

EXPERIMENTAL FAT NECROSIS: THE ACTION OF PANCREATIC TISSUE UPON SUBCUTANEOUS ADIPOSE TISSUE.

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PLATE LIII.

In a paper by the writer published in 1897* an account was given of some experiments which sought to discover the cause of the "fat necrosis" so often associated with acute disease of the pancreas. Since fat necrosis consists of a decomposition of the neutral oils contained in fat cells, the attempt was made to discover whether that change could be accomplished by the pancreatic juice, which has a ferment capable of decomposing neutral oils. To this end a ligature was placed about the gastro-splenic portion of the pancreas near the duodenum. In some cases as many as possible of the veins leaving the pancreas were tied and the substance of the gland was lacerated with a sharp hook. The object of the operation was to prevent the outflow of the pancreatic juice into the duodenum and to permit it to act upon the adjacent adipose tissues. The operation was performed upon twenty animals, mostly cats. In five cases well-marked fat necrosis ensued. There were three indecisive and twelve negative results. In three of the five cases where positive findings were obtained an accidental micrococcus infection was associated. It was not, therefore, considered justifiable to attribute the fat necrosis to the action of the pancreatic juice alone.

In order to discover whether bacteria played any important role in the production of fat necrosis, operations similar to those described have since been undertaken, where infection with pyogenic bacteria was brought about intentionally.

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Cat 37. Large, fat. The gastro-splenic portion of the pancreas and some of the veins issuing from it were ligated, the pancreas was lacerated with a sharp hook, and its surface was smeared with a quantity of the staphylococcus pyogenes aureus from a fresh culture on agar. Upon the following morning the cat was found dead. The autopsy showed beginning peritonitis and widely spread fat necrosis in the adipose tissue near the pancreas and in the omentum, in the form of a thin, flat layer, including the most superficial cells just below the peritoneum. The staphylococcus pyogenes aureus was recovered in cultures from the peritoneum, liver and spleen. Examination of the other viscera yielded nothing noteworthy. Microscopic sections showed well-marked beginning pancreatitis.

Cat 44. Medium sized, fairly well nourished. The gastro-splenic portion of the pancreas was ligated close to the duodenum, also several veins leaving it; it was lacerated with a sharp hook, and the surface was smeared with the streptococcus pyogenes from a culture made recently from pus on agar. On the fourth day the animal was found dead. Autopsy revealed peritonitis, especially near the pancreas. A part of the tail of the pancreas was disorganized and necrotic. There were numerous evidences of fat necrosis in the retro-peritoneal fat of the pancreatic region and in the omentum, and a few scattered spots appeared in the mesentery and meso-rectum. Some of the areas were large and flat, others were localized. The lungs showed pneumonia and fibrinous pleurisy. Smears from the peritoneum yielded a streptococcus and a large bacillus. The streptococcus alone was recovered in cultures. The evidences of acute pancreatitis were very marked in microscopic sections.

Cat 45. Large, well nourished. The gastro-splenic part of the pancreas was ligated; there were no laceration and no ligation of the veins, but the surface was smeared with the staphylococcus pyogenes aureus. The animal remaining healthy in appearance was killed at the end of a week. A single small area of fat necrosis was found close to the pancreas. Localized opacities due to thickening of the peritoneum were seen, but there was no general peritonitis. Pancreatitis was well marked.

The proportion of positive results was larger from these than from the first series of operations, but the number of cases was too small to permit one to draw far-reaching conclusions. The extensive fat necrosis in Cats 37 and 44 seemed as likely to have been due to the laceration of the pancreas as to the introduction of the pyogenic cocci, though the latter may have served to hasten or intensify the result.

It will be noticed that in Cat 37 extensive fat necrosis occurred within twenty-four hours after the operation on the pancreas. As following this line of procedure did not seem likely to lead to a solution of the problem under consideration, it was abandoned. Furthermore, on account of the instability of the fat-splitting ferment, injections of pancreatic extracts appeared to offer an unpromising prospect.

In an experiment described in the article already mentioned a large piece of fresh cat's pancreas was inserted into the abdominal cavity of another cat, with the view of observing the effects of its ferments on the peritoneal adipose tissues. Although fat necrosis followed, an accidental infection of the peritoneum and of the animal's own pancreas also took place and nullified the results. Owing to the danger of thus confusing the possible effects of disease of the animal's own pancreas with those of the pancreas artificially introduced, the peritoneal adipose tissue was deemed an unsuitable location, and efforts were then made to insert pieces of pancreas into the subcutaneous or intermuscular fat. As before, the cat was the animal selected. Numerous attempts to cultivate bacteria from the fresh cat's pancreas planted upon agar and kept in the incubator found it to be sterile in each case. In most of the following experiments the pancreas used was tested for its fat-splitting power, always with positive results. The locations chosen for the operations were the inguinal regions and a point a little below the sternum. The skin of the vicinity was shaved and washed with alcohol and an antiseptic solution. The instruments and silk used were sterilized with heat. The pancreas was taken fresh and with great care from the abdomen of another cat. It was inserted into the adipose tissues through a small incision, and was held in position by a silk suture, not passing through it, but uniting the edges of the tissues at the bottom of the wound. The superficial incision was closed separately with silk and collodion.

Cat 27. *Thin.* A piece of fresh pancreas from another cat was introduced through a small incision into the subcutaneous adipose tissue of the right inguinal region. After four days a large abscess had resulted, undoubtedly from accidental infection. The animal was killed and the abscess removed entire for histological examination. Besides

the ordinary changes of suppuration, the walls of the abscess showed areas of fat cells presenting a reddish blue stain with hamatoxylin and eosin and contents having the homogeneous appearance seen in undoubted fat necrosis. Some cells exhibited large needle-shaped crystals. Owing to the occurrence of suppuration the result was considered indecisive, but as probably indicating fat necrosis.

Cat 30. Healthy, young animal, quite fat. Portions of fresh pancreas from another cat were introduced into the subcutaneous adipose tissue of the right inguinal region and into the adipose tissue below the sternum behind the linea alba. After four days the points of introduction had become immense abscesses. The animal was killed and both abscesses were removed entire. The histological examination gave the same result as in the last case.

Cat 33. Healthy young cat, well nourished. Three localities were utilized for the introduction of cat's pancreas into the adipose tissues: the middle line below the sternum, the middle line above the symphysis pubis, and the right inguinal region. After five days abscess formation had begun, so that the animal was killed and the abscesses were excised. The histological examination detected no fat necrosis.

Cat 35. Large, well nourished. A piece of fresh cat's pancreas was introduced below the sternum in the same manner and with the same precautions as previously, except that a portion of adipose tissue was excised to make room for the piece of pancreas. After three days an immense abscess had resulted. The animal was killed and the abscess removed. The examination of sections with the microscope gave the changes to be expected for suppuration, with alterations in the fat cells similar to those already mentioned for cats 27 and 30.

The experiments just described seemed to show the futility of continuing along the lines followed in them. Although cultures were not made from the abscesses encountered, it was sufficiently evident that they proceeded from infection. In cases related hereafter, where identical results were obtained, the micro-organisms causing the suppuration were demonstrated. (Cats 43 and 50.) It was impossible to determine how far the changes in the tissues were to be attributed to bacteria, and how far to the working of the pancreatic ferments. As has been remarked, the infection probably took place from organisms on the skin, as the pancreas was removed with such care as to render it above suspicion. The writer has been led to

suspect that the pancreas may be possessed of irritating properties which make the tissues in contact with it extremely liable to infection.

In order to observe the effect of perfectly sterile pancreas on the tissues the following technique was devised, which can be recommended when it is desired to insert solid substances hypodermically. A cannula of glass of the size and shape represented in the accompanying figure was made from ordinary glass tubing. It was sterilized in a Petri dish, and the portion of pancreas to be used was placed in the large end, with a bit of sterile black silk. The pancreas and silk were forced along to the small end with a stiff platinum wire. The skin having been shaved, cleaned and rendered aseptic as far as possible, a small incision was made through it. The adipose tissues underlying it were nicked. The small end of the cannula was then forced into the adipose tissue three or four centimetres. The pancreas and bit of silk were pushed out of it by the wire, depositing them in the desired locality, after the withdrawal of the cannula. The surface wound was closed with one or two sutures. The success of this plan was very gratifying. In seventeen experiments made according to it upon nine cats, eleven were satisfactory in excluding bacteria, while infection occurred in but four instances and two experiments in another animal indicated doubtful infection. The black silk served admirably to identify the point of introduction.

Cat 38. Large, fat. Pieces of fresh pancreas one to two millimetres in diameter were inserted into the adipose tissues just below the sternum and in the right inguinal region, after the manner just described. On the twelfth day there had been no evidence of inflammation, and the wounds in the skin were nearly closed. The animal was killed. The points where the pancreas had been inserted were at once located by means of the black silk. Agar tubes were inoculated from both, and placed in the incubator. These tubes remained sterile. Serial sections of both regions were cut; those from below the sternum were negative; in the inguinal region a small area of fat necrosis was found.

Cat 39. Large, fat, not very vigorous. Pieces of fresh cat's pancreas



one to two millimetres in diameter were inserted through the sterile cannula into the adipose tissues of the right groin and below the sternum. The wounds of the skin healed rapidly and without incident. The animal was killed three weeks after the operation. Cultures were made from both points, which remained sterile. The examination of sections showed no fat necrosis.

Cat 40. Small, thin. A small piece of fresh cat's pancreas was placed in the fat tissue of the right groin through the cannula. The wound remained healthy. The cat was killed at the end of six days. Cultures from the locality remained sterile. Examination of sections did not demonstrate fat necrosis.

Cat 42. Small, not fat. By means of the sterile cannula small bits of fresh cat's pancreas were introduced into the adipose tissue in the sternal and left inguinal regions. After the lapse of seventeen days the points in question were examined. A positive result was obtained in the left groin, where a small spot of fat necrosis was found. Agar tubes from this area showed no growth. No bacteria could be found in sections stained by the Gram-Weigert method and with methylene-blue.

Cat 43. Large, fat. Through a glass cannula, bits of fresh cat's pancreas were inserted in the adipose tissues in three places: the right and left inguinal regions and the region below the sternum. At the end of three days there was much swelling and slight fluctuation. The cat was killed. Smears were made and agar tubes inoculated from all three localities, and the spots were excised. Suppuration was evident in all, and gas formation, as well, below the sternum. The pancreas taken in this case was from the same source as that used for Cat 42 and had produced no growth on agar. As Cat 42 had shown no suppuration, the source of infection in Cat 43 was probably the skin. The cause of the suppuration proved to be an undetermined micrococcus. Examination of sections showed probable fat necrosis in both inguinal regions, but none in the locality below the sternum.

Cat 47. Small, sickly, not fat. Pieces of fresh pancreas of a young cat were placed in the right and left inguinal adipose tissues through the glass cannula. The cat died unexpectedly two nights later. Autopsy showed the cause of death to be bronchitis and broncho-pneumonia, probably having no relation with the operation. The edges of the wounds had parted slightly, but there was no swelling in the neighborhood, nor any of the macroscopic evidences of suppuration. Cultures from both sides yielded a small bacillus, resembling *B. coli communis*. The weather during this period was excessively hot, and the results of the cultures were not deemed positive evidence of ante-mortem infec-

tion of the points of introduction. On both sides, in the neighborhood of the inserted bits of pancreas and for two to three millimetres in the track taken by the cannula, the adipose tissue had a strikingly white, opaque, cheesy appearance. Examination with the microscope, fresh and in sections, demonstrated the histology of fat necrosis.

Cat 48. Large, moderately fat. The fresh pancreas of a half-grown kitten was used. Small bits were inserted into the adipose tissues of the right and left inguinal regions through the glass cannula. At the end of five days the cat, appearing healthy, was killed. The regions where the pancreas had been placed were not swollen; but the adipose tissues for two or three millimetres around presented a white, cheesy appearance. Examination of the cheesy matter fresh, and of the region in sections, showed the characters of fat necrosis. Smears and sections stained for bacteria and culture tests all gave negative results.

Cat 49. Medium sized. Bits of fresh pancreas from a full-grown cat were introduced by means of a sterile glass cannula into the adipose tissues of both inguinal regions. After eight days, appearing healthy, the cat was killed. The skin wounds were in good condition. There was slight swelling where the pancreas had been placed, but no suppuration. There were, however, numerous minute, curd-like, white spots on the edges of the adjacent adipose tissues. Agar tubes inoculated from the region remained sterile. Smears stained for bacteria gave negative results, as did the subsequent examination of sections of the tissue for bacteria. Inspection of the curd-like masses and study of sections of the tissues gave the histology of fat necrosis.

Cat 50. Small. Received a bit of fresh cat's pancreas in the inguinal region through the sterile glass cannula. At the end of eight days swelling and fluctuation indicated pus formation. The pus contained an undetermined diplococcus. Nevertheless, the histological characteristics of fat necrosis were demonstrated in fresh preparations and in hardened and stained sections.

Summary.—Thirteen cats were used for the subcutaneous introduction of pancreas into the adipose tissues in twenty-four places. At nine points fat necrosis ensued; in nine there was no fat necrosis; in six the result was doubtful. The doubtful results were in cases in which suppuration complicated the issue. At eleven points there was no infection, two were doubtful, and eleven became infected. Selecting the eleven in which there was no infection and the two classed as doubtful, where infection was probably post-mortem, in all

thirteen, we find eight points at which fat necrosis occurred and five from which it was absent.

General description of the lesions at the site of introduction of the pancreas.—In those cases which resulted favorably and where the autopsies were performed only a few days after the subcutaneous insertion of the pancreas, the adipose tissues in that vicinity, which was identified by the piece of black silk, acquired a white, opaque and cheesy character.

The opacity was found to reside in certain fat cells; while adjacent fat cells presented a normal appearance. The altered cells exhibited a brown tinge, and their contents consisted of minute linear crystals. Ring-shaped plates were seldom seen, while they were common in the necrosis obtained in the cat after operations on the pancreas. Fragments of similar opaque material were seen which appeared to have arisen from the breaking up of such altered cells. Loose crystals and granules were also abundant.

The amount of material was too small to permit the application of micro-chemical tests in a perfectly satisfactory manner in all instances. When they could be employed the reactions were those characteristic for fat necrosis, including sometimes the demonstration of calcium salts.

The tissues were hardened in alcohol or in formaldehyde and embedded in celloidin. Sections stained with hæmatoxylin and eosin gave satisfactory pictures. The region where the pancreas had been placed was readily discovered by means of the black silk which had been introduced with the piece of pancreas. The pancreas itself could not be identified with certainty. Fragments of disorganized tissue associated with the bits of silk, staining with eosin, not stained by the Gram-Weigert method, and exhibiting neither nuclei nor definite structure, were supposed to represent the pieces of pancreas.

The altered fat cells were found on the surface of the cavity containing the silk and these fragments of tissue (Plate LIII). Their contents stained intensely, partly with hæmatoxylin, partly with eosin. The contents consisted in some cases of homogeneous material, in some cases of needle-shaped or fine feathery crystals, plainly vis-

ible in balsam preparations. They often contained granules, which stained intensely with hæmatoxylin, of very variable size, usually round, but sometimes quite irregular in form. Similar granules occurred between the cells. Some of these granules may have owed their intense stain to the presence of calcium salts. Some of them appeared to have arisen from the breaking up of nuclei. Nuclei belonging to the necrotic fat cells could not be discovered. The altered fat cells were of normal size or slightly enlarged at most. Necrotic cells were found, which were almost completely disintegrated, and with them loose crystals and fine granular material.

Between and around the necrotic fat cells polynuclear leucocytes appeared, usually so as to make an extensive accumulation, though sometimes in relatively small numbers. The interstices of the neighboring unaltered adipose tissues also contained leucocytes, which, with the granules above mentioned, occasionally constituted a well-marked zone around the region of operation. Larger cells with undivided nuclei, probably endothelial, were visible in smaller numbers, especially around the fibres of silk. Fragmentation of nuclei was often seen. A fine reticulum, stained by the Gram-Weigert method, which was observed sometimes in the area of most intense necrosis, suggested the formation of fibrin. In no case was there marked evidence of hæmorrhages. Where the time after operation was longest (12 days in Cat 38, 17 days in Cat 42) the silk and area of necrotic cells were enclosed in richly cellular, new-formed fibrous tissue. In the same cases there was less accumulation of leucocytes than in the more recent ones.

It will be seen that in all essential respects the histological characters of subcutaneous adipose tissues which are subjected to the direct action of the excised pancreas may be identical with the peritoneal fat necroses observed after ligation, laceration, and other operations on the pancreas. The probability, therefore, becomes very strong that fat necrosis may be produced by the unaided action of the ferments of the pancreas.

DESCRIPTION OF PLATE LIII.

Fig. 1.—Cat 47. Left inguinal adipose tissue, showing unaltered and necrotic fat cells, accumulation of granules and leucocytes at point of junc-

tion, black silk and dark fragment of tissue close to it, probably pancreas; two days after insertion of pancreas. Hæmatoxylin and eosin. Photo-micrograph by H. M. Hill. Spencer $\frac{3}{8}$ -inch objective. $\times 70$.

Fig. 2. Cat 42. Part of the area of fat necrosis from the left inguinal region; seventeen days after insertion of pancreas. Photo-micrograph by H. M. Hill. Spencer $\frac{3}{8}$ -inch objective. $\times 70$.

Fig. 3.—Cat 48. Left inguinal region, showing isolated fragment of adipose tissue, almost wholly necrotic, and leucocytes. Hæmatoxylin and eosin. Low-power water-color sketch by Miss E. M. Porter.

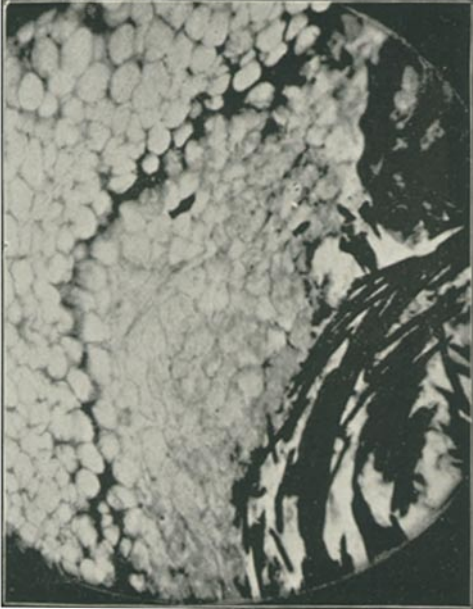


FIG. 1.



FIG. 2.

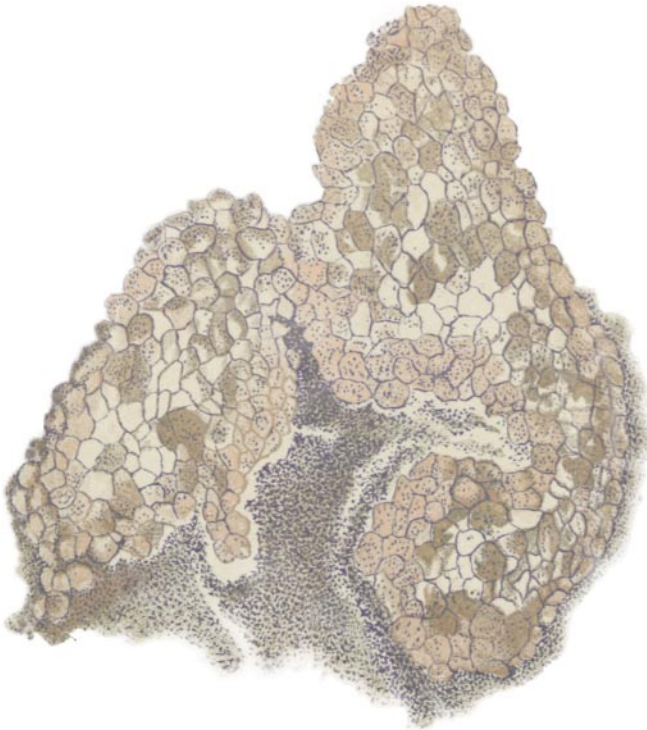


FIG. 3.