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## THE EXPERIMENTAL PRODUCTION OF FAT NECROSIS: FAT NECROSIS ABOUT THE PANCREAS OF THE HOG.

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THE relationship of fat necrosis to affections of the pancreas, and especially to pancreatitis, has been demonstrated by Fitz,<sup>1</sup> but the significance of this connection is not clear. The essential change in fat necrosis is a decomposition of the neutral fat molecule into its component fatty acid and glycerine. Frequently, if not always, the fatty acid unites with calcium to form a new compound, which is a soap. It is remarkable that the fat-splitting ferment of the pancreas accomplishes this very decomposition of neutral fats. Certain experiments reported within the last few years seem likely to put us in the way of demonstrating the exact nature of the connection between fat necrosis and affections of the pancreas.

Hlava,<sup>2</sup> excited a hemorrhagic pancreatitis with fat necrosis in a cat by injecting the diphtheria bacillus into the pancreas after laparotomy.

Langerhans,<sup>3</sup> suggested that the decomposition of neutral fats which occurs in this process might be accomplished by the fat-splitting ferment of the pancreas. He announced that he had succeeded in producing fat necrosis in a rabbit. This was the only positive result among twelve animals (rabbits and dogs) experimented upon. He made use of a suspension of rabbit's pancreas in distilled water, which was injected into the adipose tissues. His positive experiment is open to the objection that bacterial contamination was not excluded with certainty.

Hildebrand,<sup>4</sup> and his student, Dettmar, placed a ligature about the gastro-splenic portion of the pancreas, to prevent the discharge of its secretion through the duct, in two cats; and in six others they performed the same operation and also ligated the veins leaving the organ. In all cases fat necrosis was found about the pancreas. They were also successful in producing it three times by introducing portions of the pancreas of one cat into the abdominal cavity of another. In one instance they obtained it after removing a piece from the organ and leaving the distal portion without ligature. Hildebrand, furthermore, injected pure trypsin into the abdominal cavity, and found that hemorrhages into the peritoneum resulted. He suggested that the hemorrhages, so frequent in pancreatic affections, might be due to the action of trypsin, while the fat-splitting ferment was responsible for fat necrosis.

<sup>1</sup> Fitz: Proceedings New York Pathological Society, 1890. Boston Medical and Surgical Journal, 1889. Medical Record, 1889. Medical News, Philadelphia, 1889. Transactions Association American Physicians, 1890.

<sup>2</sup> Hlava: Sborník Lékařský (Archives bohèmes de médecine) vol. iv, 1890 (synopsis in French).

<sup>3</sup> Langerhans, R: Experimenteller Beitrag zur Fettgewebsnekrose. Festschrift, R. Virchow, Berlin, 1891.

<sup>4</sup> Hildebrand: Centralblatt für Chirurg., 1895, vol. xxii, p. 297; also report Cong. Deutsche Gesellsch. für Chirurg., same journal. Dettmar: Inaug. Diss., Göttingen, 1895.

Dr. Whitney, of the Harvard Medical School, allows me to state that he ligated the pancreas in a number of dogs and produced fat necrosis in one of them. No account of his work has been published.

Rosenbach, and his pupil Jung,<sup>5</sup> with a similar object in view, introduced trypsin and at other times portions of pancreas, into the abdominal cavities of rabbits. Out of four trials with pieces of pancreas they obtained fat necrosis once, using dog's pancreas.

As the work of Hildebrand has seemed to have been the most productive of results, the writer has attempted to verify his conclusions. The animals selected were two dogs, one rabbit, and seventeen cats. They were anesthetized with ether. The operations consisted in the placing of a ligature about the gastro-splenic portion of the pancreas near the duodenum, or the duodenal portion of the pancreas, or both. In most cases as many as possible of the veins leaving the pancreas were also tied. In the majority of cases a solution of continuity in the pancreas was effected. In one instance the gastro-splenic portion of the pancreas was cut through on the distal side of the ligature. An aseptic technique was followed, and the results were good with the exceptions noted below. The peritoneum and skin were closed separately with silk sutures. The wounds in the abdominal walls frequently furnished slight purulent discharges. Owing to the freedom allowed to the animals it was found impossible to secure primary union in every instance. Animals that died during the first twenty-four hours after operation were not included in this report.

The operations and their results in detail were as follows:

Dog. Weight 7 lbs., 1½ oz. A silk ligature was tied about the gastro-splenic portion of the pancreas. The animal, while apparently in perfect health, was killed at the end of one week; weight 8 lbs., 6½ oz. There was no peritonitis and no fat necrosis.

Dog. Large, fairly nourished. The gastro-splenic portion of the pancreas was ligated. The dog was killed after ten days, somewhat emaciated. There were adhesions and suppuration about the ligature, but no general peritonitis. There was no fat necrosis.

Rabbit. Moderately fat. The gastro-splenic portion of the pancreas was ligated. The animal remained in good condition, and was killed after three weeks. There was no peritonitis. There were traces of fat necrosis close to the ligature.

From the seventeen cats there were ten negative results. In two there were minute areas probably of fat necrosis. In five there was well-developed fat necrosis.

<sup>5</sup> Jung: Inaug. Diss., Göttingen, 1895. Ref. in Centralblatt für Chirurg., 1895, vol. xxii, p. 310.

One of the five exhibited disseminated fat necrosis. In detail, the operations and results were as follows:

Cat 11. Weight 6 lbs., 14 oz. The gastro-splenic portion of the pancreas was ligated. The animal was killed at the end of one week; weight 6 lbs., 5 oz. There was no fat necrosis nor peritonitis.

Cat 8. Weight 4 lbs., 1 oz., but fat. The gastro-splenic portion of the pancreas was ligated. The animal, while apparently healthy, was killed after one week; weight 4 lbs., 2½ oz. There was no peritonitis. Small nodules of fat necrosis were discovered at the interlobular pancreatic fat and in the omental fat. There was one nodule in the perirenal fat (Fig. 1), and one in the mesorectum. No bacteria could be demonstrated by staining methods in or near these areas.

Cat 6. Weight 7 lbs., 10 oz., well nourished. The gastro-splenic portion of the pancreas was ligated. The cat was killed after two weeks; weight 6 lbs., 13 oz. There was no fat necrosis nor peritonitis.

Cat 7. Weight 7 lbs., 4 oz., fat. The gastro-splenic portion of the pancreas was ligated. The cat was killed after three weeks; weight 5 lbs., 13½ oz. A few minute spots, probably of fat necrosis, were found in the omental fat. Staining methods showed no bacteria in or about them. There was no peritonitis. This was one of the two cases classed as doubtful.

Cat 4. Weight 4 lbs., 8½ oz., poorly nourished. The gastro-splenic portion of the pancreas was ligated. The cat remaining healthy was killed after four weeks; weight 4 lbs., 2½ oz. There was no fat necrosis nor peritonitis.

Cat 3. Weight 6 lbs., 3 oz., fat. The duodenal portion of the pancreas was ligated. The cat was killed after one week, then seeming to be in good condition. There was no peritonitis. Traces of fat necrosis appeared close to the ligature. This was the second of the two cases classed as doubtful.

Cat 13. Weight 5 lbs., 15½ oz. Both the gastro-splenic and the duodenal portions of the pancreas were ligated, and as many as possible of the veins leaving the gastro-splenic portion were tied, and the continuity of the tissue of the pancreas was broken by a sharp hook passed into it beneath the peritoneum. The animal died after four days, being much emaciated. The autopsy was unavoidably delayed till about thirty-six hours after death. There was no peritonitis. There were several thin, flat areas of fat necrosis on the surface of the omentum. The mucous membrane of the pyloric end of the stomach showed a number of small round excavations having all the characteristics of peptic ulcers. All the peritoneal surfaces were abundantly covered with large bacilli, stained by Gram's method. No relation in their distribution to the spots of fat necrosis could be made out. Similar bacilli were present in large numbers on the mucous membrane of the stomach and duodenum, extending into the subperitoneal tissues and about the pancreas. In view of the long time that elapsed before the autopsy was made they were not regarded as of importance.

Cat 18. Both the gastro-splenic and the duodenal portion of the pancreas were surrounded with ligatures, as well as the veins leaving the former. The cat died after forty-eight hours. There was no fat necrosis nor peritonitis.

Cat 19. The operation and result were similar to those in Cat 18.

Cat 21. Weight 4 lbs., ½ oz. The gastro-splenic portion of the pancreas was ligated and as many as possible of the veins issuing from it, and the substance of the organ was broken with a hook. The animal died after four days; weight 3 lbs., 11½ oz. The autopsy was made in less than twenty-four hours. The peritoneal surfaces were covered with a fibrinous exudate. Beneath the peritoneal exudate a few thin, flat, white areas were seen, which proved to be necrotic adipose tissue. The exudate contained great numbers of diplococci and fewer large bacilli, both staining by Gram's method. The diplococci alone were recovered in cultures. They grew feebly, not liquefying gelatine, and

very quickly died out. In sections, no grouping of the micro-organisms with reference to the spots of fat necrosis could be demonstrated.

Cat 22. Weight 4 lbs., 13½ oz., not fat. The gastro-splenic portion of the pancreas and the veins leaving it were ligated, and its substance was injured as in the other cases. The wound in the abdominal wall suppurred. The cat was killed on the fifth day after the operation; weight 4 lbs. At the autopsy, which was made immediately, the writer was astonished to find in the abdominal cavity a condition of disseminated fat necrosis comparable to that occurring in man. In the vicinity of the pancreas, the retro-peritoneal and omental fat was swollen, white and opaque, over an extent reaching twelve millimetres or more from the pancreas, and radiating from it in irregularly shaped masses. Smaller areas of similar character were scattered in great numbers through the omental fat and that of the mesentery along its entire length, even to the mesorectum. They occurred also in the perirenal fat. There was no peritoneal exudate. The animal was nevertheless the subject of an infection, proceeding no doubt, from the wound in the abdominal wall. The pus in this wound contained a variety of organisms, among them diplococci and large bacilli. Although examination of cover-slips was negative, cultures from the spots of fat necrosis and from the liver yielded diplococci or short streptococci, growing at room temperature, not liquefying gelatine, forming minute white circular colonies on agar, and staining by Gram's method. Cultures from the pancreas and the blood of the right heart were negative. The distribution of the necrotic spots, especially of those in the mesentery, made them appear suspiciously like minute abscesses, but the microscope revealed their true character. In a section of the mesentery, diplococci were found in and about one of the areas of fat necrosis, and also, in smaller numbers, at points in the mesentery remote from it. Otherwise, the examinations of sections of the necrotic areas for bacteria were negative.

Cats 27, 29, 31, 32 and 36 were good-sized and healthy. In each case the splenic portion of the pancreas was constricted by a ligature near the duodenum, as many as possible of the veins leaving it were tied, and the tissue of the pancreas was disturbed as in the other cases. The animals all made good recoveries. Their abdomens were opened at periods varying from eight to seventeen days. There was no peritonitis, and no fat necrosis in any case.

Cat 34. Large and rather fat. The splenic portion of the pancreas was ligated near the duodenum, and the pancreas was then cut completely through on the distal side of the ligature, with the intention of allowing its secretion to flow into the peritoneal cavity. The animal died at the end of a week. The autopsy was made eighteen hours after death. The peritoneal sutures were found to have given way. The abdominal cavity contained a quantity of pinkish-white turbid fluid. The peritoneal surfaces were congested and covered with a thick layer of fibrin. The fat tissue of the omentum appeared swollen and edematous. Irregular white patches of necrotic fat tissue were seen in the omentum and in the vicinity of the splenic portion of the pancreas, especially near the cut extremity. Smears made from the peritoneal exudate showed it to contain many large bacilli, and minute cocci. Cultures made from the peritoneum, the viscera, and the blood gave only minute white colonies of diplococci, staining by Gram's method, lancet-shaped, and like the diplococcus of pneumonia in form. Sections of the necrotic fat tissue and of the pancreas exhibited great numbers of large bacilli and diplococci, by the Gram-Weigert method, mostly on the surfaces, and not showing any special relation to the areas of fat necrosis. Sections of the pancreas demonstrated necrosis of the fat cells about it and in the interlobular septa in a striking manner.

In all of the animals studied, the diagnoses were based on the microscopic examination of the tissues, which is indispensable. Many pictures of great beauty illustrating the condition were obtained. The tissues

hardened in alcohol were found to be very well adapted for histological purposes. A five-per-cent. solution of formalin in normal salt solution preserved the macroscopic appearances more satisfactorily, but the writer has given up using it when the minute structure is to be studied. One-per-cent osmic-acid solution makes an excellent hardening agent, as the necrotic areas are not stained black by osmic acid. The areas of fat necrosis were sometimes rounded and nodular, about one to two millimetres in diameter; sometimes they were broad and thin. They occurred exclusively in the fat adjacent to the peritoneum, usually in immediate contact with it. A disposition of the necrotic process to affect the vicinity of the ligatures about the pancreas or the veins was noted, but it was not constant or very marked. In recently killed animals the recognition of the areas was easy, owing to their opacity, contrasting with the relatively transparent normal fat. When a peritoneal exudate was present, it was often difficult to recognize them beneath the layer of fibrin.

In frozen sections the areas were opaque, contrasting with the neighboring fat (Fig. 2). A brown tinge was often visible. Such complete disorganization of the adipose tissue as occurs in the human subject was in no instance observed. The areas consisted of rounded bodies similar in outline to the fat cells. In most cases the contents of these bodies were minute needle-shaped crystals. Frequently the crystals were arranged in a radial manner at the circumference of the circle. The central portion was then empty or contained oil droplets. Such bodies in transverse section appeared as rings (Fig. 4). Calcium salts were demonstrated in some nodules in abundance. The contents of the necrotic cells in balsam preparations usually appeared homogeneous, although their crystalline nature was sometimes discernible. They reacted variously with stains, in some instances showing an affinity for eosin, in others staining deep blue with hematoxylin. The more intense hematoxylin stain noted in some nodules the writer supposes to have been due to an abundance of calcium salts. The nuclei of the necrotic cells could not be identified. The amount of cell infiltration about the nodules of fat necrosis was quite variable. In some examples it was very slight, more often it formed a distinct band about the circumference where it was mingled with granules most of which stained deeply with nuclear dyes. Frequently it passed widely into the surrounding tissues. The cells were in large part polynuclear leucocytes. In part they were larger, mononuclear elements rounded or spindle-shaped in form, with round or oval nuclei. They generally were of moderate size, though quite variable in this respect. Fragmentation of nuclei was a prominent feature in this zone, the fragmentation being most extensive in the part immediately adjacent to the necrotic fat.

In and around the areas of fat necrosis numerous granules and rounded masses occurred, staining with carmine, hematoxylin, and by the Gram-Weigert method. Some of these were evidently fragmented nuclei; others were hyaline in character; others, which stained very deeply with hematoxylin, were supposed to contain calcium. These granules were often so numerous as to obscure the nuclei at the margin of the area. They rendered the search for bacteria in sections difficult and unsatisfactory.

The changes taking place in the pancreas were

studied only with reference to the question in hand, and the account of them must be brief. The ligatures about the pancreas were found enclosed in a zone of leucocytes or good-sized mononuclear cells, or both, and often also of fibrous tissue. The cell infiltration frequently passed into the interlobular connective tissue of the pancreas and over its surface. In the animals that were allowed to live longest there were marked atrophy and induration of the ligated extremity of the organ, which the microscopic examination showed to be due to an atrophy of the acini and an abundant formation of fibrous tissue between the lobules, and even between the acini. Desquamation of the epithelial lining of the ducts was of frequent but not invariable occurrence. There was less dilatation of the ducts on the distal side of the ligature than one might have expected to encounter. The writer is unable to say with confidence that there was more interstitial pancreatitis or any other characteristic morbid condition of the pancreas in the animals that showed fat necrosis than in those that did not.

In nearly all cases sections of the liver, spleen and kidneys were examined, but no alteration was discovered that appeared to have any relation to the question in hand. No tendency to hemorrhages of a marked or constant type was noted in any of the tissues. The urine was examined for sugar in the majority of cases, and none was found.

Although the writer has been less successful than Hildebrand, his work renders it evident that ligation of the pancreas in the cat may lead to fat necrosis. As far as he is aware, Cat 22 of his series exhibited the most extensive fat necrosis that has been recorded as having been produced by artificial means. The simultaneous existence of a diplococcus infection, in this animal and in Cats 21 and 34, is noteworthy. It is significant that the areas of fat necrosis were observed only in close connection with the peritoneal cavity, and especially in the neighborhood of the pancreas, which would make contact of the pancreatic juice with these areas intelligible. The circumscribed character which they usually exhibited and their occurrence at points remote from the pancreas are difficult to account for. It seems unjustifiable at present to say more than that extensive injury to the pancreas can cause fat necrosis.

The writer has been conducting experiments with a view to testing the direct action of the excised pancreas upon fat tissue. He believes that he has succeeded in producing fat necrosis in this manner. The conditions under which the change is effected are not yet clear, and any account of this work at present would be premature. A single one from this series may be related here, however, not to illustrate the direct action of the ferment artificially introduced, but to show the effects of a pancreatitis excited by accident.

Cat 15. Large and healthy. The abdomen was opened, a piece of pancreas 20 m. m. in length just removed from another cat was fastened to the omentum with a silk ligature, and the abdomen was closed. The animal died after six days. The autopsy was made twenty-four hours after death. A fibrinous exudate covered all the peritoneal surfaces. The effect of the piece of pancreas introduced upon the adjacent omentum was not clear. A small area of fat necrosis was discovered on the surface of the left kidney, and others were seen in the omentum. Section of the animal's own pancreas showed an acute pancreatitis, apparently originating by extension inwards from the in-

ected peritoneum, which the exposed condition of the pancreas in the cat makes possible. Fat necrosis was seen in a large part of the fat tissue in immediate contact with the inflamed pancreas, and the connection between the two was demonstrated in a convincing manner. Cover-glass preparations, sections and cultures showed a large bacillus and a small diplo- or strepto-coccus, both staining by Gram's method, in the peritoneal exudate, and in the pancreas.

In this case, the conditions seem to be practically the same as those obtained by Hlava when pancreatitis and fat necrosis followed from the injection of the diphtheria bacillus into the pancreas. The possibility of their occurring ought to be borne in mind when pieces of pancreas or pancreatic extracts are introduced into the peritoneal cavity in experimental studies.

In connection with this work the writer has examined the pancreas and peritoneal adipose tissues of about forty cats. Quite early one was encountered exhibiting spontaneously minute white spots in the omental fat, not near the pancreas however, which on section resembled closely very small areas of fat necrosis. Recently a similar condition has been found in a second cat (Cat 37).

The animal was very fat. The abdomen was opened with a view to operating on the pancreas, when the adipose tissue of the omentum was observed to contain about ten irregular, opaque, white areas, approximately one-fourth of a millimetre in diameter. They were not observed in the vicinity of the pancreas. Two of these areas and a small bit of the pancreas were removed for examination, and the abdomen was closed again. Sections of the pancreas exhibited nothing remarkable. One of the suspicious areas in the omental fat, under the microscope proved to be opaque; after slight pressure it broke into irregular, translucent masses with a brown tinge, and made of fine, radiating crystals. Upon the warm stage, after the addition of glacial acetic acid, the brown masses dissolved; at the same time there was a noticeable evolution of oil droplets, apparently from the brown masses. The small size of the suspected areas made removal beforehand of the free fat with boiling alcohol and ether impossible. Subsequent neutralization and addition of oxalic-acid solution, produced an abundant precipitate of calcium oxalate crystals. Thin sections stained in hematoxylin showed the area partly surrounded by good-sized mononuclear cells, which, along with a small amount of fibrous tissue, partly penetrated its interior.

The condition seemed to be one of fat necrosis arising spontaneously in the cat and probably not recent (Fig. 3). If it depended upon any morbid state of the pancreas, that had apparently subsided. The animal made a good recovery. It is now, after ten weeks, still healthy. The intention is to observe the progress of events after the lapse of a longer period.

#### FAT NECROSIS IN THE PANCREAS OF THE HOG.

Balser,<sup>6</sup> who was the first to describe fat necrosis in the human subject accurately, was also the first to study its appearance in the hog in detail. He found it in the fat tissue in or about the pancreas in nearly all Hungarian swine, frequently in Algerian, and in a few German swine. He found in the necrotic nodules bodies resembling the fungus of actinomycosis. In order to determine whether or not fat necrosis occurred in American hogs the writer examined the pancreas of one hundred hogs. It was impossible to learn anything more concerning the animals than that

they were raised either in Ohio, Indiana, Illinois or Michigan, and were apparently sound and healthy. The pancreas of the hog is surrounded by a quantity of adipose tissue, which is also abundant between the lobules. The fat cells are very large. Little white flecks consisting of single fat cells or groups of fat cells are often seen in the pink parenchyma. Not rarely one meets with dark red spots in the parenchyma, several millimetres in diameter, apparently the result of hemorrhage. The organ, taken as fresh as possible, was cut in slices one to three millimetres thick, and each slice carefully examined. In two cases fat necrosis was found.

In one of these the number of areas of fat necrosis was not large and they were confined to a limited region. In the other they were numerous and were scattered throughout the organ. They were not conspicuous, being distinguished by their more yellow color, which contrasted with the white, normal fat. They appeared much like minute abscesses, but were somewhat harder than the normal fat. In shape they were irregular. Their various dimensions were one to two millimetres only. They nearly always impinged on one side against a portion of the parenchyma. Frozen sections showed them to be made of fat cells, rendered opaque by the presence of numerous needle-shaped crystals, frequently arranged in the form of a ring about the circumference of the cell. They contained an abundance of calcium. Sections stained in hematoxylin and eosin gave about the appearances already described for the cat (Fig. 4). The contents of the areas exhibited usually a strong blue stain. The borders were surrounded by a band of connective tissue with numerous connective-tissue cells, and a small number of polynuclear leucocytes. Leucocytes also occurred in small numbers among the cells of the necrotic areas. Often they were in rounded clumps, corresponding to the outlines of a fat cell, and suggesting that they might have migrated into the interior of a necrotic cell. The ray fungus described by Balser after using the Ehrlich-Biondi stain was not found; nor were the hemorrhages about the necrotic areas. Examination of sections for bacteria was negative. Cultures were not made. The parenchyma of the pancreas was not remarkable. None of the other organs were examined.

#### SUMMARY.

Among two dogs, one rabbit and seventeen cats operated on in the manner described there were twelve negative, and three partly successful results. In five cases fat necrosis of a marked type followed. In three of the latter a diplococcus infection was associated.

A peritoneal infection in a cat was observed to lead to pancreatitis; which in turn produced fat necrosis.

Nodules, somewhat similar to those obtained artificially, which were seen twice appearing spontaneously in the omentum of the cat, were possibly old fat necroses.

Fat necrosis in and around the pancreas of the hog was found in two out of one hundred specimens examined.

#### EXPLANATION OF PLATES.

FIG. 1. Cat 8. Borax carmine. Fat necrosis in the perirenal fat, showing the outlines of the affected cells faintly, and cell-infiltration about the necrotic area (low power).

FIG. 2. Cat 22. Frozen section. Fat necrosis in the omentum, showing the opacity of the area, and the outlines of the altered cells in it (low power).

FIG. 3. Cat 37. Hematoxylin and eosin. Supposed area of fat necrosis in the omentum (x 80).

FIG. 4. Hog. Hematoxylin and eosin. Small area of fat necrosis close to the pancreas (x 80).

[The writer is indebted to Dr. F. C. Busch, Assistant in the Pathological Laboratory of the University of Buffalo, for these photomicrographs.]

<sup>6</sup> Balser: Verhandl. d. XI Cong. f. innere. Med., Wiesbaden, 1892, p. 450.

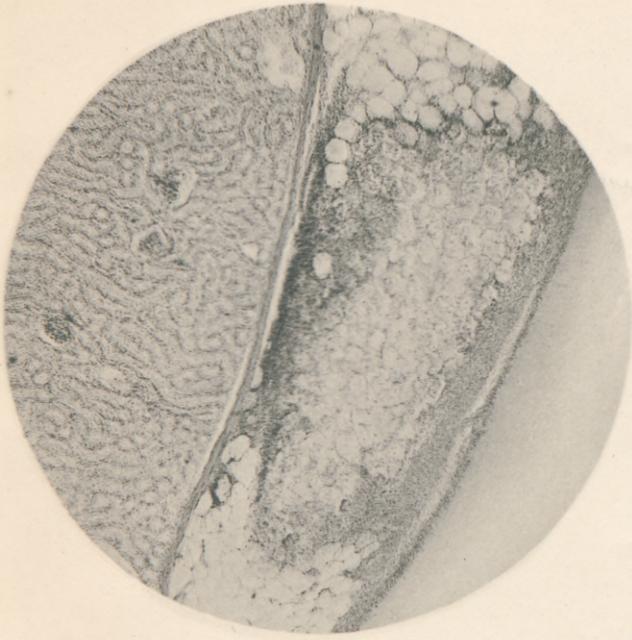


Fig. 1.



Fig. 2.

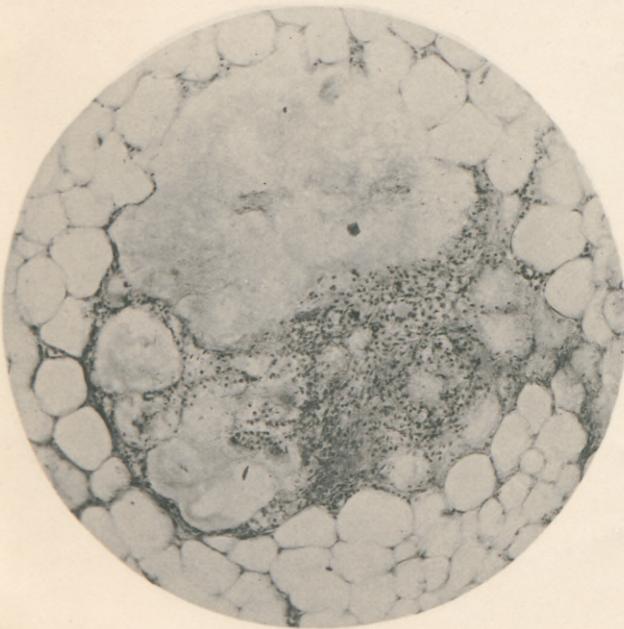


Fig. 3.



Fig. 4.





