ON THE OCCURRENCE OF THE FAT-SPLITTING FERMENT IN PERITONEAL FAT NECROSES AND THE HISTOLOGY OF THESE LESIONS.

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The relation which exists between certain diseases of the pancreas and fat-necrosis has been emphasized especially by Fitz in his comprehensive studies of pancreatitis. According to this author* disseminated fat-necrosis in the abdominal cavity is comparatively infrequent in the suppurative form of pancreatitis, while it is relatively common in the hæmorrhagic and the gangrenous varieties. Balser,† who first directed especial attention to this condition, of which he gave a full description, found it in 5 out of 25 autopsies either within the interacinous tissues of the pancreas or in the adjacent or distant adipose tissues. Since this time, fat-necrosis has been observed by a large number of pathologists in cases in which gross disease of the pancreas has been wanting at autopsy. In these instances the necroses are limited, as a rule, to the immediate neighborhood of the gland, although at other times they have been found more widely disseminated when such disease of the pancreas existed. Still more recently, and now that acute suppurative or necrotic pancreatitis has become a field for surgical interference, the condition has been encountered at the operating table.

The coincidence of disease of the pancreas with the occurrence of fat-necrosis in the peritoneum on the one hand and the proximity of the necrotic areas to the pancreas on the other hand lent support to the view, quite early advanced, that a relation of cause and effect must exist between the gland and this pathological process. The first attempt, which may be regarded as at all successful, to prove this

* Acute Pancreatitis. The Middleton-Goldsmith Lecture, 1889.

† Virchow's *Archiv*, xc, 520, 1882.

assumption by experimental means was made by Langerhans.* In his studies† of the histology of the lesions he had satisfied himself that a decomposition of the fat-molecule had taken place, liberating the fatty acids which, after a time, combined with calcium to form insoluble lime soaps. He therefore experimented by injecting sterile pulp of the fresh pancreas into the perirenal tissues of a rabbit with the result of setting up an inflammatory process in which evidences of fat-necrosis were detected. In his repetitions of this procedure he did not always obtain similar results, but although he could claim only a single successful experiment out of twelve attempts, he nevertheless felt justified in considering that "the fat-splitting ferment by acting directly upon the tissues sets up a suppurative inflammation, while its less intense action brings about a decomposition of the oil drops within the fat cells resulting in fat-necrosis."

The experiments which have followed those of Langerhans have consisted in the introduction of portions of fresh pancreas into the peritoneal cavity of rabbits and dogs, the isolation of the pancreas from its main veins so as to bring about the production of passive congestion, thus favoring, as it is thought, the passage of the secretion into the blood, and the separation of one-third to one-half of the head of the pancreas from the tail, which is then permitted to pour its secretion directly into the peritoneal cavity. These experiments, which have been more or less successful, have been made by Dettmer,‡ Hildebrand,§ Körte‖ and Williams.¶ The facts which they established are: (1) The secretion of the pancreas may enter the peritoneal cavity without setting up diffuse inflammation, a conclusion previously arrived at by Senn; (2) in a certain number of instances the free, sterile pancreas or the pancreatic secretion causes focal fat-necrosis;

* Experimenteller Beitrag zur Fettgewebsnekrose, Virchow's Festschrift, 1891.

† Virchow's *Archiv*, cxxii, 252, 1890.

‡ Experimenteller Beitrag zur Lehre von den bei Pancreatitis haemorrhagica beobachteten Fettgewebsnekrosen und Blutungen, Inaug. Diss. Göttingen, 1895.

§ Ueber Experimente am Pankreas zur Erzeugung von Fettnekrosen, *Centralbl. f. Chirurgie*, xxii, 297, 1895.

‖ *Berliner Klinik*, 1896, Heft 102.

¶ *Boston Medical and Surgical Journal*, 1897, I.

and (3) the element of infection plays an insignificant, if not an entirely unessential, part.

In order to connect more certainly the pathogenic action of the pancreatic secretion with the fat-splitting ferment, and in view of the fact that this ferment itself is not obtainable in a form suitable for experiment, the effects of trypsin were tried. It was found, especially by Dettmer,* that pure trypsin caused a sero-hæmorrhagic exudation into the peritoneal cavity, but was without power to set up fat-necrosis. The diastatic ferment having already been excluded, it was regarded as highly probable that the fat-splitting ferment was the active agent.

My present studies have dealt with two problems. The first was the demonstration of the presence of the fat-splitting ferment in the areas of necrosis and its absence from the normal fat in a suitable human case. The second was to confirm this observation by making similar tests upon animals in which fat-necrosis had been experimentally produced. In the second series of cases experiments were made with a view of determining how long the ferment remained in the tissues in demonstrable conditions or amounts.

I may say in this place that the successful experimental lesions were subjected to microscopical study and the existence of necrosis was proven. The histology of the experimental lesions is also considered in this communication.

The method which was uniformly followed in the demonstration of the ferment is essentially that of Claude Bernard, for the details and practical carrying out of which I am indebted to Dr. Abel, to whom I wish to express my obligations. It consists in preparing a perfectly neutral fat, which I did from fresh butter by dissolving it in ether, adding a few drops of NaOH and washing out with successive quantities of distilled water. The separated ethereal solution of neutral butter was evaporated over a water bath and the tests were made at once. For this purpose, in each instance, I employed (1) a piece of the pancreas itself, (2) one or more focal necroses, and (3) a piece of adipose tissue equal in size to the pancreas or necroses, this piece

* Op. cit.

being taken at a distance from the latter. The pieces of tissue were placed in 90 per cent pure alcohol for from one-half to two hours, pressed in filter paper, reimmersed in the alcohol for a short period, again pressed and then allowed to become air-dry. They were then cut into morsels, which were incorporated with the neutral fat in watch-glasses which were then covered and placed in the thermostat at the body temperature.

For the demonstration of the action of the ferment two methods were employed. The first was the detection of the liberated fatty acids by the sense of smell. The second consisted in dissolving the butter, etc., in pure absolute alcohol and adding tincture of litmus to the alcoholic solution. It was quite remarkable how well the two tests agreed with each other. The exact details will be given in the individual protocols.

It would seem that the reaction takes place quickly if the ferment is present in an active condition. In not a few instances a distinct and even marked reaction was obtained within six minutes from the time of placing the watch-crystals in the thermostat.

The animals used for experiments were cats and dogs. The former appeared to be better adapted for the purpose. The operations consisted of (1) ligation of all the veins proceeding from the pancreas, which was quickly followed by a vivid congestion of the gland, and the laceration of the organ by means of a sharp hook; (2) the same operation, in addition to which a strong ligature was thrown about the splenic half of the organ; (3) ligation of the veins, tying off of the splenic half, and section beyond the ligature; (4) the isolation of the duct of Wirsung, its ligation where it entered the duodenum and section. The effects of congestion alone were tried in two cases without marked success.

The object in these experiments was to produce necrosis at a distance from the gland which would serve for the tests mentioned. It was also considered desirable to avoid all infection of the peritoneal cavity. The most satisfactory results were obtained after ligating the veins and lacerating the pancreas. Tying off one-half of the organ was soon abandoned, inasmuch as it was found that the ligated part

sometimes suppurated and the necroses were not more extensive than in other cases. Cutting the duct of Wirsung (one case) did not lead to extensive necroses. The focal areas were found in the pancreas and mesenteric fat close to the site of operation and were small in size. The duct in the cat is hardly larger than a capillary tube and, it is believed, quickly becomes obliterated after section. By the use of rigid surgical precautions, except in two cases of suppuration about the ligature around the pancreas, in one instance only (a dog) was a general peritonitis encountered. Notwithstanding a sero-purulent exudate in the abdominal cavity, this animal appeared to be in perfect health when killed 3 days after the operation.

The necroses varied in extent. They were always to be detected about the pancreas in the interacinous and peripancreatic adipose tissue. They were usually present in the fat of the gastrohepatic omentum and more variably in the great omentum and mesenteric fat. When in the mesentery the necroses were likely to be in the duodenal and upper jejunal portions and in that of the descending colon. They were never discovered in the perirenal tissue. Wherever adhesion took place between the omentum and the lacerations on the surface of the pancreas, there fat-necroses were always found. In size the necroses varied from a pin's head or less to a pea. The smaller ones were yellow or white and opaque, the larger often hæmorrhagic. The latter were usually selected for the tests.

With one exception all the animals were killed, chloroform being used for this purpose. The animal which succumbed spontaneously was a full-grown but rather poorly nourished cat, which died two days after the operation. There were in this instance many areas of necrosis in the pancreas and mesentery and fewer in the omentum. In part they were hæmorrhagic. The other animals were killed on the 2nd, 3rd, 4th, 6th and 8th days respectively. Positive reactions for the ferment were obtained in all except the two longest periods mentioned.

In order to determine whether in the long periods the ferments were present at an earlier date only to disappear later on, the animals were subjected to a second operation (Protocols C, D and E), at which

time necrotic foci were excised and tested, the peritoneal cavity being again closed. These tests were made on the 3rd day after the first operation. The cats were killed on the 6th and 8th days respectively. The first tests were positive; the second negative in all instances.

When we come to consider the histology of the experimental lesions we shall find that a great difference exists in those of the first three and those of the 6th and 8th days. While in the former the process is very acute, in the others the evidences of cicatrization are most pronounced.

The conclusions which may be drawn from the foregoing observations are: (1) In peritoneal fat-necrosis the fat-splitting ferment is demonstrable at certain stages of the pathological process; (2) it is present in greatest amount in the early stages and may disappear in the later ones when healing is well advanced; (3) although it cannot be affirmed that steapsin is the direct cause of the necrosis of tissue, such an assumption is rendered highly probable by its constant occurrence in the diseased areas, its absence from the healthy fat, and the nature of the pathological changes; (4) the escape of the pancreatic secretion into the peri- and para-pancreatic tissues is the origin of the necroses, and this escape is facilitated chiefly by lesions of the pancreas itself, but also by disturbances in its circulation.

That necrosis of fat cells may result from other causes is certainly not excluded by these findings. The genesis of similar lesions found in the marrow of the bones by Ponfick* in the subcutaneous fat (Chiari) and pericardial fat (Balser, Chiari) is not immediately apparent. That micro-organisms may, in these cases, play a part is, in my opinion, very probable indeed. Ponfick's case was one of empyema in which, besides the lesions in the medulla of the bones, areas of softening, not due to vascular occlusion, existed in the myocardium.

The histology of the fat-necroses, as it occurs in human beings, has been studied by Ponfick, Balser, Fitz, Chiari,† and especially by Langerhans.‡ The experimental lesions also have been subjected to

* Virchow's *Archiv*, lvi, 541, 1872.
† *Prager Medicinische Wochenschrift*, viii, 1883.
‡ Virchow's *Archiv*, cxxii, 252, 1890.

microscopical examination and compared with those found in man. The main facts to be gleaned from these observations are as follows: The pathological process begins with a decomposition of the neutral fat contained within the fat cells; the fluid constituents are eliminated and the solid fatty-acid crystals remain behind. These, after a time, unite with calcium to form insoluble lime salts. The pathological products are found within the protoplasm of cells whose nuclei have already lost or are preparing to lose their affinity for staining agents. The general form and characters of the cells are retained for a time; but in later stages the lime compounds occur free under the form of granules, rings and hyaline masses (Schollen), which as a whole appear white and non-transparent. Thus it happens that a small number of cells, a whole lobule of adipose tissue or even several adjacent lobules may give rise to a necrotic focus which now becomes the seat of a reactive (demarcating?) inflammation.

Pathological alterations within the blood-vessels may be slight or entirely wanting, and are, at best, so inconstant and variable as to be excluded as representing the exciting cause of the lesions. In the same way the part played by micro-organisms can be only an insignificant one and is probably limited to an occasional instance. At any rate all evidences of constancy of relationship between bacteria and the necrotic foci are thus far wanting.

One of the objects of my study of the histology of the experimental lesions was to compare them with those occurring in the course of disease in man. As a basis for this comparison two human cases were studied, in one of which the necrotic lesions were widely disseminated (Protocol A) and in the other were nearly limited to the pancreas and peripancreatic tissue (Protocol B). In the first case only was the fat-splitting ferment demonstrated in the necroses; in the other no attempt was made to detect it owing to the intimate association of the lesions with the pancreas. Still another purpose of this study was the determination of the changes which take place in the tissues coincidently with the disappearance of the ferment from the necrotic foci. The observations upon this point may be taken as a contribution to our knowledge of the healing of fat-necroses.

There is little difficulty in the human cases mentioned in confirming the results of Langerhans' researches. In addition it is to be noted that lesions may come under observation early, before the lime compounds have formed, in which case besides the changes peculiar to fat-necroses, those of an acute inflammatory nature are also present. This fact is true of one of our cases (Protocol A) in which none of the disseminated foci of necroses had been incrustrated with lime. The white, opaque central mass is composed of confluent cells, derived from the adipose tissue, the individual outlines of which have been lost and in which nuclei either complete or as fragments are entirely wanting, but which are occupied by fatty-acid crystals. The addition of concentrated sulphuric acid and then water does not bring these crystals out more distinctly, but quickly decomposes the fat in the unaltered cells, causing a separation of an abundance of crystalline fatty acids. At the end of twenty-four hours no further change has taken place; sulphate of calcium crystals have entirely failed to make their appearance.

Just outside the completely necrotic focus is a zone of infiltration in which cells and fragments of cells and nuclei are found in large amounts. This presents somewhat heterogeneous characteristics; it constitutes in part the zone of reaction—demarcating inflammation—and encloses many ring-shaped and globular hyaline bodies which are characterized further by their peculiarly intense staining with hæmatoxylin. Among the foreign elements in this mantle, polymorphonuclear leucocytes and cells of the lymphoid type are to be distinguished. Beginning at this point and extending for a variable, but often considerable, distance into the surrounding tissue, there are evidences of an inflammatory invasion consisting of œdema, hæmorrhage and especially of fibrin. Many swollen and necrotic cells (emigrated and tissue elements) are present in this part. It is noticeable that the rich network of fibrin tends to extend along the connective tissue septa, against which many of the necrotic foci abut. The diffusion of the simple inflammatory lesions, in contradistinction to the definitely circumscribed nature of the specific necrotic areas, is a remarkable feature of the pathological process.

Évidences of proliferation of the fixed tissues are abundant; even in such early stages as just described they are discernible; but later (Protocol B) they are much more marked. There is nothing peculiar about the manner of this new growth, except that it is peripheral. The necrotic centres do not become invaded but gradually shrink and finally would appear to be completely replaced by connective tissue. At the time of great activity of the tissues the acute inflammatory process is no longer to be made out; red blood-corpuscles, serum and fibrin have disappeared.

The pancreas itself suffers in two ways; it undergoes necrosis in the same way as the adipose tissue. This is observed on the surface (extension by continuity) and deeper down near the interlobular connective-tissue bands. Again, it is invaded by a new growth of connective tissue not limited strictly to the fields of necrosis. Finally, and in this way, small nodules characteristic of intestinal pancreatitis with loss of parenchyma come to be formed.

The acute lesions of the experimental cases approach very closely to the corresponding lesions in man, while the older ones in animals pass through more typical stages of healing, and apparently also in a shorter period of time. The central masses of necrotic cells are indistinguishable in the two forms, while the peripheral zone of infiltration is perhaps greater in the experimental cases. The elements composing this zone differ somewhat. The great majority of preserved cells exhibit single, deeply-staining nuclei. Fibrin appears somewhat variably; necroses 72 hours old (Protocol C) may be devoid of it, while again it may be abundant in about the same period or even later (Protocol D and E). The pancreas itself may suffer severely or may escape entirely. In the first instances large necroses exist within the parenchyma. The venous radicles are occupied by light fibrinous thrombi.

Cicatrization is already under way on the sixth day (Protocol D), but on the eighth it is well advanced (Protocol C). The new cells are developed at the periphery of the focus of necrosis, the proliferation extending well beyond the immediate site of injury. The necrotic area gradually shrinks, becoming smaller and smaller without being

invaded to any extent either by wandering or fixed cells. Multinuclear (Giant) cells occur either at the most recent edge next the necrosis or at some distance in the adipose tissue. At this time (8th day) the outermost zone of granulation tissue is already fibrillated. If the area has been hæmorrhagic, blood pigment (hæmatoidin) is still present and is enclosed in the new cells.

The central mass of necrosis is not completely calcareous, and it is exceptional to obtain the micro-chemical reaction for calcium.

From the following protocols more detailed information may be obtained.

Protocol A. Woman, aged 50 years. Well nourished; well developed panniculus. Sudden death; symptoms of œdema of the lungs. Anatomical diagnosis: Gall-stones in common duct; dilatation of duct above the concretions; dilatation and distention of gall bladder; jaundice. Disseminated peritoneal fat-necroses. Moderate chronic diffuse nephritis.

The under surface of the omentum shows a considerable number of foci of fat-necrosis, surrounded by hæmorrhagic or hyperæmic zones. The nearer the pancreas is approached the more numerous become these foci. The greatest number in the omental fat are found on the left side, passing down almost to the crest of the ilium. Where the necroses are most abundant near the pancreas, adhesions, easy to break down, exist between this organ and the omentum. Similar adhesions between the omental foci and the abdominal wall, near the iliac crest, are met with, and hæmorrhage has taken place freely into the pouch formed in this manner. The pancreatic interstitial fat especially in the splenic portion and the mesentery also show foci of necrosis. The pancreatic duct is not dilated; its contents are, however, thick and opaque. The lymphatic glands in the neighborhood of the pancreas are swollen and reddened; the general peritoneal glands appear to be normal.

The following tissues were tested, in the manner described, for the fat-splitting ferment: (a) a bit of dog's pancreas, (b) a bit of pancreas from this case (controls), (c) several of the focal necroses with the adipose tissue immediately surrounding them, (d) adipose tissue from a distance. Results: Positive reaction in ten minutes in all except d; more marked reactions at the end of 30 minutes. No development of rancidity in d at the end of the experiments—after one hour.

Cultures made from the viscera after returning to the laboratory (the autopsy having been made at a distance) gave the following results: Heart's blood, liver and gall-stones were negative. Pancreas, spleen and

fat about the pancreas and kidneys gave a bacillus belonging to the group of B. coli communis.

Protocol B. Man, aged 56 years. Heavy periodical drinker. Large, well-developed, obese. Anatomical diagnosis: Biliary (hypertrophic) cirrhosis of liver; ascites; chronic diffuse nephritis; lobular pneumonia; general passive congestion of viscera; multiple foci of fat-necrosis in the pancreas.

The pancreas is large, grey in color and firm in consistence. There exist just beneath the capsule several whitish areas, the largest not exceeding 5 mm. in extent, which proved to be fat-necroses. They are more numerous in the substance of the organ where they appear in the form of lines and squares, and often contain a putty-like material. The addition of H_2SO_4 and water to this material yields, after a time, gypsum crystals. The ferment was not sought in these foci owing to their proximity to the pancreatic tissue.

Protocol C. March 30th, noon, operated upon two cats as follows: No. 1; tied off largest veins only, excepting omental branches; No. 2; tied off all the veins which could be found; lacerated pancreas with a sharp hook. Three days later (April 3, noon), cat No. 1 opened under ether anaesthesia; a number of small, white opaque necroses existed in the omentum near the pancreas; somewhat larger ones present in the pancreas itself. Clean peritoneum. A bit of the fat containing three small necroses (not exceeding 2 mm. each) excised; abdominal cavity closed. Cat No. 2 opened in the same manner. Peritoneum clean; omentum free except at one point, where it is tucked over a laceration in the pancreas. Numerous fat-necroses in the omentum and in the fat about the pancreas; the individual ones are much larger than those in No. 1. Two focal fat-necroses excised; one of these represents one-half of a large omental focus, the other half being left for a future experiment. A single hæmorrhagic node free from visible necrosis also excised from the omentum. Abdominal cavity closed. Tests for the ferment: No. 1 showed no reaction to the sense of smell in one hour; very faint reaction to litmus. Butter control negative. No. 2: the necroses tested separately; hæmorrhagic area also tested. The necroses reacted promptly both by odor and to litmus. Hæmorrhagic nodes gave no reaction. April 8 (8 days after first operation), both animals chloroformed. No. 1: No necroses to be found except in and near the pancreas about the ligature. No. 2: Clean peritoneum; the foci of necrosis fewer in number and reduced in size. A number of small ones (pin-point to pin-head) are found in the omentum. The remaining half of the excised necrosis is reduced to the size of a canary seed and is firm and white. Tests for ferment: (a) one focus 1.5 x 2 mm. from the lesser omentum; (b) a piece of

the great omentum having a number of necroses side by side; (c) the remains of the partly excised necrosis; (d) bit of the pancreas. Results: no reaction either to sense of smell or to litmus in (a), (b) and (c). Positive reaction (5 minutes) in (d).

Protocol D. April 22, 10 A. M., operated as follows: Cat No. 1; veins tied; pancreas lacerated. April 24, 4 P. M. (after 54 hours), peritoneum opened under ether narcosis. A number of focal areas of necrosis in or near the pancreas; other smaller ones at a greater distance. Two nodules excised (a) hæmorrhagic area from the omentum, (b) opaque area from the capsule of the pancreas. Test for ferment: (a) faint reaction of rancidity and to litmus; (b) stronger and marked reactions. April 28 (after 6 days), animal chloroformed. No distinct nodules in the omentum; several flat white areas in the pancreas. No reaction from the former.

No. 2. Smaller, poorly nourished cat. Pancreas lacerated without tying off of veins. Died during night of April 23d. Autopsy, April 24th. Peritoneum clean; the edges of the wound sticky, but no pus found. Many areas of focal necrosis in pancreas, mesentery and omentum. Bacteriological examination of cover-slips from the wound showed a few doubtful cocci; cultures from peritoneal cavity, sterile. Ferment in necroses (two tests) and in pancreas (control).

Protocol E. May 11th, noon, operated on two cats as follows: No. 1. half grown; tied off the veins and lacerated the pancreas. May 13, animal died. No necroses in the omental or mesenteric fat. Several small opaque points near the pancreas. No tests. Cause of death not made out. No. 2. Full grown animal. Tying of veins, laceration of pancreas and ligation of splenic half of the organ. Section of the pancreas beyond the ligature, permitting the splenic half with its ducts to be in free communication with the peritoneal cavity. May 14, noon, under ether anaesthesia opened the abdomen and removed (a) bit of adipose tissue from the omentum; (b) a focus of necrosis from this situation; (c) necrosis in the pancreas; (a) and (b) tested for the ferment. Results: from adipose tissue, no reaction; from necrosis, positive reaction; (c) reserved for histological study. May 17. Cat chloroformed. External wound suppurating; peritoneal cavity clean. A number of small, opaque foci of necrosis in the omentum. Owing to their small size several were united for the test. A piece of pancreas from the animal used as a control. The necroses gave no reaction; the pancreas gave positive results. The largest necrotic area was studied histologically.

Protocol F. Cat; operation March 18. Veins ligated; ligature about splenic portion of pancreas. Chloroformed March 23. The ligature about the pancreas had nearly severed the organ. It was covered with a small quantity of sticky pus-like material. Peritoneum otherwise clean.

Many foci of necrosis, the greatest number being near the pancreas on the splenic side. They involve the omental fat over the stomach and its free portion, the mesentery and the substance of the pancreas. Positive reactions for the ferment from the pancreas and from the necroses from omentum and mesentery. No reaction from adipose tissue alone.

Protocol G. Same operation as and similar results to those in *F.*

Protocol H. April 8, 11 A. M. Full-grown cat. Isolation of the duct of Wirsung at its entrance into the duodenum; ligation at the intestine and section. A small drop of clear fluid immediately escaped from the proximal end. Animal chloroformed April 12, 10 A. M. Peritoneal cavity clean. No general foci of necrosis. On the other hand, necroses exist about the ligature, in the fat between the duodenum and the pancreas, extending along the sheaths of the bile and the blood-vessels towards the liver. Duct dilated. No test for the ferment.

Protocol I. Small black dog. Operation March 16. Ligation of veins, ligation of the splenic half and laceration of the pancreas. Chloroformed March 22. External wound granulating; peritoneum clean. The largest area of necrosis is 6 cm. from the pylorus and extends from the pancreas to the intestines, following the ligated blood-vessels. At the intestine (duodenum) the necrosis spreads in the form of fine lines over the serosa. Similar white lines, showing nodosities, and suggesting strings of beads run over the pancreas itself. Test: (a) fat about kidney free from necrosis; (b) pancreas; (c) fat with lines of necrosis from duodenum. Reaction in from 6 to 8 minutes in (b) and (c). No reaction in (a). The microscopical examination of the white contents of the necroses, which had the consistence of Neufchatel cheese, showed it to be composed of a mass of necrotic and oil-containing cells (compound granular corpuscles) and fatty-acid crystals.

Protocol J. Similar experiment to *I*, except that the pancreas was not ligated. Dog killed on the 7th day. Several necroses near the pancreas. No tests.

Protocol K. Dog; operation March 24, veins tied, laceration of pancreas. Chloroformed March 26. Numerous necroses in omentum and mesentery and about pancreas. Tests positive from pancreas and necrosis (two situations); negative from fat at a distance.

Protocol L. Cat; operation April 15, 10 A. M. Ligation of veins; laceration of pancreas. Chloroformed April 17, 10 A. M. Peritoneum clean. Duodenal end of pancreas swollen and oedematous. The omentum shows several areas of hæmorrhagic infiltration with doubtful evidence of necrosis. What appears to be necrotic fat discovered along the sheaths of the portal vessels. Tests with pancreas, supposed necroses in omentum and fat at a distance certainly free; all failed to react excepting the first.