considered as the resting condition of the toad's pancreas. The animal feeds seldom: in winter not at all. It may be kept alive for a very long time without food; yet it was found that, after a certain period of fasting the pancreas of the toad, like that of the mammal, exhibited changes which cannot be considered characteristic of rest. It is not possible in such an animal to draw a definite line between the state of rest and that of starvation, but it was found that the pancreas taken a month after capture in summer, or three months in winter showed no starvation changes.

The histological method was the same as in the case of the mammalian pancreas, except that the gland was hardened entire and a series of sections cut through the whole. There was thus no danger, in this case, of error due to local variations.



The pancreas of the toad, hardened and stained as described, shows on section an appearance very similar to that of the mammal, as far as the purposes of this investigation are concerned. The most evident differences, under lower power, are the number and prominence of the ducts, the general looseness of structure, the large number of the islets of Langerhans, and the large relative size of some of them. The epithelium of the smaller ducts consists, as in the mammal, of pale cubical cells, with oval nuclei, containing small nucleoli and a scanty chromatin network. The epithelium of the ductules is very obviously continued by the centro-acinary cells, which are, in parts of the gland, so prominent, that many alveoli present the appearance of a number of secretory cells somewhat loosely arranged round a ductule. The islets of Langerhans are abundant, and vary much in size. Some of the smaller islets have the appearance of buds from the smaller ducts, being simply clumps of cells resembling those of the duct epithelium. Some of these buds show an arrangement into a double row of cells surrounding a lumen. In the immediate neighbourhood of such structures may often be found alveoli which differ from them only in the nature of the outer layer of cells, which in this case is formed of typical secretory cells, with basophil outer zone, inner zone filled with eosinophil granules, and a nucleus containing a large nucleolus and a network with large masses of chromatin. A few of the cells of the outer layer, however, have nuclei like those of the inner layer—the centro-acinary cells in this case—and their cell-substance shows only faint basophil staining and few zymogen granules. Though the transition between these two forms is sufficiently clear, it is difficult to be certain as to its direction—whether we have here a new bud from a ductule forming an alveolus, or an alveolus undergoing a change analogous to that seen in the mammalian islets, and here giving rise to the appearance of a bud from a ductule. The absence of signs of nuclear multiplication, and the presence of zymogen granules in cells with faint basophil staining and nuclei of the centro-acinary type, seem to point to the latter of the two possibilities. This view is confirmed by study of the large compound islets, consisting of several divisions, separated by septa containing blood capillaries. In such an islet one division has frequently the structure of one of the simple islets just described, and is continuous with yet unaltered or partially altered alveolar tissue. The rest of the islet usually has the structure which must be considered as typical of the islets of the resting toad's pancreas, and which is illustrated in fig. 8. Such a typical islet appears quite shut off and separate from the surrounding secretory alveoli. It consists of a number of columns or compartments of cells which stain similarly to the ductule epithelium, but are mostly elongated in form and have a palisade arrangement. The nucleus of such a cell is sometimes correspondingly elongated, having a nucleolus at each end; in other cases the cell contains two rounded nuclei, indicating that the elongated form probably represents a stage of amitotic division. A compartment of such an islet can often be found in which the cells show an alveolar arrangement into two layers round an indistinct lumen,

In such a case the cells of the outer layer have spherical nuclei, which are of the type found in secretory cells, while the cell-substance shows basophil staining, and, in a few cases, a small number of eosinophil granules. Such an appearance, I believe, represents the early stage of reconstruction of an alveolus from the islet. Fully formed and loaded alveoli are often seen, which yet appear to be included in the islet outline. An instance of this is to be seen near the upper border of the specimen in fig. 9. In spite of much searching, however, I have not been able to trace a series of intermediate stages in the formation of such an alveolus.

Two other points in the histology of the resting pancreas of the toad are deserving of mention and further investigation. The first, which has some relation to the appearance just described, is the fact that the alveoli immediately surrounding such a "resting" islet are more fully loaded with secretory granules than those in the rest of the gland. This was noticed by JAROTSKY in the mammal, and by him attributed to the formation of a prezymogen by the islets. I have also noticed it in the resting dog's pancreas, but the difference in the toad is far more striking. These fully loaded alveoli are not always seen surrounding the islet on every side. At one point the islet may be growing, by the breaking down into islet tissue of an imperfectly loaded alveolus, while on its other sides it may be surrounded by the fully loaded alveoli, which, by their concentric disposition round the islet, give the impression of having formerly formed part of it. Another process, of which the significance is not quite clear, is generally to be seen at some point of the resting pancreas of the toad. This is a vigorous nuclear multiplication, giving, under a low power, the appearance of a localised infiltration with round cells. When seen under a high power, this is seen to be due to mitotic nuclear division, the nuclei implicated being chiefly those of the alveoli and ductules, but, to some extent, apparently those of the connective tissue also. In the pancreas of the winter toad, the process was widespread, mitotic division of nuclei being found chiefly in the neighbourhood of the ducts. A patch of such nuclear multiplication, which was found, with a high power, to contain many mitotic figures, is seen near the division between the two lobes in fig. 9. Some of the cell-bodies containing these nuclei retain traces of their secreting characters, but they are in most cases lost. We have here, then, another method by which the alveolar tissue can become converted into areas filled with cells of homogeneous type.

The whole process is by no means clearly made out as yet, but enough has been seen to make it clear that there is a constant tendency, on the part of the secretory tissue of the toad's pancreas, to revert to what we may consider a more embryonic type. This must obviously be balanced by a constant reconstruction of secretory tissue, and of this process we have seen some indication. We have yet to consider the effect of the conditions which, in the case of the mammalian pancreas, were found to cause extensive change of the secretory tissue.

viii. *Effect of Prolonged Fasting on the Toad's Pancreas.*

For reasons given above, it is necessary to keep a toad for a prolonged period without food if we wish to observe the effects of inanition. Toads were taken, on two different occasions, which had been in the laboratory for 6 months and 5 months respectively. In both the changes found were of the kind expected—a widespread conversion of alveoli into tissue resembling the islets of Langerhans. In most cases this amounted merely to a wider distribution, affecting in certain parts of the gland nearly all the alveoli, of the process, described in the resting gland, of direct conversion of alveolar into islet tissue. The pancreas, of which a section is reproduced in fig. 10, was taken from a toad which had been in the laboratory from the beginning of October to the end of March. This showed a more extensive change, and contained large areas in which only traces of alveoli could be detected. In some of these areas a few mitotic figures were found. These areas had the structure of the newly formed portions of islets seen in the resting gland. No indications of the reconstruction of alveoli were found in this specimen.

If we adopt the idea of a balance of two opposing processes in the normal resting gland, we may explain this result of fasting as due, either to an acceleration of the conversion of alveoli into islet tissue, or to an inhibition of the reconstruction of alveoli from islets, or to a combination of the two.

ix. *Effect of Secretory Activity on the Toad's Pancreas.*

Secretion of the pancreas was brought about by injection of ordinary secretin solution, prepared from the dog's duodenum, into the dorsal lymph-sac. No measure of the rate of secretion was possible, but it was observed, in a toad which had received such an injection and was pithed during its absorption, that fluid could be detected, with a hand-lens, exuding from the papilla of the pancreatic duct into the duodenum. For the rest, the evidence of secretory activity was purely histological, and it was taken for granted, that the discharge of all or most of the large and easily preserved zymogen granules was sufficient indication of prolonged and vigorous activity. Such a change was produced by the injection of secretin into the dorsal lymph-sac. The secretin was given in doses varying with the size of the toad, each injection being sufficient to fill the lymph-sac. The process was repeated as often as the fluid was found to be absorbed, and was continued for 2, 3 or 4 days. In this way a large toad sometimes received as much as 40 cub. centims. of secretin solution. If the toad was killed shortly after the last injection, it was found that the ducts and tissue spaces of the pancreas were distended with fluid. The animal was, therefore, left for some hours after the last dose of secretin had been completely absorbed. In a few instances the toad received its final injection on

the evening of one day and was found dead on the morning of the next. In such cases the pancreas was only preserved, when it was clear that the animal was only recently dead, *rigor mortis* not having set in, and the muscles still responding to stimulation of the nerves. The facts that in such a case a small piece of liver gave a normal histological picture, and that the changes in the pancreas were precisely similar to those found in other specimens from toads similarly treated, but killed by pithing immediately before preparation, should suffice to exclude the suspicion of *post-mortem* change.

Eleven toads were thus injected, and the effect was of the same kind in all cases, though it varied somewhat in degree. This might be expected from the varying effect of different preparations of secretin and the varying response of different animals as seen in experiments on mammals. There was a very great increase of the tissue, which we have now frequently described, and have called islet tissue. This increase is due, as always, to a conversion of the secretory alveoli. In the specimen, of which a section is reproduced in fig. 11, the change is very extensive. The exhaustion is very complete, no zymogen granules being found in any part of the section, and a very large proportion of the whole tissue of the gland has undergone the change into islet tissue. A few alveoli retain their identity, and a large number can be seen in various stages of conversion. The condition of the whole gland, in fact, is very similar to that of the small portion of dog's pancreas shown in fig. 5. Scattered in this transitional tissue are several fully formed islets of the resting type, which were presumably present before the injection of secretin. This specimen was obtained from a winter toad. A control toad, not injected with secretin, gave pancreas sections very similar to that shown in fig. 9.

Another feature of this exhausted gland, not included in fig. 11, was a very large area studded with nuclei, which nearly all showed mitotic figures. The production of these mitotic figures is thus also clearly accelerated by the action of secretin. In fig. 12 is seen, under higher magnification, a portion from another pancreas less completely exhausted, the discharge of zymogen granules being not quite complete. In the middle of the field is seen a large area composed of faintly staining cells, with nuclei so faintly stained as to be visible with difficulty in the photograph. A few of the cells can be seen, in the original section, to retain traces of basophil staining. They show no definite arrangement, either into the alveoli of secretory tissue, or the columns of the "resting" islet. A clump of cells, seen in the middle of this area, have lost their alveolar arrangement, but retain their staining properties and a few zymogen granules. Many cells of surrounding alveoli have become assimilated to the cells of this forming islet, which has thus the appearance of eating its way into the adjacent tissue. In one corner a longitudinally cut lumen is seen, but whether it is that of a pre-existent ductule, or has arisen *de novo* in the islet-area, it is impossible to say. The cells are exactly similar to those of ductules elsewhere in the section.



The whole section contains great numbers of such areas, which, indeed, are almost continuous, very few unaffected alveoli being found.

The specificity of these effects was proved by the injection, in precisely similar manner and quantities, of an extract prepared from the lower end of the ileum. This has been shown to contain the depressor substance present in secretin solutions, but to be without stimulating effect on the pancreas (BAYLISS and STARLING). Other toads were injected with salt solution. In neither case was any perceptible effect produced.

Infusion for several hours of the whole toad with secretin, through a cannula tied into the bulbus arteriosus, caused some change of the same kind as that described, but far less in extent. The cells were probably killed quickly. Ileum extract and salt solution, similarly perfused, had no appreciable effect.

x. *Effect of Occluding the Pancreatic Duct in the Mammal.*

The statement of several observers, that occlusion of the pancreatic duct is followed by a degeneration and disappearance of alveolar tissue, while the islets remain intact, is often put forward in evidence of the independent origin and function of the islets. Though it is evident, that the disappearance of lumina and rearrangement of cells, which has here been described, would sufficiently account for the supposed immunity of the islets from the effects of duct-ligature, it seemed advisable to repeat the observations.

In the pancreas of the dog there are two ducts, of which only one is easily reached for the tying operation. The shortness of the ducts also makes it difficult to avoid the re-formation of a lumen by a process of ulceration, if the ducts are simply tied. The method used by SCHIEF, HEDON, and others, of injecting paraffin into the lower duct was, therefore, adopted. The operation was kindly performed for me by Professor STARLING. The dog was anæsthetised with morphia and A.C.E. mixture. The paraffin injected had a melting point of 40° C. The aseptic precautions were perfectly successful, and the dog was kept for 4 weeks after the operation. Only one such experiment was made.

In the rabbit there is only a single duct. It is quite easy to doubly ligature this, and remove about  $\frac{1}{4}$  inch of duct between the ligatures, thus minimising the risk of restoration of the channel. I performed this operation on three rabbits. These were anæsthetised with morphia and ether. They bore the operation well, the wounds healed without suppuration, and the rabbits were killed 3, 7 and 15 days respectively after ligature of the duct.

In the dog a cast of paraffin was found blocking the duct up to the splenic end of the pancreas. The gland looked shrunken and irregular. Microscopically this was found to be due to an interstitial fibrosis. The connective tissue was everywhere

much increased. Many lobules of practically normal alveoli containing normal islets could be found. Other lobules, however, were reduced to small central cores of pancreatic tissue, surrounded by dense layers of connective tissue. These pancreatic remnants were in some cases still obviously composed of secretory alveoli, but of alveoli which were undergoing a change very similar to that now familiar in the formation of islets. In neighbouring lobules the change had proceeded further and the remnant was very similar in appearance to islet tissue. There was no indication that pre-existent, fully formed islets had escaped destruction, but it was clear that the whole pancreas was undergoing a change which would ultimately reduce it to a mass of connective tissue, including remnants of pancreatic alveolar tissue, which, as in all circumstances of defective nutrition, would assume a form resembling that seen in the islets of the normal gland. This I believe to be the true explanation of the supposed immunity of the islets from the destructive process consequent upon occlusion of the duct.

In the rabbit the effect was somewhat different. After 3 days' ligature there was distension of the ducts and indistinctness of the alveolar cells. The alveolar cells had, to a large extent, lost their characteristic staining properties and their secretory granules. It was difficult to distinguish the islets of Langerhans; areas showing the characteristic islet arrangement and staining of cells were apparently quite continuous with the degenerating alveoli surrounding them.

After 7 days, very many of the alveoli were greatly distended, and their secreting cells flattened, so that the section appeared thickly studded with sections of ducts. Between these were other alveoli in which the cells were becoming disordered, losing their characteristic staining properties, and becoming assimilated to the islets. It was impossible to draw any sharp line of distinction between islets and alveoli undergoing these changes, or to determine whether the islets present in the pancreas before the operation had survived. There was a slight degree of interstitial fibrosis.

After 15 days the fibrosis had proceeded much further. A large part of the pancreas was replaced by fibrous tissue. In this were embedded the remains of pancreatic lobules. In these could now be seen no traces of normal alveolar structure. Many of the alveoli by distension of the lumen and alteration of the epithelium has been converted into structures resembling branching and tortuous ductules, of which the lumina were not so large as those seen 7 days after ligature. In some cases continuous with these, in others quite separate and surrounded by a capsule formed of the invading fibrous tissue, were large areas having the structure of islets of Langerhans. They were more numerous and much larger than the islets seen in the normal rabbit's pancreas, but in no case showed an alveolar structure. All showed the radial arrangement of cells to blood capillaries, which was noticed in the fully formed islet of the rabbit's pancreas. Though the shrinkage, due to fibrous contraction, might account for the great number of these structures, on the assumption that they were pre-existing islets which had survived the

destructive process, such a theory will not account for their large size. A comparison with the pancreas 7 days after ligature, in which islets were with difficulty distinguished at all, renders inevitable the conclusion that these islets are, at least in part, formed from portions of the alveolar tissue of the pancreas, in the course of the changes following ligature of the duct.

In no case was I able to observe the proliferation of duct epithelium, with nuclear mitosis, described by SSOBOLEW and LAGUESSE, and connected by the latter with a formation of new islets.

It is of interest to note that a formation of islets from alveolar tissue, similar to that which I have described, was observed by M. B. SCHMIDT in a case of obstruction of the human pancreatic duct by a calculus. In this case the obstruction was associated with severe diabetes. He observed similar changes as the result of senile atrophy.

#### *xi. Summary and Discussion.*

The results of this investigation may be summarised as follows :—

1. The islets of Langerhans are not independent structures of separate origin to the rest of the pancreas, but are formed by certain definite changes in the arrangement and properties of the cells of the ordinary secreting tissue. The changes are of such a kind as to assimilate all the cells to those forming the epithelium of the ductules and the centro-acinary cells, thus bringing about a reversion to embryonic type. The lumina disappear in this process, and all the cells are brought into more intimate relation with the blood capillaries. Such changes have been observed both in mammals and Amphibia.

2. In the pancreas of the toad some evidence was found of cell-multiplication in the islets, and of reconstruction of alveoli from them. Such evidence is at present wanting in the case of mammals.

3. The change from the secreting to the "islet" condition is greatly accelerated, both in mammals and Amphibia, by exhaustion of the gland by means of secretin. True exhaustion of the mammalian gland was not found possible unless the animal was also bled. This suggests that secretin stimulates both anabolic and katabolic activity of the pancreatic cells, and that anabolism must be otherwise depressed if the exhaustion effect is to be produced.

4. The proportion of islet tissue to secreting tissue is also increased by prolonged fasting. In other words, disappearance of the stored material of the secretory cells, whether by discharge into the duct, to produce the secretion, or by absorption into the blood and lymph, when the nutrition of the body fails, is attended by increased formation of islets from secretory alveoli.

5. Occlusion of the duct causes a disappearance of most of the pancreatic tissue in the course of a few weeks. That which escapes destruction assumes a form

resembling the islets, but the already existing islets exhibit no special immunity from the destructive effects of the operation.

Several points still require elucidation. In the first place, the evidence of reconstruction of alveoli from islets is as yet inadequate.

It is quite evident that, if islets are in constant process of formation from alveoli, there must be a constant disappearance of islets and new formation of alveoli to maintain the balance between the tissues. In the toad there is evidence of reconversion of islets into alveoli, and though there is no direct evidence of such a process in the mammal, it seems unlikely that the course of events should differ fundamentally from that in the toad.

The scattered and localised nature of the process of islet formation presents no real difficulty of explanation. It is quite in accordance, indeed, with the markedly different conditions of activity, apart from islet formation, which can be seen in different lobules, and in different and even neighbouring alveoli of the same lobule. In the normal pancreas the whole gland does not secrete simultaneously with equal vigour, or at least does not equally maintain, in all its alveoli, the balance between output and new formation of secretory granules. We have seen that by artificially producing a simultaneous exhaustion of the whole gland or a whole lobule, we get no longer a localised process of islet formation, but an approach to a conversion *en masse*.

The difficulty of the rich blood supply of the islets has already been discussed by PISCHINGER, who attributed it to a weakened resistance to injection—a lowering of capillary pressure, caused by shrinkage of the cells forming the islet. It is not necessary, however, to suppose that the change is merely one of this kind. The observations of the rich blood supply of the islets have been made on the full-formed islets of the resting gland. An islet like that shown in fig. 12, in the earliest stage of its formation, has a blood supply differing in no respect from that of the rest of the gland. Whether the development of the blood supply in such an islet as that in fig. 8 is due merely to widening of capillaries with the rearrangement and decrease in volume of the cells, or to new formation of capillaries, I have not been able to determine. The toads in which I hoped to study these changes, by keeping for some time after treatment with secretin, did not survive. No *à priori* impossibility, however, can be urged against either process.

In regard to the supposed internal secretion of the islets, these observations furnish no evidence in either direction. It may be, as LAGUESSE has suggested, that the passage from alveolar to islet form denotes a change of polarity in the cells, which, having previously secreted into the lumen, now secrete into the blood vessels. I have never been able to discover the "endocrine" granules, which he and others have described, and it might be suggested with equal plausibility that the process of *formation* of the islets, involving the absorption of zymogen granules, nuclear chromatin and other cell-constituents, is the essential factor in the internal secretion of the pancreas. Be that as it may, the one function of the islets which is indicated



by these actual observations is that of pancreatic growth, the islet stage in the toad, at any rate, being that in which cell-multiplication takes place—an observation which tallies with the embryological account given by LAGUESSE.

The pathological observations, on the selective action of certain forms of inflammation and degeneration, need not indicate more than that pancreatic tissue in the islet stage shows weakened resistance to certain morbid processes.

The evidence as to the relation of such changes to diabetes is at present too inconsistent to do more than furnish support to preconceived theories of the function of the islets.

In conclusion I wish to express my gratitude to Professor STARLING for kind permission to work in his laboratory. I am deeply indebted to him, and also to Dr. BAYLISS, for constant advice and personal assistance, and for the supply of much valuable material.

#### FIGURES.

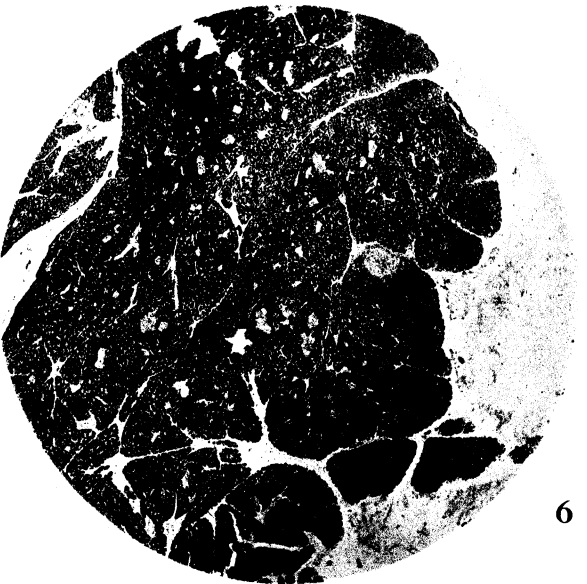
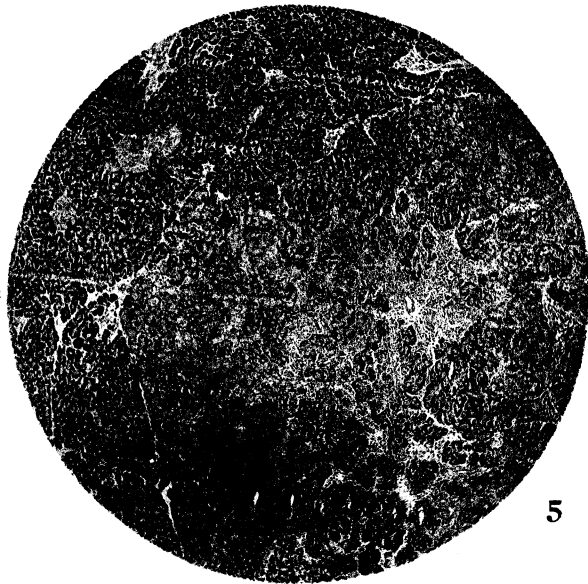
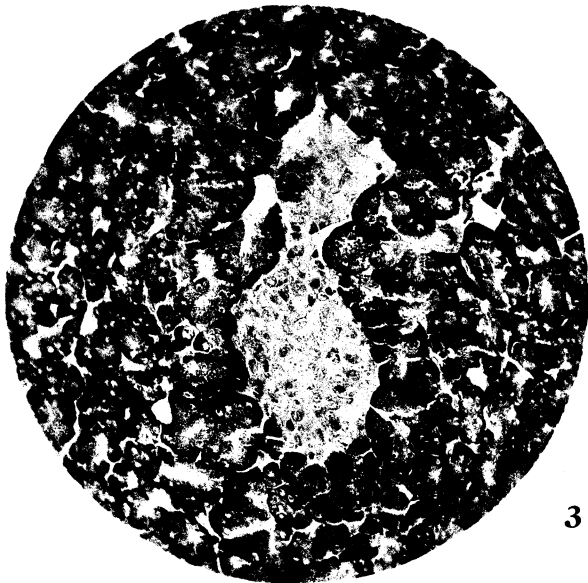
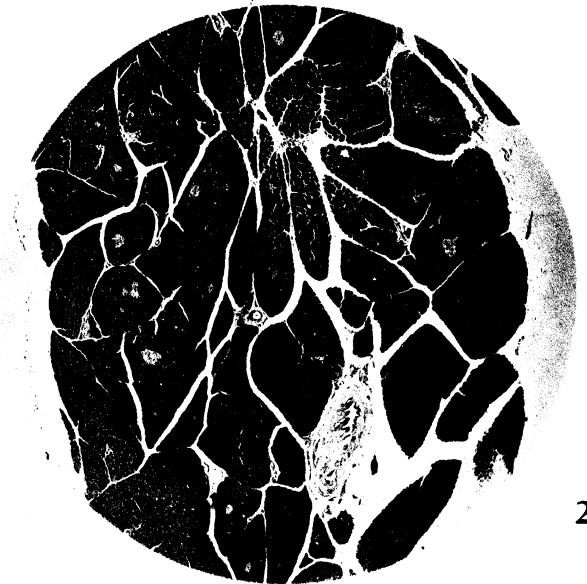
Where not otherwise stated the preparations were hardened in the corrosive sublimate and formaldehyde mixture, and stained with eosin and toluidene-blue.

#### PLATE 2.

- Fig. 1. Pancreas of dog, splenic end, resting.  $\times 18$ .
- Fig. 2. Pancreas of cat, splenic end, resting, corrosive.  $\times 18$ .
- Fig. 3. Islet of Langerhans, pancreas of dog, resting.  $\times 225$ .
- Fig. 4. Pancreas of dog, exhausted by secretin, splenic end.  $\times 18$ .
- Fig. 5. Pancreas of dog, exhausted by secretin, splenic end.  $\times 36$ .
- Fig. 6. Pancreas of cat, nearly exhausted by secretin, splenic end.  $\times 18$ .

#### PLATE 3.

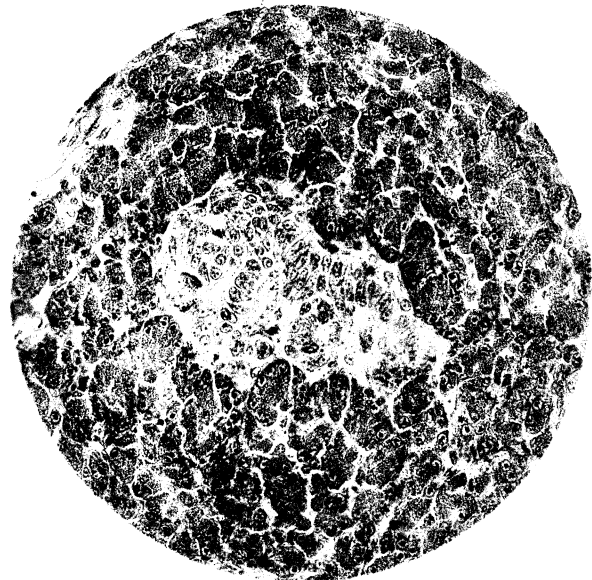
- Fig. 7. Pancreas of cat, starved.  $\times 16$ .
- Fig. 8. Islet of Langerhans, resting pancreas of toad.  $\times 225$ .
- Fig. 9. Pancreas of toad, resting.  $\times 45$ .
- Fig. 10. Pancreas of toad, starved.  $\times 45$ .
- Fig. 11. Pancreas of toad, exhausted by secretin.  $\times 40$ .
- Fig. 12. Islet of Langerhans, active formation, pancreas of toad exhausted by secretin.  $\times 250$ .



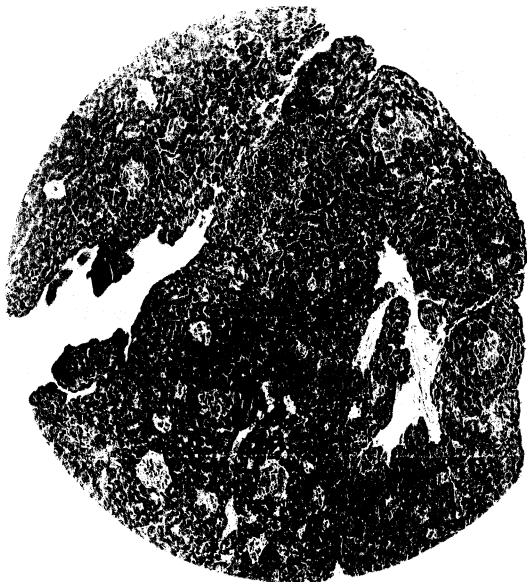




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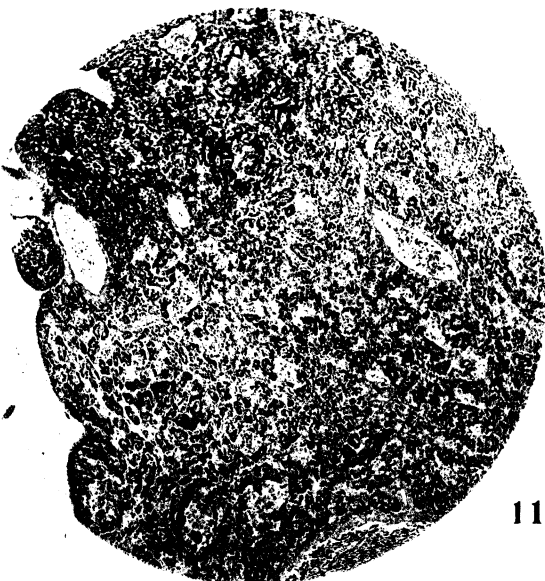
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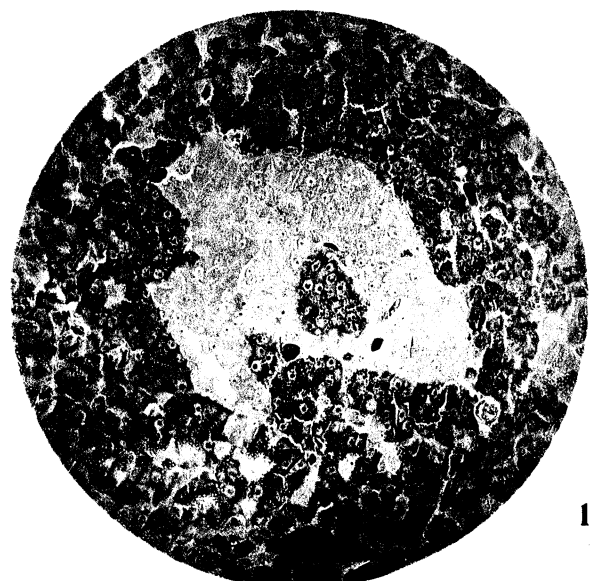
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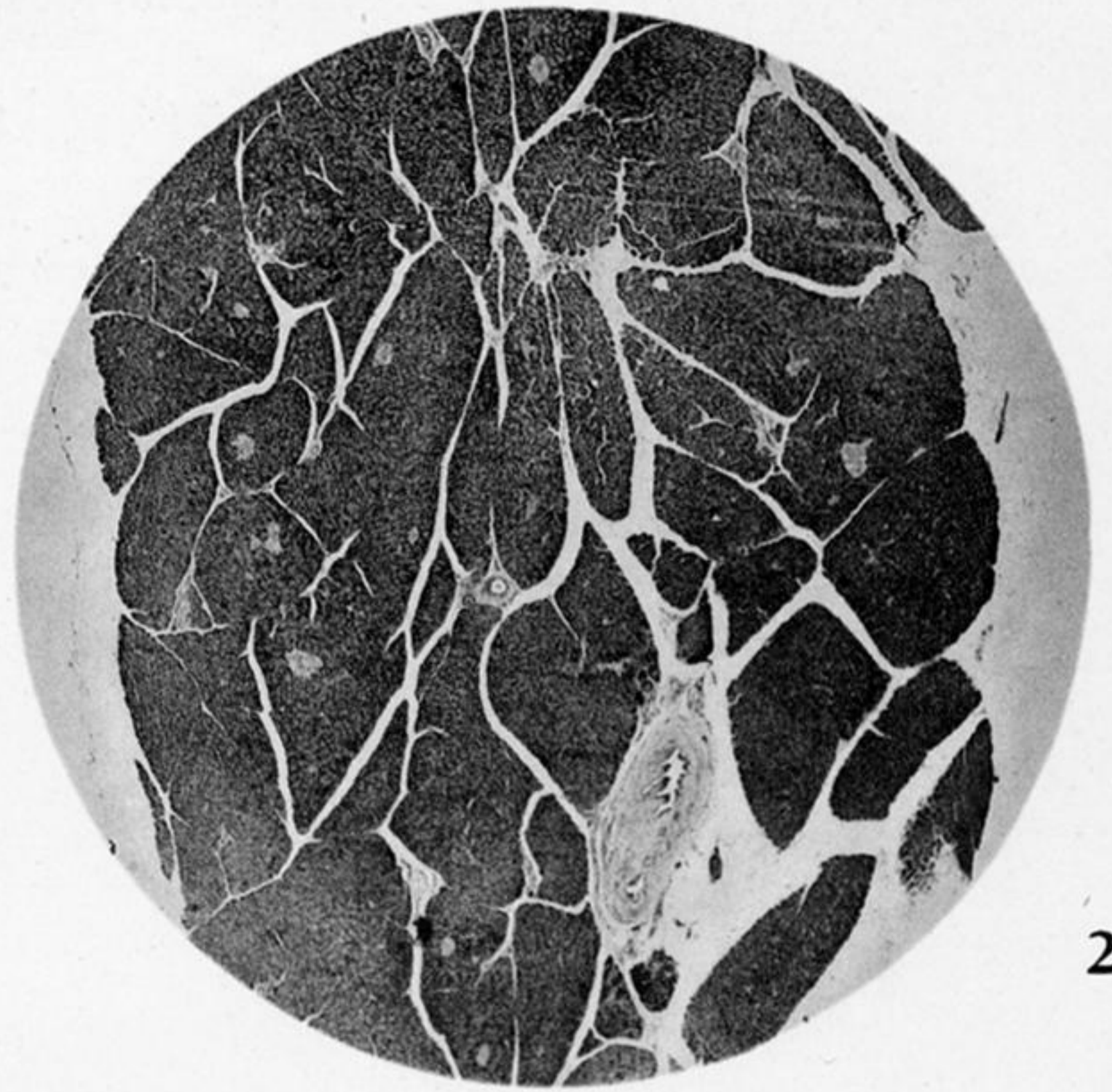
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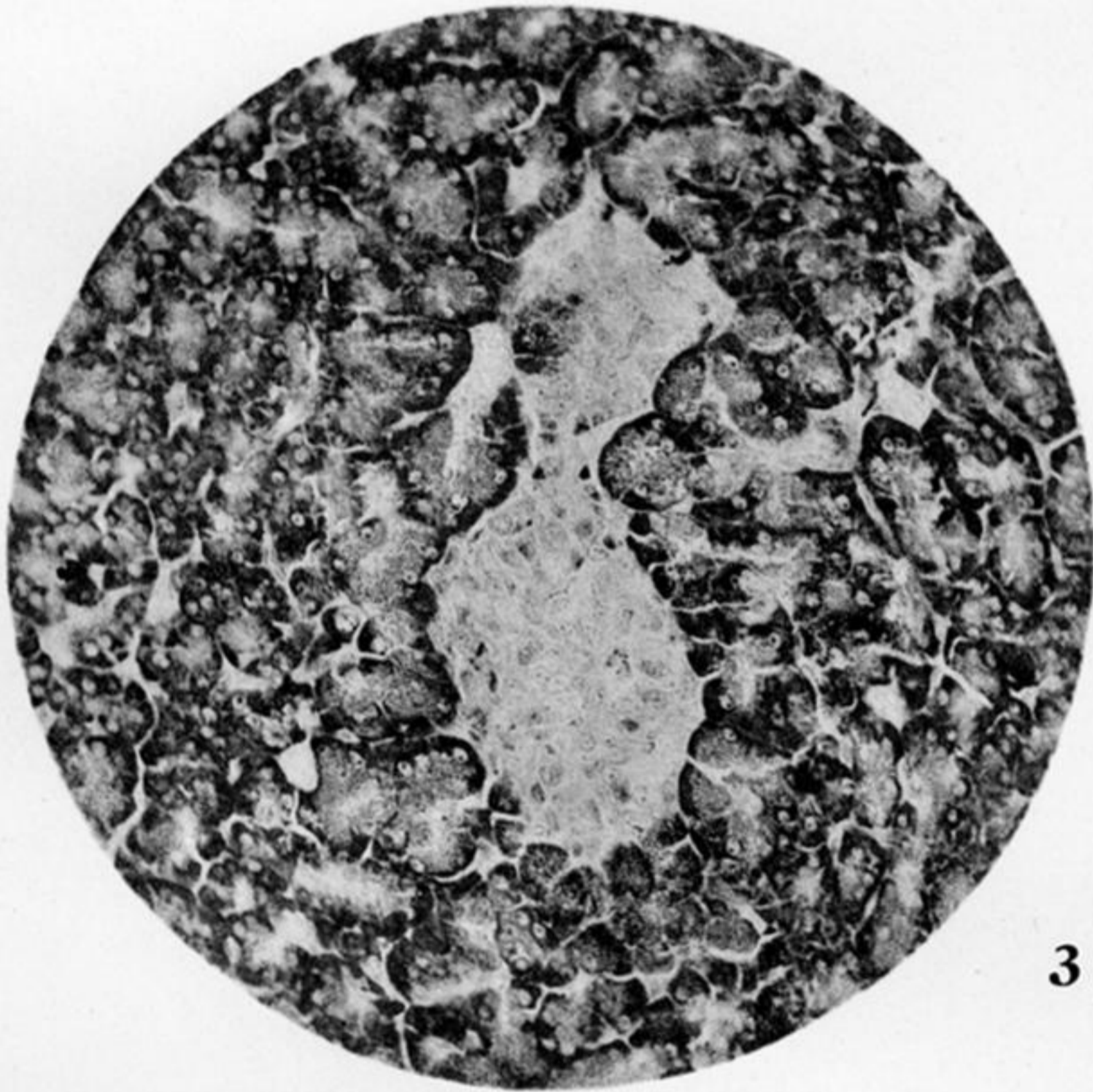




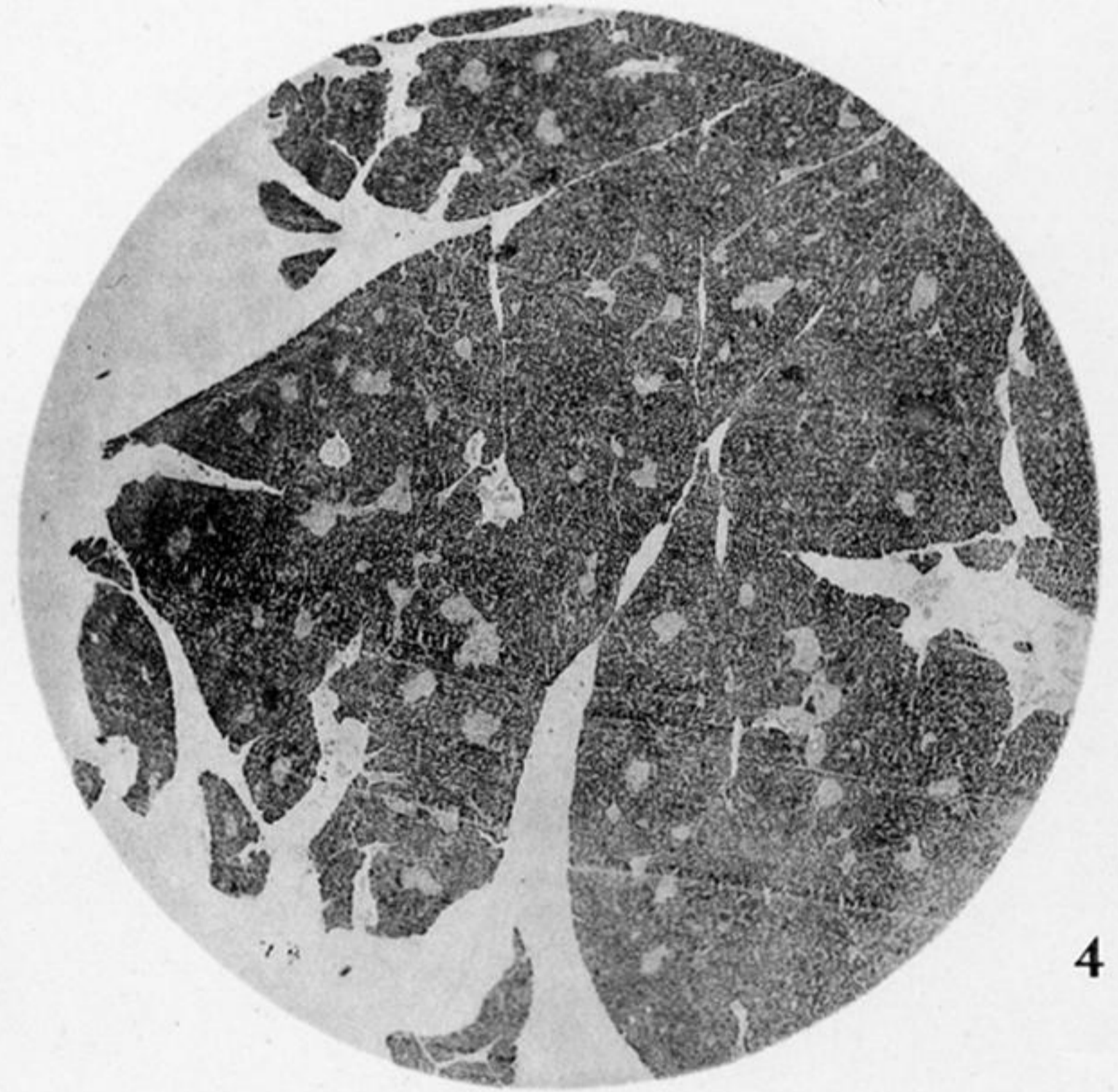
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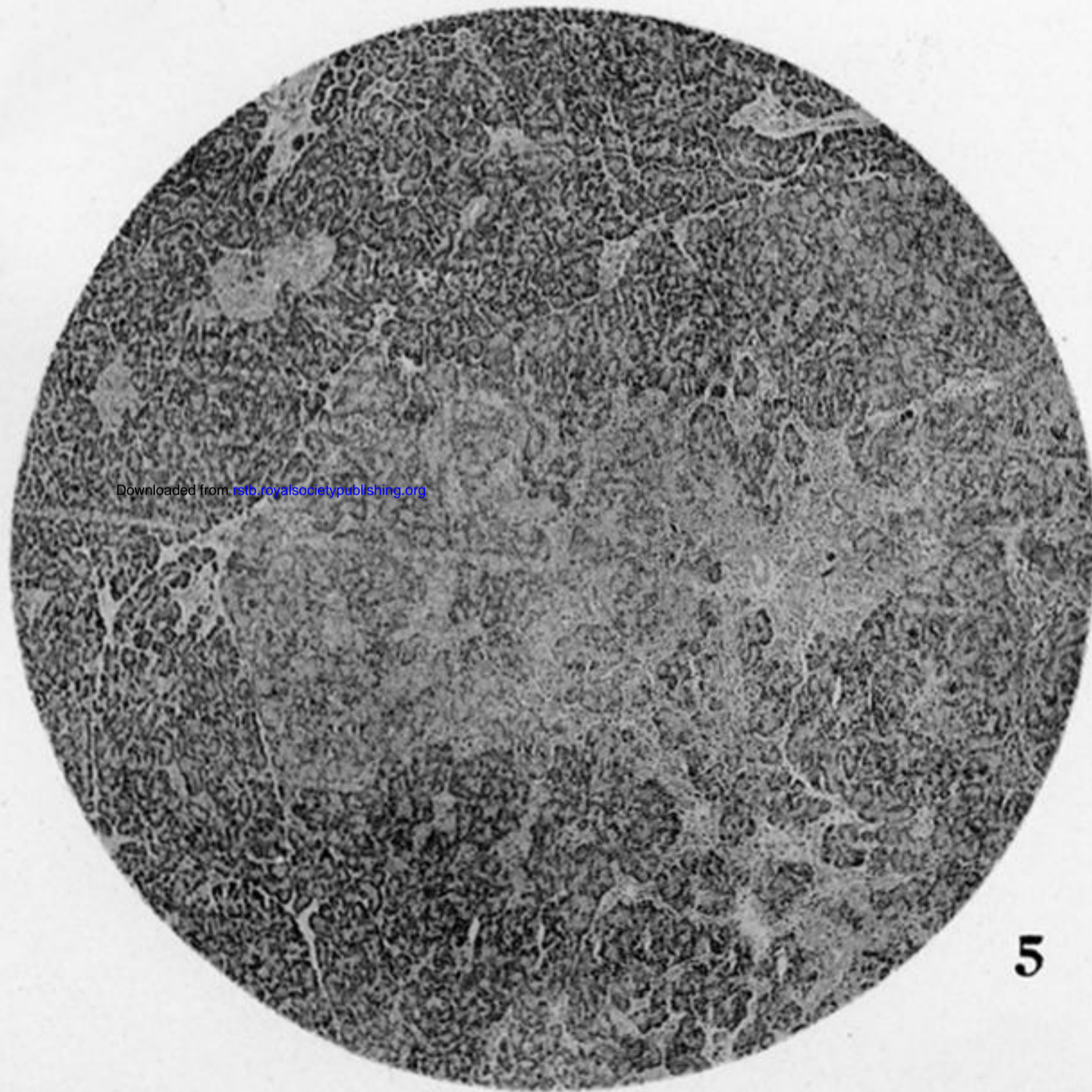
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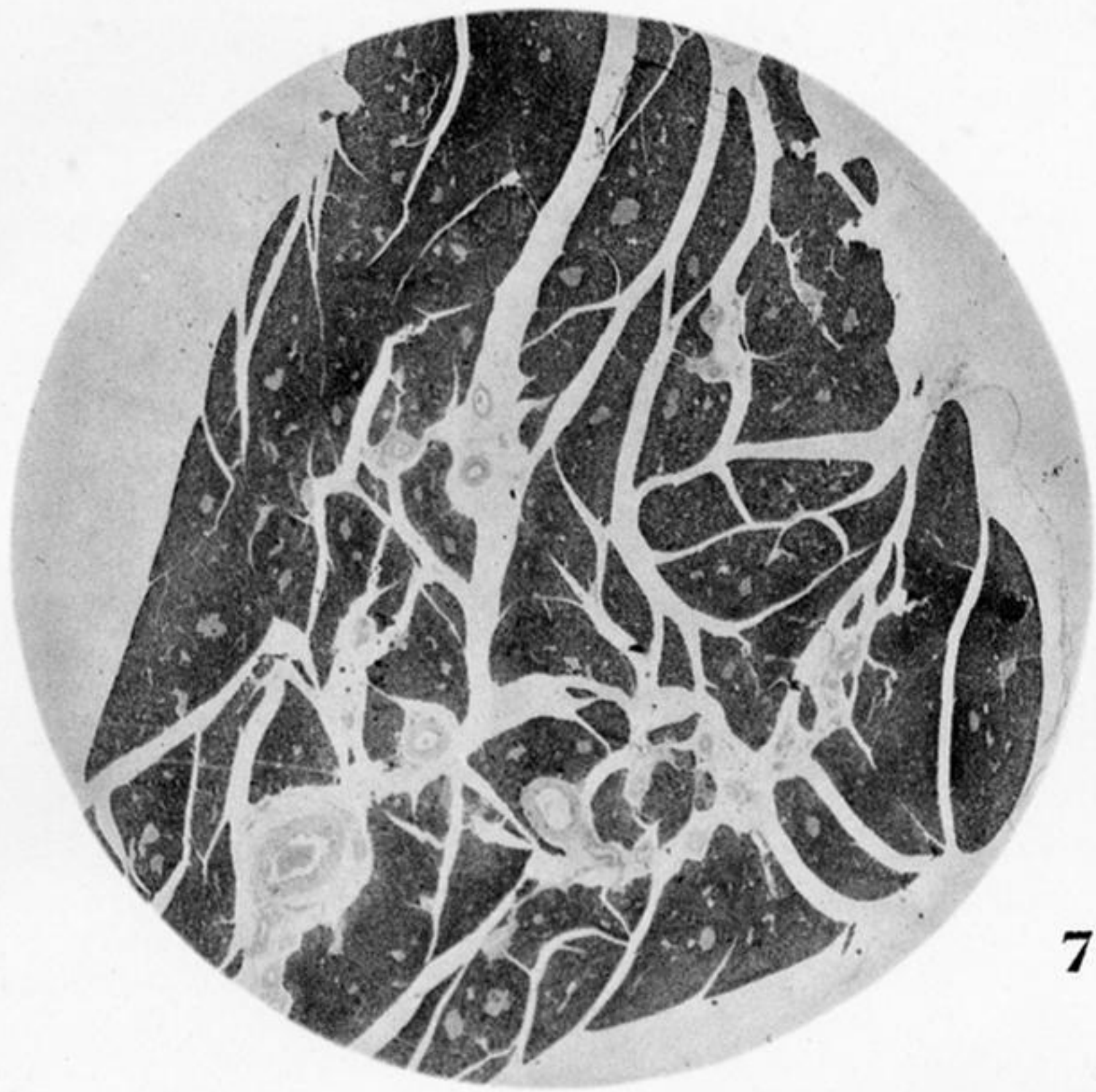


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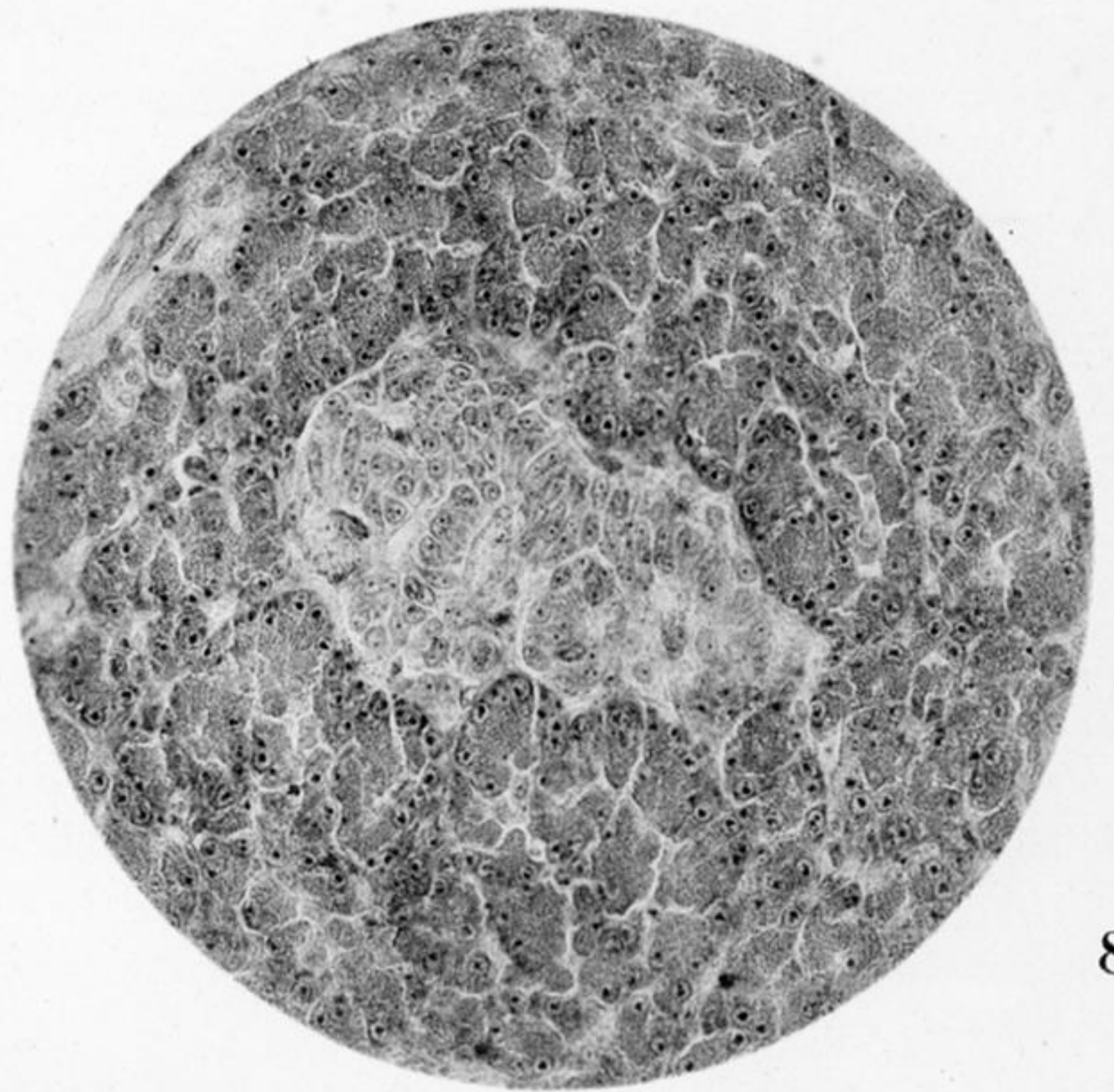
PLATE 2.

- Fig. 1. Pancreas of dog, splenic end, resting.  $\times 18$ .  
Fig. 2. Pancreas of cat, splenic end, resting, corrosive.  $\times 18$ .  
Fig. 3. Islet of Langerhans, pancreas of dog, resting.  $\times 225$ .  
Fig. 4. Pancreas of dog, exhausted by secretin, splenic end.  $\times 18$ .  
Fig. 5. Pancreas of dog, exhausted by secretin, splenic end.  $\times 36$ .  
Fig. 6. Pancreas of cat, nearly exhausted by secretin, splenic end.  $\times 18$ .

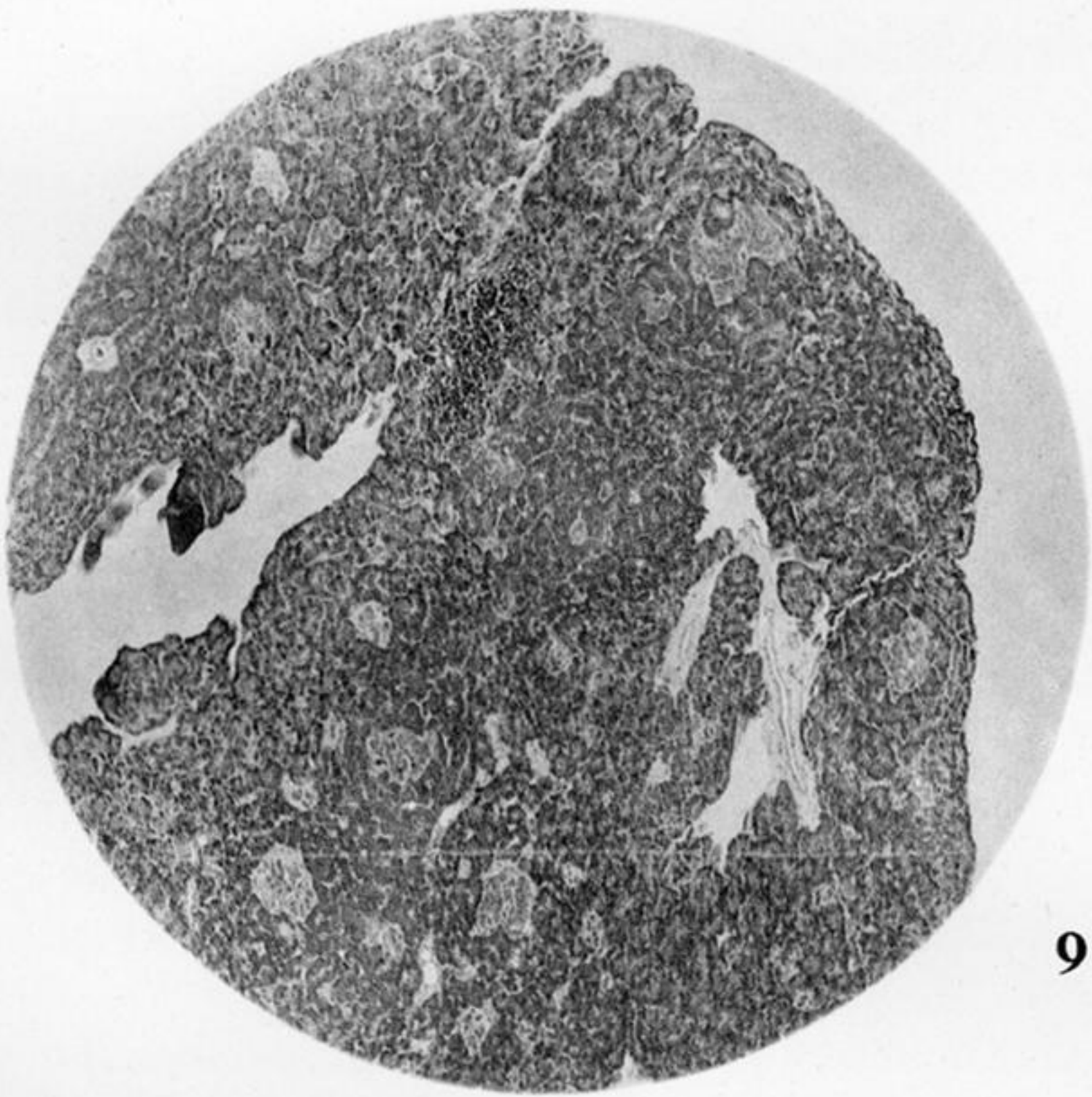




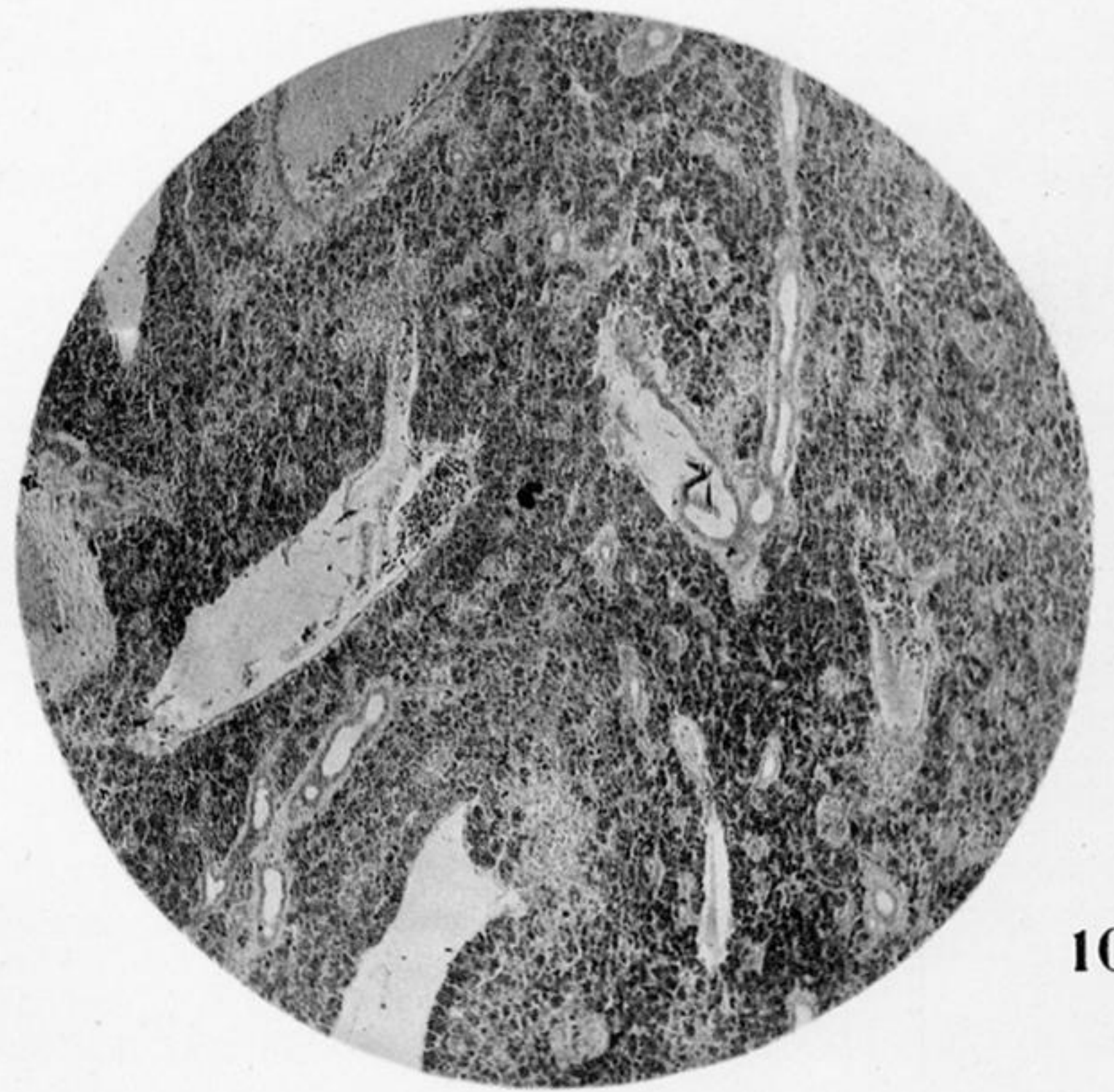
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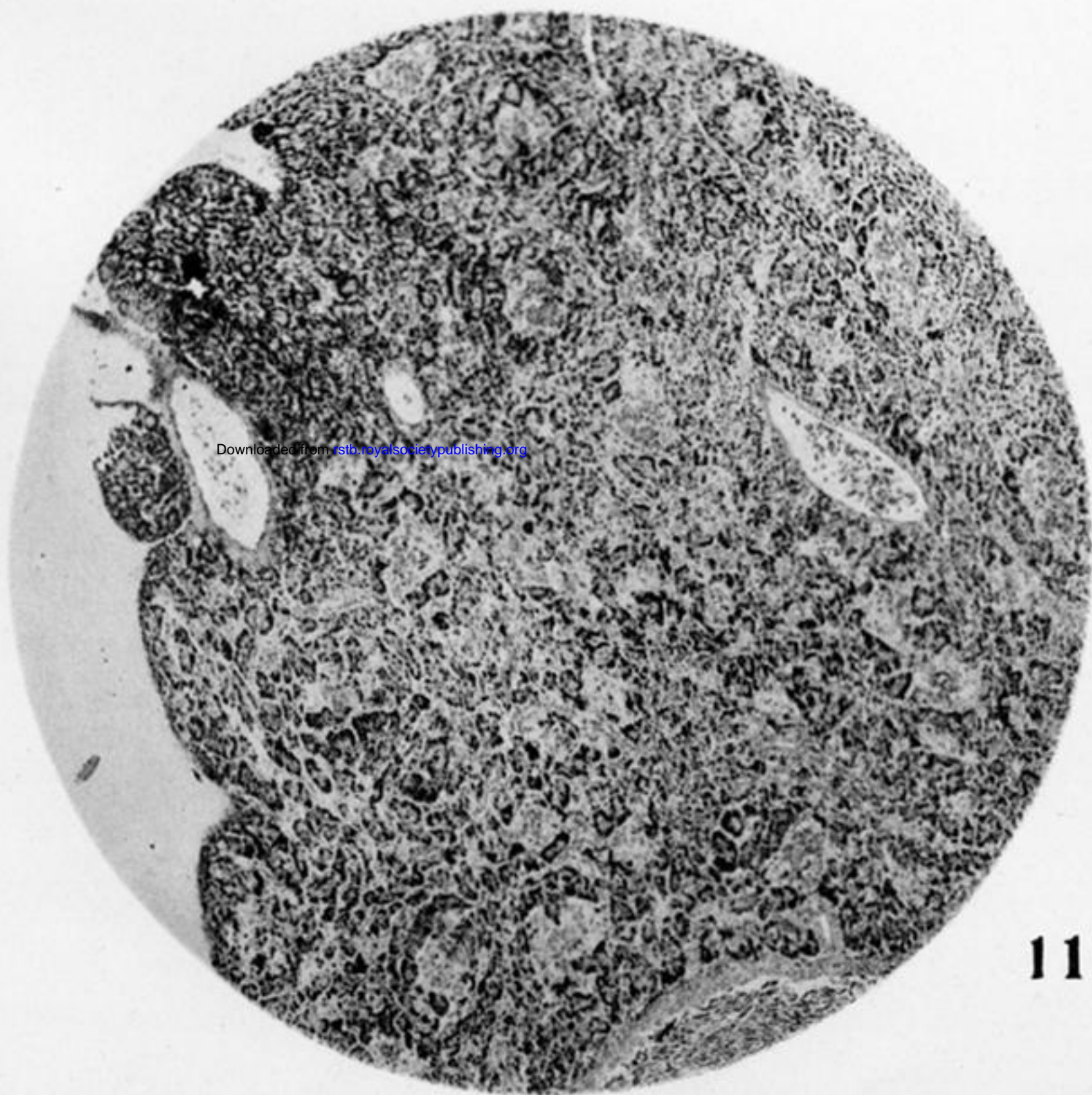
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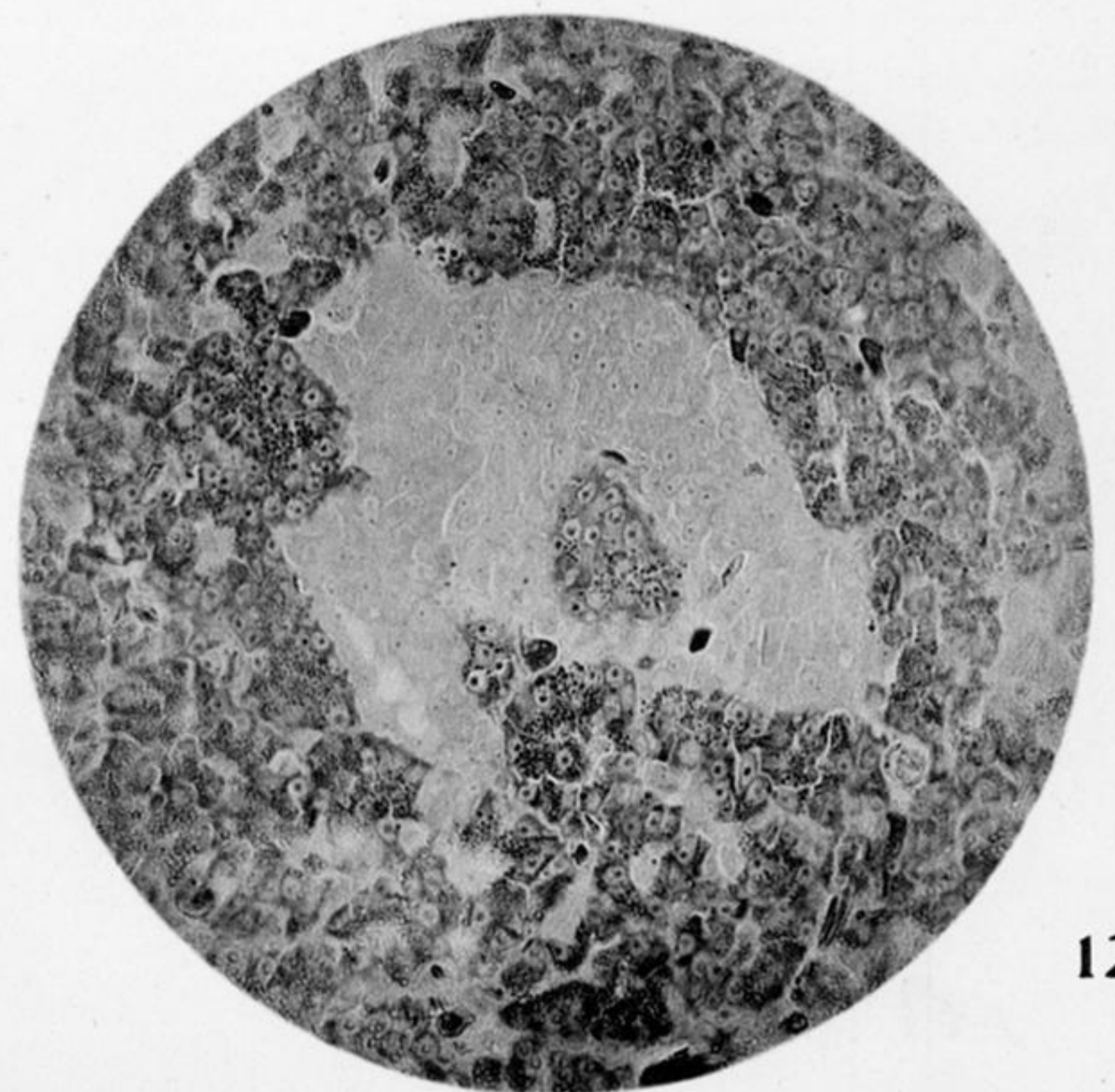
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PLATE 3.

- Fig. 7. Pancreas of cat, starved.  $\times 16$ .  
Fig. 8. Islet of Langerhans, resting pancreas of toad.  $\times 225$ .  
Fig. 9. Pancreas of toad, resting.  $\times 45$ .  
Fig. 10. Pancreas of toad, starved.  $\times 45$ .  
Fig. 11. Pancreas of toad, exhausted by secretin.  $\times 40$ .  
Fig. 12. Islet of Langerhans, active formation, pancreas of toad exhausted by secretin.  $\times 250$ .