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Contributors

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Royal College of Surgeons of England

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BY
H. GIDEON WELLS

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STUDIES IN FAT NECROSIS

H. Gideon Wells

Langerhans[1] made the first actual attempt to learn the cause of the changes of fat necrosis in 1890. Before that time Balser (1882),[2] who first called attention to the condition, and Chiari (1883)[3] had attempted to reach conclusions by study of the histology of the lesions. The former decided that overgrowth of fat tissue was at the bottom of the trouble, thinking that the new fat cells which he described as growing about the periphery shut off the blood supply of the center of the focus. Chiari took an exactly opposite view. Observing fat necrosis in the bodies of marantic patients, he considered that lack of nutrition during cachexia was the cause. From his histologic studies he concluded that the process was not at all different from retrogressive changes seen in other organs and there designated as simple necrosis and fatty degeneration.

Langerhans's first studies were of a histologic and micro-chemic nature. He observed that the necrotic masses did not float like masses of stearin, but sank quickly to the bottom. On teasing out they were seen to consist of characteristic needles of fatty acids, a few small oil droplets, and many flakes of varying size, the last being responsible for the high specific gravity. Further evidence of the change from the normal fat that had previously occupied the affected area was furnished by staining with osmic acid, which merely yielded a dirty, grayish-brown coloration. Addition of concentrated sulphuric acid to sections from which the fat had been removed with alcohol and ether caused complete solution of the heavy flakes. If this treatment was followed by the addition of water, and the specimen allowed to stand until the following day, numerous characteristic crystals of calcium sulphate settled out. From these observations he concluded that, since both calcium and fatty acids existed in the necrotic areas, they were probably combined. Further evidence was furnished by the insolubility of the calcium salts in hydrochloric acid, which showed that they were not carbonates or phosphates, and the insolubility of the fatty acids in boiling ether, which showed that they were not free. At the time Langerhans wrote the knowledge of the history of fat in metabolism was less advanced than now, and he sought support for his assumption of the formation of calcium soaps in the observations on this process as it occurs in adipocere. With this exception the chief illustration of such a process was the description of retrogressive metamorphosis in lipomas by Virchow,[4] who said that in these tumors the fat is occasionally saponified, combines with calcium and magnesium, and together with earthy phosphates forms a crumbling, mortar-like mass. By

[1] *Virchow's Archiv*, Vol. CXXII (1890), p. 252.

[2] *Ibid.*, Vol. XC (1882), p. 520.

[3] *Prager medicinische Wochenschrift* (1883), No. 30, p. 285; No. 31, p. 299.

[4] *Krankhafte Geschwülste*, Vol. I, p. 393.

studying fresh specimens, Langerhans sometimes found foci in the middle of a fat lobule consisting of fat cells full of fatty acids without calcium salts, showing that the splitting occurred first and the union with calcium later. His conception of the process after these studies he states as follows:

To collect these observations briefly, it develops that the multiple necroses of fat tissue begin with decomposition of the neutral fat contained within the cells; the fluid constituents are eliminated and the solid fatty acids remain behind. The latter combine with calcium to form calcium fatty-acid salts. The entire lobule or several neighboring lobules form a dead mass, which has the destroyed separated from the living tissue through a demarcating inflammation.

The part played by the fat cell itself in the necrosis was puzzling. He found that the nucleus did not lose its affinity for stains until after decomposition of the contained fats. He says:

Although there is no demonstration of a primary injury to the cell, I cannot hazard the conclusion that the physiological function of the cell is intact at the beginning of the decomposition of the fat, because we know so little of the rôle of the cells in the building and taking up of fat. It would perhaps be simplest to assume that in metabolism somewhere within or about the cell a harmful substance appeared and accomplished the decomposition of the fat droplets, but, unfortunately, every observation fails to support this.

The frequent association of fat necrosis with lesions of the pancreas, the frequent localization of the process to the fat about the pancreas, and the similarity of the fat-splitting that he had described with that of pancreatic digestion, led Langerhans[5] to study the relation of this organ to the disease experimentally, and in 1891 he described his methods and results. He made an extract of the pancreas of freshly killed rabbits by rubbing the gland in a mortar with splintered glass, under aseptic precautions. This was injected after filtration into twelve animals—nine rabbits and three dogs—and in one rabbit a single spot of typical fat necrosis appeared in the perirenal fat. From this one success he believed that the pancreatic juice was the cause of the fat necrosis, but of course he could not obtain any clear conception of the process.

Since that time a large variety of experiments have been tried in the study of fat necrosis, most of which have simply added a new method by which pancreatic juice can be made to enter the peripancreatic fat, or the subcutaneous fat (Williams) and the abdominal cavity, when, all are agreed, fat necrosis frequently, but not invariably, results. Experiments in which extracts or dried preparations of the pancreas have been used, rather than the secretions of the living gland, have been made by Jung and by Dettmer.

Jung[6] placed a gelatin capsule containing "trypsin" in the abdominal cavity of one rabbit, and pieces of dog's pancreas in three others. All showed fat necrosis, which he thought was due to the fat-emulsionizing and fat-splitting ferment, but the greater part to the proteid-splitting enzyme. The experiment upon which Jung based his idea of the importance of trypsin was the one mentioned first, in which a prepara-

[5] *Festschrift zur Feier des 71. Geburtstages Virchow's.* [6] Dissertation, Göttingen, 1895.

tion of pancreas in a gelatin capsule was placed in contact with the omentum. As this preparation, which he calls "trypsin," seems to have been merely dried and pulverized pancreatic tissue, his assumption of a pure tryptic action is quite unwarranted, since the other ferments are not excluded.

Dettmer[7] first tried the effect of causing pancreatic juice to enter the abdominal cavity, which was accomplished variously by ligating the pancreatic duct, or the vessels, or both, by placing pancreas tissue in the abdominal cavity, and by injecting an extract of fresh pancreas. As these caused fat necrosis, he endeavored to find what ferment was responsible, to which end he injected trypsin into still other animals. As trypsin alone did not cause fat necrosis, he concluded that the fat-splitting ferment must be responsible. The trypsin used by Dettmer was quite a different article from that used by Jung, and he describes it as follows:

> The trypsin used by us (from the firm of Merck in Darmstadt) was studied thoroughly after it was softened in water; it showed no trace of organic structure, but had a more crystalline appearance; it was designated by the firm as the proteid-splitting substance, in contrast to another preparation, called pancreatin, which contains all active elements of the pancreas. A test of our preparation for a saccharificant action gave a negative result, likewise the test for fat-emulsionizing property. Our preparation was therefore pure trypsin. We obtained with our pure preparation only hemorrhage, no fat necrosis in the Balser-Langerhans sense.

It will be observed that no test for lipolytic activity was made. The experiments performed by Dettmer were as follows:

(Experiment 1.) Injected into the peritoneal cavity of a cat "a large quantity of a watery solution of trypsin." Thirteen days later, the cat having remained well, the abdomen was opened aseptically and nothing abnormal found. About one gram of powdered trypsin was then sprinkled upon the omentum and intestines of this cat (Experiment 3). The animal died in less than twenty-four hours, and autopsy showed great hyperæmia and many hemorrhages in the omentum, but the fat itself "showed no traces of alteration." There was no evidence of peritonitis. Experiment 2 was a duplicate of Experiment 1, with likewise a totally negative result. This animal then had about 0.5 grams of trypsin placed in the abdomen, after which the wound was closed (Experiment 4). Death occurred seven days later. Autopsy showed no peritonitis; the fat was entirely unaltered, but there were a few small hemorrhages in the omentum. The parts of the intestine that had come in contact with the trypsin were superficially eroded. No histologic examination is mentioned. Dettmer's conclusions are as follows:

> These experiments show beyond question that the proteid-splitting ferment, the trypsin, is not able to produce fat necrosis. But, as fresh pancreatic juice is always able to cause fat necrosis in living fat tissue, there remains nothing more possible except that this action is due to the third ferment contained in pancreatic juice, the fat ferment.

Except for the series of experiments just mentioned, the numerous experiments performed by many different investigators have, for the most part, added nothing to the important matter of how the pancreatic juice causes this peculiar form of necrosis,

[7] Dissertation, Göttingen, 1895.

and what constituent of the juice is the active one. Their results have shown that fat necrosis can be obtained in the following ways: By injecting aqueous extracts of the fresh gland; by transplanting pieces of pancreas; by ligating the pancreatic duct, with or without laceration of the organ to facilitate escape of the pancreatic juice; by ligating the veins leading from the pancreas, with or without injury to the gland or ligation of its duct; by injection of necrotizing or infectious substances into the gland itself or into its duct; by cutting the duct or bisecting the organ, so that its secretion escapes into the surrounding tissues; by binding between glass rods, and other forms of trauma. All of these methods have close relation to the conditions of trauma, hemorrhage, and infection which ordinarily are the cause of fat necrosis as seen in man. These experiments, as well as clinical observations, taken altogether leave no doubt that pancreatic juice, and it alone, is the cause of fat necrosis, bacteria having been excluded by many negative cultural experiments. Further than this they tell little.

In contrast to them is the work of Flexner,[8] in which it was attempted to prove definitely the relation of pancreatic lipase to fat necrosis, which relation had, since Langerhans's one successful experiment, generally been assumed to be a positive one. Flexner's studies were directed to prove the presence of lipase in the areas of necrosis in both human and experimental cases, and its absence in the normal fat tissue. His method is described as follows: A neutral fat was prepared from fresh butter by dissolving it in ether, adding a few drops of NaOH solution, and washing out with water. The separated ethereal solution of neutral butter was evaporated over a water bath, and the tests were made at once. For this purpose were 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 pieces of pancreas or necroses, this piece being taken from a distance from the necroses. The pieces of tissue were placed in 90 per cent. pure alcohol for from one-half to two hours, pressed in filter paper, re-immersed in the alcohol for a short period, again pressed and 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 body temperature. The fatty acids liberated were demonstrated by the odor and by tincture of litmus, the reaction being sometimes obtained, it is stated, within six minutes. Fat necrosis was produced in cats and dogs by various methods leading to escape of juice from the gland, most satisfactory of which was combined ligation of the veins and laceration of the gland. In one case of fat necrosis Flexner was able to obtain a positive reaction in ten minutes from the necrotic tissue and from the pancreas, but not from the normal fat tissue. In animals killed on the second, third, and fourth days all gave evidence of fat-splitting enzyme in the necroses, but not in the normal fat. To test the time at which the lipase (steapsin) disappeared, pieces of necrotic tissue were excised aseptically from cats on the third day after the operation on the pancreas, and

[8] *Journal of Experimental Medicine*, Vol. II (1897), p. 413.

the animals allowed to live until the sixth and eighth days. The conclusions drawn from the experiment were:

(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.

Flexner also adds:

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.

This valuable work of Flexner's, filling out the results of Langerhans and Dettmer, seemed to leave little doubt that fat necrosis is simply a matter of escape of pancreatic juice into the fat tissue about the gland and into the peritoneum, and the lipase (steapsin) acting upon the fat causes its splitting, which leads to the peculiar and characteristic foci. To be sure, Flexner appreciated the incompleteness of the demonstration and the many unsolved questions, but this has been generally overlooked, and at the present time the teaching is usually as above stated.

The evidence upon which is based the belief in the primary importance of lipase in the pathogenesis of fat necrosis, human and experimental, is as follows: (1) the microscopical evidence of fat-splitting, consisting in the demonstration of masses of crystals of fatty acids in the areas of necrosis, together with the disappearance of the fat from these areas; (2) the association of fat necrosis with the occurrence of free pancreatic juice, the known presence of lipase in this fluid, and the inability to produce fat necrosis with the tryptic ferment (Dettmer; the experiments of Jung seem to be of little or no value); (3) the demonstration by Flexner and by Opie of a fat-splitting ferment in the areas of necrosis, while it could not be demonstrated in the normal fat tissue.

The course of events is believed to be about as follows: (1) an injury to the pancreas by infection, trauma, occlusion of ducts (Opie), or what not; (2) escape of more or less normal pancreatic juice either free into the tissue or into the lymph stream; (3) dissemination of the fluid, in a manner not always easy to explain; (4) action of the lipase upon the fat cells, leading to splitting of their contained fat into glycerin and fatty acid; (5) glycerin diffuses, leaving behind the solid fatty acids; (6) necrosis of the cells; (7) eventually the fatty acids combine with

[9] A full corroboration is furnished by E. L. OPIE, *Contributions of the Pupils of William H. Welch* (Baltimore, 1900), p. 859, who, using Flexner's method, obtained reactions with foci remote from the pancreas, namely the pericardial and subcutaneous fat, in animals in which widespread dissemination of the lesions had been produced.

calcium salts and are precipitated, while a new growth of connective tissue encapsulates the area and diminishes its size.

All this is simple and clear-cut, but unfortunately far from proved. This is particularly so when the part supposedly played by lipase is considered in the light of recent developments in the study of fat and fat metabolism. J. H. Kastle and A. S. Loevenhart[10] have demonstrated, not only that lipase is present in all the tissues of the body in which there is any utilization or storage of fat, but also that lipase acts reversibly upon fat and its components, building up fat from the fatty acid and alcohol, as well as splitting fat into these substances. This reversibility of enzymes had previously been shown for maltase by A. C. Hill; and for the ferment which synthesizes benzoic acid and glycocoll into hippuric acid, by Schmiedeberg, who, however, did not appreciate the significance of the reactions that he had observed. The work of Kastle and Loevenhart may be briefly summarized as follows:[11] Lipase will cause solutions of ethyl alcohol and butyric acid to unite to form the ester, ethyl butyrate. On the other hand, in solutions of ethyl butyrate lipase causes a splitting. In either case the end result is the same, namely, a mixture of fat, fatty acid, and alcohol; in other words, lipase simply acts to establish an equilibrium between these substances, and the effect of the enzyme is merely to hasten this equilibrium which would be attained more slowly without its aid. Subsequently it was found that all the tissues in the body that were tested for lipase showed its presence, most notably in the liver, active mammary gland, blood, lymph, and intestinal mucosa, as well as, of course, the pancreas. Of particular interest is the fact that the liver is about two times more active, weight for weight, than the pancreas of the hog, and of course in its total bulk many times more. Subcutaneous fat was found to be both lipolytic and lipogenetic, as also are the pericardial and perinephritic fat, showing the presence of lipase in adipose tissue.

The history of fat in the body may now be considered to be as follows: The lipase in the stomach does not act, because of the presence of hydrochloric acid. In the intestines lipolysis occurs, with production of a mixture of fat, fatty acid, and alcohol—usually glycerin. But, as the fatty acid and glycerin are diffusible, while the fat is not, they are separated from the fat by absorption into the wall of the intestine. Hence an equilibrium is not reached in the intestine; so the splitting continues until practically all the fat has been decomposed and the products absorbed. When this mixture of fatty acid and glycerin first enters the epithelial cells lining the intestines, there is no equilibrium, for there is no fat absorbed with them as such. Therefore the lipase, which Kastle and Loevenhart showed was present in these cells, sets about to establish equilibrium by combining them. As a result we have in the cell a

[10] *Chemical News*, Vol. LXXXIII (1901), Nos. 2150-2155; and also *American Chemical Journal*, Vol. XXIV (1900), p. 491.

[11] For a more complete discussion of fat metabolism see A. S. LOEVENHART, "On the Relation of Lipase to Fat Metabolism—Lipogenesis," *American Journal of Physiology*, Vol. VI (1902), p. 231; also H. GIDEON WELLS, "Reversibility of Enzymes and its Application to Physiological and Pathological Processes," *Journal of the American Medical Association*, January 25, 1902.

mixture of fat, fatty acid, and glycerin, which will attain equilibrium only when new additions of the two last substances cease to enter the cell. Now another factor also enters, for on the other side of the cell is the tissue fluid, containing relatively little fatty acid and glycerin. Into this the diffusible contents of the cell will tend to pass to establish an osmotic equilibrium, which is quite independent of the chemical equilibrium. This abstraction of part of the cell contents tends to overthrow chemical equilibrium again, there now being an excess of fat in the cell. Of course, the lipase will, under this condition, reverse its action and split the fat it has just built into fatty acid and glycerin. It is evident that these processes are all going on together, and that as the composition of the contents of the intestines and of the blood vessels varies the direction of the enzyme action will also vary. In the blood serum, and also in the lymphatic fluid, there is more lipase which will unite part of the fatty acid and glycerin, and by removing them from the fluid about the cells favor osmotic diffusion from the intestinal epithelium, thus facilitating absorption.

Quite similar must be the process that takes place in the tissue cells throughout the body. In the blood serum bathing them is a mixture of fat and its constituents, probably nearly in equilibrium since lipase accompanies them. If the diffusible substances enter a cell containing lipase, *e. g.*, a liver cell, the processes of building and splitting will be quite the same as in the intestinal epithelium. The only difference is that here the fatty acid may be removed from the cell by being utilized by oxidation or some other chemical transformation.

With this explanation of the physiological processes of fat metabolism in mind, a reconsideration of the subject of fat necrosis leaves one in considerable confusion. (1) Since lipase is a normal constituent of fat tissue, how can it be that the presence of lipase of pancreatic juice is responsible for the changes of fat necrosis? (2) The splitting of fat occurring normally and constantly in the fat cells, is it possible that the products of this decomposition or the lipase itself can cause the death of the fat cells? (3) The presence of masses of free fatty acids in the fat tissue does not at all agree with the idea of reversible action of lipase. If the action of lipase is to cause a balance between the fats and their acids and alcohol, the presence of added lipase in the fat tissue should have little effect; the only one conceivable would be an increased accumulation of fat, in no wise different from that due to increased ingestion of fats. (4) Ferments are not diffusible, yet one not infrequently finds the lesion deep in the fat tissue when the lipase has simply been introduced into the peritoneal cavity. Fat necrosis not infrequently occurs as a widespread process, invading not only the entire peritoneal fat, but also the subcutaneous, pericardial, and subpleural fat; it is difficult to explain this distribution and localization of a ferment. The focal character of the lesion is equally perplexing.

All these questions and facts leave much doubt as to the genesis of fat necrosis through action of lipase, while they offer no substitute explanation. The facts of which we are sure, and upon which must be based the study of the problem, are the fol-

lowing: (1) An actual necrosis of fat tissue occurs which is sharply circumscribed; (2) within the necrotic area splitting of the fats takes place, leading to the presence of free fatty-acid crystals in large masses; (3) these lesions are present in man, only associated with escape of pancreatic juice from its normal channels, and have been produced in animals always by similar processes; (4) the etiological factor may be distributed to points remote from its original site.

The questions to be answered are: (1) Is a ferment responsible for fat necrosis, or can it be produced by agents that are not ferments? (2) If a ferment, is it lipase or one of the other ferments of the pancreatic juice or of the tissues? (3) If lipase, is it the lipase of the pancreatic juice or of the fat tissue itself that is acting? (4) Is the necrosis of the tissue primary and the splitting of the fat secondary, or, as seems to be commonly accepted, is the splitting of the fat the first step and the necrosis a result of this lipolysis? (5) How is the peculiar dissemination of fat necrosis brought about, and why is the process focal?

IS A FERMENT CONCERNED IN FAT NECROSIS?

It is quite remarkable that in the experiments so far performed no attempt has been made to determine this point by the usual method for detecting ferment action, that is, the susceptibility of ferments to heat. Probably this is because nearly all experiments so far have been made by causing an injured or transplanted pancreas to yield its secretion *intra vitam*, under which circumstances study of the acting fluid was not easy. To determine the actual presence and agency of a ferment, it was necessary to secure an extract that could be manipulated outside of the body, and that would produce fat necrosis with a considerable certainty. In view of Langerhans's results, but a single focus in twelve experiments, the simple preparation made by triturating fresh pancreas with water did not offer a good prospect, but, as it was the simplest, it was tried first. The results were surprising, in that the extract of fresh hog's pancreas never failed to cause a decided fat necrosis in cats, dogs, and rabbits. Probably Langerhans's poor success was due to the much slighter activity of the pancreas of the rabbit, which he used, as compared with that of the hog. Loevenhart found that the hog's pancreas gives a much more actively lipolytic extract than does that of the dog, and it is quite probable that the herbivora would have a still weaker pancreatic juice. The extract used was made as follows: 50 grams of hog's pancreas, removed about three hours before the experiment, was washed quickly in sterile water, then in 95 per cent. alcohol, and again in sterile water, to remove as many as possible of the bacteria on the surface of the gland, necessarily present from the handling at the slaughter-house. It was then minced in a sterilized meat-chopper, collected in sterile dishes, and triturated with sterile quartz sand in 100 c.c. of the solution to be used. In one series the fluid was 1 per cent. sodium carbonate, and in another 0.4 per cent. acetic acid. The gland had of itself a slight initial acidity to phenol-phthalein. After standing a short time to permit the sand to settle out, the

fluid was strained through gauze, which permitted a considerable amount of pulp to pass into the filtrate. From 10 to 50 c.c. of this fluid was then injected into the abdominal cavity of cats, dogs, and rabbits, after the various indicated manipulations had been performed.

Protocol 2.—Dog, received intraperitoneal injection of an alkaline solution of fresh hog's pancreas. Killed after five days, and fat necrosis found.

2–11–'02. Injected intraperitoneally 50 c.c. of emulsion of fresh hog's pancreas in 1 per cent. Na_2Co_3.

2–16–'02. Appears perfectly well, and has been so ever since the first day after the injection. Killed with chloroform. *Autopsy:* Omentum lies over the upper half of the abdominal cavity, adherent by firm hemorrhagic fibrinous adhesions to the parietal wall and to the intestines. A mass of intestines is adherent beneath the omentum, and in the coils are a few small pockets of pus. Scattered about in the omental fat are many irregular areas, pinhead size and larger, appearing somewhat more yellow than the fibrin masses. There are a few such areas in the mesentery. The areas are not all near the point of greatest inflammation, but some are in fat that appears otherwise normal. Pancreas and other viscera appear normal. *Cultures* from the abdominal fluid yielded a bacillus in morphology like B. coli communis. *Histologically* the areas described are seen to be typical foci of fat necrosis, with considerable leucocytic demarcation.

Protocol 51.—Cat, received intraperitoneal injection of an alkaline solution of fresh hog's pancreas. Death after about twelve hours; typical fat necrosis.

4–22–'02, 11:30 A. M. Injected intraperitoneally, under chloroform anæsthesia, 30 c.c. of emulsion of fresh hog's pancreas in 1 per cent. Na_2CO_3.

4–23–'02, 8 A. M. Found cold and stiff. Probably had not survived the injection more than twelve hours. *Autopsy* showed an extensive fibrinous deposit over all the peritoneal surfaces, with a considerable quantity of turbid, pinkish fluid. In the omental fat were several areas that seemed to be typical fat necrosis. Pancreas and other viscera showed no change. *Cultures* from the fluid yielded the staphylococcus pyogenes aureus, and an undetermined bacillus. *Histologically* there were found in the omentum, in addition to typical minute foci of fat necrosis, areas of more general necrosis affecting all of one side of the omentum, the other side being unaffected, a sharp line of demarcation between the necrotic and the unaffected tissue being present. There were some places where the necrosis extended entirely through the omentum.

Protocol 56.—Cat, injected with alkaline emulsion of pancreas. Death after eighteen hours; result positive.

4–29–'02, 1:45 P. M. Ten c.c. of a 1 per cent. solution of Na_2CO_3 containing emulsion of fresh hog's pancreas injected intraperitoneally.

4–30–'02, 9 A. M. Found dead, but body still warm. About one ounce of turbid fluid found in the peritoneal cavity, the surfaces being cloudy and hyperæmic. A few foci of necrosis seen in the pro-peritoneal fat, retroperitoneally, and in the mesentery; more in the omentum, but nowhere very abundant. The largest are the size of a pinhead. There are also stripes of fat necrosis in the omentum extending along the fat trabeculæ. A few foci in the pericardial fat in the lower part, but none in the fat above the heart. Pancreas and other organs show no changes. *Cultures:* an agar slant developed a profuse growth, but was not worked out. *Histologically* typical fat necrosis in foci and *en masse* as in 51.

Protocol 50.—Dog, injected with acid extract of pancreas; death in about twelve hours; result positive.

4 22 '02, 1:30 P. M. Injected with 50 c.c. of an acid extract of fresh hog's pancreas, made by triturating the gland substance with 0.4 per cent. acetic acid; after straining through cheese-

cloth, the fluid was allowed to stand one and three-fourths hours at room temperature, to permit the acid to have whatever effect it might upon the enzymes present.

4–23–'02, 8 A. M. Found dead and cold; could not have survived the injection more than twelve hours. *Autopsy:* peritoneal cavity contains turbid, fibrin-flecked fluid. Omentum slightly adherent over intestines and studded with clear white spots, from pin-point size to confluent areas one or more centimeters in diameter. The mass of fat attached to the anterior abdominal walls is studded with similar spots, without evidence of inflammation. Smaller numbers were also present in the retro-peritoneal, mesenteric, and pericardial fat tissue. *Cultures* all gave growth of an organism resembling, on agar slants, the growth of B. mucosus capsulatus, but it was not followed out. *Histological* examination shows typical fat necrosis.

Protocol 53.— Cat, received acid extract of pancreas, death in about twelve hours; result positive.

4–22–'02, 2:30 P. M. Injected 20 c.c. of an emulsion of fresh hog's pancreas in 0.2 per cent. acetic acid, after it had stood for about two and three-fourths hours.

4–23–'02, 8 A. M. Found dead and cold; must have died not much later than twelve hours after injection. *Autopsy:* peritoneal cavity contained much turbid fluid and fibrin. The animal was emaciated and the fat was small in amount, but in the omentum were occasional fairly typical foci, and a few others in the mesentery, but none found elsewhere. *Histologically* the fat necrosis was found to be typical, but more extensive than appeared to the naked eye, the entire surface of the omentum in considerable areas showing a necrosis for a depth of one or two cells.

Protocol 60.— Cat, received injection of pancreas emulsion in acid; death after eighteen hours; result positive.

4–29–'02, 4 P. M. Injected into peritoneal cavity 20 c.c. of an emulsion of fresh hog's pancreas in 0.4 per cent. acetic acid which had stood two hours.

4–30–'02, 10 A. M. Found dying and chloroformed. Typical fat necrosis, moderate in abundance, distributed generally throughout the omentum, mesentery, and retro-peritoneal fat, the largest areas being not larger than a pinhead. There were also several typical areas in the pericardial fat, but none above the heart. The contents of the peritoneal cavity were slightly acid to phenol phthalein. *Cultures* showed abundant growth of unidentified organisms. *Histological* examination shows most extensive fat necrosis, often passing entirely through the omentum in large areas, and notable for the absence of leucocytic invasion.

Protocol 52.—Rabbit, injected with pancreas emulsion, alkaline, that had been boiled. Inflammation, but no fat necrosis.

4–22–'02, 3 P. M. Injected with 30 c.c. of the same emulsion as No. 51, that is, in 1 per cent. Na_2CO_3, after it had been heated to boiling in the water bath for twenty minutes.

4–23–'02, 10 A. M. Seems well; killed. Abdominal cavity contains a considerable amount of fibrinous exudate, not greatly different in amount and nature from that seen in the animals injected with unboiled extract, but no traces of fat necrosis. *Cultures* remained sterile. *Histological* examination of the omentum, which was preserved entire and examined throughout, showed no fat necrosis, merely acute inflammation.

Protocol 58.—Cat, injected with alkaline pancreas emulsion that had been heated to boiling. Killed after three days; result negative.

4–29–'02. Injected 20 c.c. of pancreas emulsion in 1 per cent. Na_2CO_3 that had been heated 10 minutes at 100° C.

5–2–'02. Has been quite well. Killed by chloroform. Peritoneal cavity shows a few threads of fibrin, otherwise no evidence of inflammation, and no signs of fat necrosis. *Microscopic* examination showed a slight leucocytic infiltration, but no fat necrosis.

Protocol 61. —Cat, received alkaline pancreas emulsion that had been heated to boiling. Killed at the end of three days. Result negative. A duplicate of No. 58.

It will be seen that in not a single case did the unboiled hog's pancreas, whether in alkaline or acid solution, fail to produce a distinct fat necrosis, while the injections of similar preparations that had been boiled failed to cause any such process, macro- or microscopically. It is thus certain that in the fresh hog's pancreas there is some substance that is destroyed by boiling, and without which the pancreatic extract is unable to cause fat necrosis. The chief criticism of these experiments is that bacteria were not excluded — there were culturable organisms in the peritoneum of all the animals with fat necrosis, while cultures from the animals injected with boiled extract were sterile, showing that the bacteria were present in the pancreatic extract as prepared. The treatment necessary to secure an active emulsion of pancreas renders it difficult, if not impossible, to maintain sterility, but fortunately it was found not to be necessary to obtain pancreas extract in this way to produce fat necrosis. It was found that the ordinary commercial "pancreatin" prepared by various firms was invariably effective, even although the preparation used was several months old.[12] There was never a failure to produce fat necrosis by injecting intra-abdominally into a cat or dog as much as 10 c.c. of a 5 per cent. emulsion of this preparation, and often the process was very extensive, even more so than any described from the operative methods used by various experimenters. It made no difference whether the solution was alkaline, acid, or neutral, as with fresh pancreas. Experiments made to ascertain the minimum amount of pancreatin that would cause fat necrosis showed that amounts as small as 0.05 gm. of commercial pancreatin might sometimes produce a few foci, and again sometimes fail entirely.[13]

Because of its simplicity and effectiveness this method can be recommended for teaching purposes, as it gives a ready method for demonstration of the appearances of fresh fat necrosis as seen by the surgeon. The frequency with which surgeons mistake this condition for miliary tuberculosis leads one, in fact, to urge that this demonstration be frequently made.

Pancreatin seems to be free from pathogenic bacteria, probably because of its dry condition; at least cultures from animals with fat necrosis produced by this preparation were quite generally sterile. This, therefore, excludes bacteria as a causative agent in these experiments, an exclusion not absolutely essential, since many investigations have already shown the non-bacterial nature of fat necrosis. Marx[14] reviews the literature of this subject and finds that in human beings the results have been variable, colon bacilli being often found, and sometimes cocci, especially when accompanying marked pancreatitis. The very variability of these results is in favor of the accidental nature of the presence of bacteria. Further, in acute peritoneal infections of other than pancreatic origin fat necrosis is not found. In experimental fat necrosis Opie found that pieces of necrotic fat were sterile.

[12] Preparations put out by the following firms were tried, and all gave positive results: Armour & Co.; Truax, Greene & Co.; Parke, Davis & Co.; Fairchild Bros. & Foster; and Merck (not the scale preparation).

[13] In these, as in all subsequent experiments, unless otherwise specified, the preparation of Armour & Co. was used, not because of any particular activity, but because it was first used and it seemed desirable to use the same preparation throughout to make the results comparable.

[14] *Virchow's Archiv*, Vol. CLXV (1901), p. 290.

As with the fresh pancreatic extract, boiling was found to prevent the production of fat necrosis, without fail, no matter what the reaction of the fluid. It now remained to ascertain the temperature at which this property was destroyed.

THERMAL DEATH POINT

A series of experiments was performed, in which 1 gm. pancreatin in 20 c.c. distilled water was kept for a period of 5 minutes at temperatures within a narrow range; then, after cooling, 10 c.c. containing about 0.5 gm. was injected intraperitoneally. Abstracts of the protocols follow:

Protocol 79.—Medium-sized dog, injected intraperitoneally with 10 c.c. of emulsion of distilled water and Armour's pancreatin, 0.5 gm., that had been heated five minutes between 55° and 60° C. After three days it was killed and abundant and typical foci were found in the omentum and in the fold of the anterior peritoneum.

Protocol 82.—Cat, received intraperitoneally 0.5 gm. pancreatin in water that had been heated to 70°, and then slowly cooled to 60° during fifteen minutes. Killed after two days. At one point in the omentum were found a few minute white points, grouped together, which microscopic examination showed to be areas of fat necrosis, with considerable infiltration with leucocytes.

Protocol 81.—Dog, received emulsion of pancreatin that had been heated for five minutes at from 68° to 70°. Killed after two days, and in the omentum are perhaps a dozen minute, pin-point-sized spots which are seen microscopically to be foci of fat necrosis, marked by intense invasion of leucocytes.

Protocol 87.—Twenty c.c. of water containing about 1 gm. of pancreatin was poured through an abdominal wound in a cat, upon which had been performed a laparotomy for the purpose of removing part of the omentum, in connection with another experiment. The mixture had been heated for about five minutes at from 65° to 71°. Killed after two days, and about the site of the ligature on the omentum the fat was found streaked with white, which in some places formed considerable areas of changed fat. More remotely in the omentum and in the mesentery were a few very minute white spots. All these white portions of the fat were found under the microscope to be necrosed fat.

Protocol 107.—A cat received 10 c.c. of emulsion of pancreatin that had been heated to from 71° to 74° for five minutes. Killed after two days and no traces of fat necrosis could be found.

Protocol 91.—Small dog, received an injection of 10 c.c. of pancreatin emulsion that had been heated during five minutes at between 75° and 79°. Killed after two days, and no fat necrosis could be found.

Protocol 94.—Through a laparotomy wound in a small dog was poured 15 c.c. of an emulsion containing 0.5 gm. pancreatin, which had been heated at from 80° to 81° for four minutes. Killed after two days and no fat necrosis could be found.

As will be seen, at temperatures not over 60° the pancreatin is still quite active, but less so than when unheated. Two experiments at temperatures reaching 70°, but staying for the greater part of the time a few degrees lower, gave a few foci that were characterized by early invasion of leucocytes and healing. A third preparation of the same temperature used in an animal that had suffered an injury to the omentum immediately before caused a much more marked necrosis, chiefly about the site of the

injury, where the action of the agent seemed to have been favored. Temperature above 71° invariably destroyed the property of the pancreatin to cause fat necrosis. Probably the death point of the active agent is not a fixed one, but will vary within slight limits in different preparations and in solutions of different reactions. This point has not as yet been investigated farther.

The agent contained in extracts of fresh hog's pancreas and in dry commercial pancreatins is, therefore, destroyed by heat; in the case of the latter the maximum temperature that can be withstood for five minutes in an aqueous solution is not above 71°. This observation seems to indicate that we have here to deal with a ferment, almost certainly, one is tempted to say, for the best-known ferments suffer loss of activity at just about such temperatures. The close agreement of the experiments pointing to a reduction of activity at temperatures from 65° to 71°, with entire loss of power above this point, offers a hopeful field for study to ascertain the exact nature of the ferment, if it is a ferment, that causes fat necrosis. Experiments on this feature are being performed, and interesting results have been obtained, which will be published in a subsequent paper, on their completion.

CAN SUBSTANCES OTHER THAN FERMENTS CAUSE TRUE FAT NECROSIS?

Since the idea of enzyme reversibility did not agree with the facts known concerning fat necrosis, more particularly the presence of large amounts of fatty acids in the cells where normally fat exists, it seemed possible that some substance that interfered with equilibrium might be present, accounting for the continued splitting without synthesis. The pancreatic juice, in addition to the ferments, contains a considerable amount of alkali carbonate. Loevenhart has found that lipase cannot synthesize the ester from sodium butyrate and ethyl alcohol, and on investigation of the literature he found much evidence that in normal metabolism the fatty acids do not form soaps. Munck has showed that soaps are highly toxic; sodium oleate, although less toxic than the palmitates and stearates, caused death of rabbits in doses of 0. 13 mg. per kilo when injected intravenously. On the other hand, free oleic acid is not toxic. Pflüger has shown that 1 per cent. Na_2CO_3 saponifies the higher fatty acids very slowly and incompletely at 37°, so that presumably there is but little chance of such a combination occurring in the body in a fluid of the alkalinity of the blood. However, it was quite natural to suspect that a solution so strong as the pancreatic fluid might cause a union of alkali with fatty acid when freshly split, as in living fat. If this occurred, since the soap cannot be synthesized into fat by lipase, it would be impossible for equilibrium to be attained, and splitting would go on until no fat remained. This would at least account for the removal of the fat from the necrotic area, although it would not explain the presence of crystals of free fatty acids observed in the necrotic cells. Accordingly the effect of alkaline solutions upon the fat tissues was tried. One per cent. and 2 per cent. Na_2CO_3 solution, sterilized in the autoclave, when injected intraperitoneally caused nothing at all comparable to fat necrosis,

although it did produce a considerable degree of acute inflammation without bacterial aid. Pancreatic extract and pancreatin did not cause fat necrosis when boiled in a solution of 1 per cent. Na_2CO_3 and injected with it, as mentioned previously, and weak acetic acid solutions were equally inert. Injection of 2 per cent. Na_2CO_3 directly into the subcutaneous fat of dogs caused no necrosis, but a diffuse hemorrhagic extravasation with œdema and leucocytic invasion. One per cent. Na_2CO_3 containing inactive pancreatin, injected into subcutaneous fat, caused an extensive inflammation with great leucocytic infiltration, but without any necrosis of tissue and nothing at all resembling fat necrosis. Strong solutions of NaOH naturally caused much greater changes. Injection of 1 c.c. of a 10 per cent. solution subcutaneously in the tissues of a dog produced a large area of gangrene very quickly; in one hour the area was well defined, of a dark red color, with sharp borders, and on section the subcutaneous tissue was hemorrhagic and softened. Microscopically stained specimens showed that there is no sharp line of demarcation between the dead and living tissue. The fat suffers just the same as the tissue in which it lies. After twenty-four hours the skin is black and soft, but still intact; beneath it is undermined by liquefaction of the subcutaneous tissue, replacing which is an oily, purulent-appearing fluid. This fluid contains so many red cells after cutting into it that it cannot be well examined until cleared up with weak acetic acid. Then it is seen to consist largely of fat droplets of varying size, in and about which are many crystals of radiating clusters of needles, each individual crystal being shaped like an icicle. The crystals are found only where the red cells are being dissolved by acetic acid, and perhaps are the result of the action of acid on soaps. Sections taken from the edge of the zone of necrosis after seventy-two hours show in hardened and stained preparations irregular areas consisting of a group of fat cells full of a solid, eosin-staining substance. In every respect these areas resemble fat necrosis, except that they are not in as regular groups and are not so well demarcated. Leucocytes that appear normal are seen within the necrotic areas, and occasionally a line of leucocytes resembling the line demarcating areas of true fat necrosis can be found. Such pictures, which are infrequent, offer no distinct differences from the early stages of fat necrosis.

The possibility that the formation of calcium soaps played an important part by precipitating out fatty acids and destroying the equilibrium suggested that perhaps an alkaline calcium solution might cause conditions resembling fat necrosis. Subcutaneous injections of saturated solution of $Ca(OH)_2$ caused merely an acute inflammatory reaction (the solution was not sterilized), without alterations, in the fat tissue, and without formation of any discernible accumulation of insoluble calcium salts after twenty-four hours.

As fat tissue contains lipase, the question arose as to the possibility of it by itself causing the changes in the fat. To study this, portions of the omentum of dogs were shut completely out of all relation to the surrounding fluids and tissues by inserting a part of the omentum in a sterilized rubber finger-cot, and then deprived of nutrition

by a ligature tied tightly about the base of the cot. In this way necrosis was produced in the fat, presumably without injury to any enzyme it might contain, without disturbing its chemical equilibrium, and without the influence of outside factors. Within three days the pieces sloughed off at the point of ligation and were found loose in the abdominal cavity, containing a soft and brownish fat. Sections of this fat showed it to be totally necrotic, with opaque, thick fibrils of connective tissue, and with but a few abnormal nuclei surviving. The fat cells were completely empty in stained and hardened sections, and there was no resemblance whatever to fat necrosis. Frozen sections stained by sudan III showed that the fat was still present in the fat cells, staining well, apparently in a perfectly unchanged condition.

The part played by fatty acids in the necrosis was studied by placing lumps of sterilized palmitic acid and stearic acid in the subcutaneous fat tissue, and in a fold of the omentum in contact with fat tissue, where it was retained by a suture that formed a pocket. After two and four days the pieces with the surrounding tissue were taken out and hardened in formalin. Miscroscopic examination showed that the lumps of fatty acids had acted like perfectly inert foreign bodies, causing no changes but proliferation and a slight leucocytic infiltration of the tissues immediately adjacent.

ACTION OF PANCREATIN POST MORTEM AND IN VITRO

Not infrequently at autopsy necrosis of the fat immediately about the pancreas, and more particularly the fat lying between the lobules of the gland, has been observed (Chiari).[15] At times cellular reaction has established the vital nature of the fat necrosis, but in other cases the necrosis is more diffuse and without cellular reaction, suggesting a change in the fat of *post-mortem* origin, quite analogous to the common self-digestion of the gland. After death the digestion of the gland destroys its consistence to such an extent as readily to permit of the escape of its fluids beyond its confines, and the question of the possibility of this pancreatic fluid producing fat necrosis after death becomes of interest. If fat necrosis is simply the reaction of fat tissue to certain enzymes, it is reasonable to expect pancreatic extracts to cause the process after death, or even *in vitro*, if the temperature is kept favorable for enzyme action. The following experiments were performed:

EXPERIMENTS IN VITRO

Pieces of fat omentum were placed in a 1 per cent. Na_2CO_3 solution containing pancreatin, and kept at body temperature in the incubator. At intervals portions were placed in formalin and examined histologically, both in frozen and imbedded sections. It was found that within half an hour the cells on the surface had lost their staining entirely, and the membrane surrounding the fat cells had assumed an increased affinity for eosin and had become thickened. The appearance was exactly similar to the change seen in fat tissue in the earliest stages of fat necrosis (see subsequent description) before the necrosed fat cells become filled with the characteristic opaque material. The changes that follow from longer exposure simply consist of a loss of nuclear stain throughout the fat tissue, and later an entire loss of even the connective tissue structure.

[15] *Zeitschrift für Heilkunde*, Vol. XVIII (1898).

POST-MORTEM EXPERIMENTS

Protocol 75.—Injected 20 c.c. of 1 per cent. Na_2CO_3 solution containing 1 gm. of pancreatin intraperitoneally in a dog, immediately after it had been chloroformed to death. The body was left at room temperature over night. At autopsy there were found throughout the peritoneal fat many groups of small ecchymotic spots, about some of which were minute pale areas, barely visible, that suggested fat necrosis, but they were too small and indistinct to be positively identified as such. Sections of the omentum (in all cases in this work the tissues were hardened in formalin and imbedded in celloidin or frozen) show areas in which the fat tissue has lost its staining properties. Some of the fat cells in these areas contain finely granular material, more are empty; never is the amount comparable to that seen in the fat cells of fat necrosis. Occasionally the contents take the eosin stain, but never the hæmatoxylin. The areas are at the margins of the fat, and in size and outline are much like foci of fat necrosis. In fact, the chief difference observable is in the small amount of granular material in the fat cells, absence of any blue stain, and absence of leucocytes.

Protocol 85.—Dry pancreatin was sprinkled upon the anterior fold of fat tissue in the abdomen of a dog that had just been killed, and the fat tissue was folded over it. The body was sewed up and kept at room temperature for seventeen hours. *Autopsy:* At the place where the pancreatin was placed there was a soft, cheesy mass of fat toward the surface; deeper in there were minute, barely visible opacities in the fat, too small to be identified. Sections showed that much of the fat, particularly on the surface, had lost its nuclear staining, and the fat cells contained a granular material which in some instances stained bluish, in other with eosin. In places this change is seen extending in a short distance from the surface, resembling fat necrosis strikingly. As a matter of fact, there is no essential difference between these changes and true fat necrosis. The differences are of degree rather than of kind.

Protocol 117.—Wads of cotton saturated with an emulsion of pancreatin were placed in the omentum, and held in place by sutures of silk, tied loosely to prevent interference with circulation. The dog was kept alive under anæsthesia for one hour, and pieces were cut out at the end of one-half hour and one hour. The dog then died, and the body was left at room temperature for eighteen hours. Sections of the tissue at one-half hour showed merely death of the peritoneum and some of the superficial fat cells, none of which contained any solid intracellular material. At one hour the necrosis had extended deeper into the fat tissue, without solidification of the contents of the fat cells. The sections of tissue removed after the body had been dead for eighteen hours showed that the outer layers of fat cells were necrotic,as in the other specimens; all of these and many deeper ones that were not necrotic were full of a solid exudate. It looked no different from the *intra-vitam* reaction, except that the process seemed to have extended somewhat since death with the development of the characteristic solid cell contents.

Protocol 78.—Dry pancreatin was dusted over the omentum of a cat that had just been laparotomized for another purpose. Five hours later it was found that the abdominal wound, which had been sutured, had ruptured and the omentum was strangulated through the opening, apparently for some time, as it looked gangrenous. In some places in the omentum where it was not gangrenous were what appeared to be foci of necrosis, still very small and indistinct. Sections through the gangrenous part showed the entire surface to be necrotic, while the deeper cells appeared normal. It resembled ordinary necrosis of fat except that the surface cells were full of granular material resembling exactly the solid cell contents of fat necrosis. In other words, this tissue shows a mixture of simple necrosis of fat,and true "fat necrosis" which might be expected because of the conditions present, namely, gangrene and pancreatin. The sections look exactly like fat necrosis, except in the diffuseness of the process and the absence

of limitation or inflammatory reaction; on the other hand, it looks exactly like the sections of *in-vitro* digestion, except in the solid material that fills some of the superficial cells. It illustrates well the relationship of these processes.

These results seem to indicate that each of the processes that occur within the fat cells in fat necrosis may be produced experimentally outside the body, or within the body after death, with pancreatin. The typical anatomical picture is not reproduced, however, either macroscopically or minutely. The difference is always essentially one of degree rather than kind, suggesting that the characteristic grouping depends upon certain factors of circulation, or other processes inseparable from life.

STUDY OF THE SUCCESSION OF CHANGES DURING THE DEVELOPMENT OF FAT NECROSIS

Previous experimental work, depending generally upon the extravasation of the secretion of the pancreas, has given little opportunity for study of the order of events that take place in the production of the typical foci of necrosis. The method of using pancreatin solution makes this very simple, and such study is of great interest. A number of experiments were performed as follows: A dog was anæsthetized and the abdominal cavity opened aseptically; the omentum was drawn out, and at points separated as widely as possible small wads of cotton, saturated with a 5–10 per cent. suspension of pancreatin in distilled water, were placed within a fold of the omentum, and retained there by a suture, tied loosely to prevent disturbances of circulation. The omentum was then returned to the abdominal cavity, and the abdominal walls were united by heavy through-and-through sutures, held together with artery forceps instead of by tying. The animal was kept anæsthetized for the duration of the experiment, and at stated intervals the sutures were loosened, the omentum brought out, and a piece containing one of the cotton wads ligated off and removed, after which the omentum was returned to the abdominal cavity. The portions of fat in the removed piece of omentum that had been in contact with the cotton were placed in formalin for study. Sometimes the animal was permitted to come out of the anæsthetic after a few hours, and killed twenty-four hours later to study the later changes, but generally it was chloroformed to death after five or six hours. Several such experiments were performed, and the results were practically the same in all, the differences being but trivial and chiefly quantitative. From them the manner of development of the typical focus of fat necrosis was found to be about as follows:

Fifteen minutes.—The changes at this time consist of a general engorgement of the capillaries between the fat cells, with, at places, hemorrhages. (The amount of hemorrhage varies greatly in different cases, and as it is much more abundant in these experiments than in simple injection experiments, probably much of it is due to the unavoidable manipulation of the omentum.) The peritoneal endothelium is swollen, and in many places necrotic, as shown by a loss of visible nuclei while the cytoplasm becomes granular and often disintegrated. Where the endothelium is destroyed the underlying connective tissue is frequently swollen, the fibrillar outlines obscured, and the eosin staining increased in intensity.

Thirty minutes.—The changes noted at fifteen minutes have become more extensive, and in a few places the fat cells nearest the affected surface have lost their nuclei and have undergone changes in their walls indicating necrosis. There is a slight leucocytic infiltration beginning throughout the fat tissue. The necrotic fat cells are entirely empty in hardened sections. In frozen sections they contain what appears to be normal fat, staining by sudan III, and without crystals.

One hour.—Many of the small vessels contain hyalin thrombi, and the infiltration of leucocytes has become more extensive. There are more necrotic cells than at thirty minutes, but they still contain normal-appearing fat, without crystals. Where the areas of hemorrhage are near the surface, when the surface is necrotic, the red cells have lost their outline and appear as a homogeneous yellow mass.

Two hours.—The changes have increased only in degree, with the exceptions that the leucocytes have to a considerable extent migrated to the margins of the areas of necrosis, and in some places form a wall about part of the area.

Three hours.—By this time the accumulation of the leucocytes at the boundaries of the necrosed tissue have become more dense, and many of the areas are well walled off, so that the resemblance to typical fat necrosis is readily seen. About this time is first seen the presence of an eosin-staining material in a few of the most superficial fat cells, which has not been dissolved out by the alcohol and ether used in imbedding in celloidin. Some of the cells are completely filled with this substance, while others are but partly so; no crystals can be found in this solid substance. In frozen sections the contents of these superficial cells are opaque, structureless, and no crystals are present; when stained by sudan III this material stains a faint yellow, in some cells not at all, with a few interspersed fat granules that stain deeply. The cells farther from the surface contain fat that stains well. Areas of hemorrhage, which are marked in some instances and slight in others, show considerable disintegration of the erythrocytes with formation of pigment, especially when the tissue between the blood and the surface is necrotic.

Four hours.—There are no essential differences between the condition at this time and at three hours, except that, because of more extensive solidification of the contents of the fat cells and more demarcation by leucocytes, the picture of true fat necrosis is often completely produced. I have never been able to find crystals as early as this. In some four-hour specimens can be found fat cells located at the margin of the encompassing wall of leucocytes in which solidification has taken place, where the solid material takes a blue stain with hæmatoxylin about the same in intensity as ordinary nuclear staining. The blue staining substance seems to form in fume-like projections from the wall of the fat cell, often inclosing clear spaces in the meshes it forms. This seems to mark the earliest stage in the formation of calcium salts, and from its location at the edge of the necroses it seems due to union with calcium salts diffusing in from the outside.

Five hours.—By this time the necrosis is extensive, and most of the fat cells that have been in immediate contact with the cotton are necrosed for a depth of from one to three rows, often deeper in places. The various stages described are merely the maximum advance for that length of time; the slighter degrees are also present in the same specimen at the same time. Occasionally single foci, consisting of but one or two fat cells that are necrotic and walled in by leucocytes, are seen deep in the tissue, and, as shown by serial section, in no way connected with the surface; sometimes these isolated areas have no evident relation to the vascular structures, but not infrequently they are on the margins of large lymph spaces which are usually widely dilated during this inflammatory process. Rarely at this time are found fatty-acid crystals in frozen sections, and still more rarely in celloidin preparations. After six hours they generally become quite abundant. When they are present in frozen sections they can usually be found in the celloidin preparations, but always in much smaller numbers.

From the sixth hour on the changes vary greatly in different cases, depending on factors to be discussed later, but by this time the production of true and typical fat necrosis has been accomplished. To summarize these studies the changes in the evolution of focal fat necrosis are as follows:

First the structures in immediate contact with the pancreatin suffer, and necrosis of the peritoneal endothelium and the underlying connective tissue may occur in a very few minutes. The process seems to extend by contiguity into the fat cells nearest, and they die as did the peritoneal connective tissue. At the same time the reaction of acute inflammation goes on, and leucocytes migrate from the vessels, both deep and superficial. They tend to accumulate about the areas of necrosis, and by the end of three hours there is usually a distinct walling off. About this time the contents of the fat cells that are nearest the surface whence the process is extending undergo a change whereby their contents become solidified, the resulting substance being so little soluble in cold alcohol and ether that it passes through the ordinary process of celloidin imbedding; this substance does not stain by sudan III, and it seems entirely to replace the fat, so that when the process has filled the cell there can be found at the most but a few minute fat granules in the affected cell. Crystals that can be recognized as fatty acids have been found first about the fifth hour, but they usually are not abundant until a few hours later; they also escape solution in alcohol and ether, at least in part, and they seem to replace the solid, non-crystalline substance that first replaces the fat. After the fourth hour a basic-staining substance begins to appear in some of the cells that are solidified and nearest the still intact portions of tissue.

The subsequent changes vary greatly. In ordinary cases the amount of necrosis does not increase much after about the eighth hour, and it may stop even earlier. What will follow after this will depend entirely upon the conditions that exist. If a large amount of very active pancreatin is brought into the peritoneal cavity, the areas of necrosis will be much larger than when the amount is smaller, and the healing will take place with corresponding slowness. It is often possible to find healing as far advanced after forty-eight to seventy-two hours in some instances as it is after eight to ten days in others. The process seems to be as follows: After the area of necrosis has been fairly well walled off by leucocytes, further extension does not occur, and the process from that time on is essentially a healing one. The leucocytes nearest the surface suffer much karyorhexis, and often large masses of nuclear detritus are formed within and between the fat cells. The solid material that fills the fat cells seems to be fatty acids that have not crystallized. This is based upon the fact that they do not stain with sudan III, but replace the fat without observable intermediary steps or infiltration from outside, and that later they seem to be directly replaced by the crystals whose structure shows them to be fatty acids. One observation that is difficult to explain, particularly on this basis, is that the solid fat cells are larger than those about them and appear to be swollen; this is possibly due to the looser formation of the fatty acids. Calcification, if the presence of the blue-staining material is to be so inter-

preted, proceeding as it does from the intact tissues, is more complete and earlier in small areas. In minute foci all the cells may be full of the blue-staining material within twenty-four hours, while with large areas this substance may be found only about the edges, and never extends into the center of the foci. The amount of leucocytic invasion varies — when the foci are small the infiltration is usually great and rapid, and anything that increases the irritation, as handling or infection, seems to increase their numbers. Within twenty-four hours the small foci may be so filled with leucocytes that almost nothing can be seen of the cell contents which have been replaced by leucocytes and their débris; oftener it requires forty-eight to ninety-six hours to produce this condition. By the end of forty-eight hours there is a noticeable proliferation of new connective tissue cells at the margin of the foci, and this varies at first inversely with the amount of leucocytic invasion; with the large foci healing is chiefly by proliferation. These new cells are large, spindle, or oval, and when they have formed a considerable band about the focus of necrosis the leucocytes are found chiefly within it, apparently accomplishing absorption of the dead fat cells and their contents. (It was this zone of new connective tissue that Balser thought represented new-formed fat cells, and to which he attributed the production of necrosis through their shutting off the blood supply.) Giant cells were never seen. Occasionally leucocytes were seen partly surrounding the ends of crystals, resembling phagocytosis, and phagocytosis of nuclear fragments was frequent. Eventually the tissue is entirely replaced by the new connective tissue, containing new vessels, and the peritoneum heals over with endothelium, rarely forming adhesions. Fibrinous exudate is not usually present on the surface of areas of necrosis unless there is infection.

The areas of hemorrhage bear no particular relation to fat necrosis; sometimes they become necrotic and sometimes not. When they do become necrotic, the red blood-corpuscles soon lose their form, and a diffuse yellow mass results, with some pigment. Within three or four days the only trace left of blood is a great amount of yellow granules of pigment in areas which otherwise resemble typical fat necrosis.

The nuclear changes that occur in the cells that become necrosed are chiefly those of karyolysis, but it is quite different with the invading leucocytes, which become fragmented, often to a great extent. The small spherical granules that are left take a very intense stain, almost black, suggesting the possibility that the trypsin has split off the proteid portion of the nucleo-proteid, leaving the densely-staining nucleic acid by itself.

DURATION OF FAT NECROSIS

The length of time that the foci exist visible to the naked eye within the abdominal fat will evidently vary greatly with the size of the lesions. When they are small they disappear very quickly, as is shown by the following experiment:

Protocol 80.—7–1–'02. Injected into the abdominal cavity of a dog 10 c.c. of water containing 0.5 gm. of a preparation of pancreatin that was not very active. After the first day the dog seemed well.

7-5-'02. Opened the abdomen aseptically, under chloroform anæsthesia. In the fat of the fold of peritoneum that lies in the anterior median line of the dog's abdominal cavity were found perhaps a dozen typical foci of fat necrosis, none larger than half the size of a pinhead. A portion of the fat tissue was ligated off and removed for study. It showed typical foci, partly replaced by young connective tissue, and invaded throughout by leucocytes.

7-12-'02. Killed by chloroform. Absolutely no trace of fat necrosis could be found. A piece of tissue removed from the proximal side of the ligature, where there had been areas of necrosis seven days before, and examined histologically, showed a number of small foci of hyalin connective tissue, containing many small blood vessels and numerous large connective tissue cells.

In this case healing had been so complete in eleven days that nothing was visible to the naked eye, but the original foci had been minute. On the other hand, when large areas are produced the healing changes may not be very far advanced in eight or ten days, and it would probably be several weeks before they would be invisible, if ever. There are many cases in human beings, where foci were still abundant, in which death has occurred at much greater intervals after an operation at which the fat necrosis was detected, than in the experimental animals. However, in these cases the pancreatic juice was escaping more or less continuously, and the lesions found at autopsy may have been formed later than those seen at operation.

Fat necrosis, *per se*, seems to be of no great moment as regards the life of the subject. It is not the fat necrosis that causes the symptoms or the fatality, but the lesions of the pancreas that produced the fat necrosis, or the resulting shock, peritonitis, or gangrene. Fat necrosis is merely one of the results of escape of pancreatic juice from the gland, and means nothing more to the surgeon than that such escape has occurred. As has been observed by surgeons, a patient may recover from extensive fat necrosis without anything having been done to the pancreas itself. An animal may have extensive fat necrosis and yet appear perfectly well.

TIME OF APPEARANCE OF FAT NECROSIS

A few human cases have been observed in which it was possible to note that extensive fat necrosis could be produced within a comparatively few hours. H. Marx[16] reports a case in which laparotomy was performed upon a case of hemorrhagic pancreatitis, and no traces of fat necrosis found. Death occurred twenty-seven hours after the operation, and at autopsy, six hours after death, there was abundant fat necrosis, "areas the size of a lentil" being found. Marx also quotes a case reported by Simmons in which similar findings were made at operation and at autopsy, thirty-six hours apart. A few experiments were made upon animals to determine the minimum time. Since experiments showed that changes were visible microscopically as early as fifteen minutes after application of pancreatin and distinctly recognizable fat necrosis was present at the end of three hours, it was merely a question of how early the changes are in sufficient amount to be visible to the naked eye as the typical sub-endothelial white spots. The experiments made with wads of cotton could not be used for this purpose because there was so much hemorrhage and hyperæmia as to obscure the early changes, besides

[16] *Virchow's Archiv*, Vol. CLXV (1901), p. 290.

not being at all comparable to the ordinary conditions of fat necrosis. However, even here there could be seen at the end of four hours a number of minute white streaks that were highly suggestive of fat necrosis.

One experiment consisted of placing a large lump of cotton saturated with 5 per cent. emulsion of pancreatin upon the omentum, and examining the omentum under it at frequent intervals, the animal being anæsthetized. In something less than three hours and twenty minutes a few very small foci could be seen. Of more value was the following experiment: Ten c.c. of a 1 per cent. Na_2CO_3 solution containing 0.5 gm. of pancreatin was injected into a dog. After five and a half hours it was killed with chloroform, and abundant small foci were present in the omentum opposite the point of injection, and a few small areas in the mesentery. Histologically there was found abundant typical fat necrosis.

In another dog, that died somewhere about twelve hours after injection, very extensive necrosis was present throughout all the abdominal fat, and even in the pericardium.

It would seem that the areas become visible to the naked eye when the solidification of the contents of the foci renders them opaque and white, which is generally about four hours after the pancreatin comes in contact with the tissues.

MANNER OF EXTENSION

Fat necrosis is not only found upon the surface of fat tissue lying in contact with the peritoneum, but the areas may be found deep within the intra-abdominal fat tissue; furthermore, they may be found outside the abdominal cavity, in the pericardial and mediastinal fat, and in the subcutaneous tissue. This has been observed in the human cases and also in the domestic animals. As it is difficult to understand the relation of the escape of pancreatic juice to such remote lesions, some have thought that this remote process may be of a different nature. E. L. Opie[17] found, however, that equally widespread fat necrosis could be produced experimentally in cats after the pancreatic ducts had been ligated for twenty days or more. As in human cases, the distribution was chiefly in the pericardium and subcutaneous fat, but foci were also observed along the carotid and subclavian arteries. Animals that survived but a few days did not show fat necrosis outside of the abdomen, except one animal which received pilocarpin after the ducts had been ligated, in order to increase the pancreatic secretion, and which showed foci in the pericardial fat four days later. Opie hardly explains the way in which the agent causing fat necrosis reaches the pericardial fat, and the fat about the great vessels in the neck. He observed that the process occurred only in animals whose pancreatic duct had been ligated for long periods, and that the foci in the pericardium showed a much younger state than those in the omentum. Also the process was less extensive more remote from the pancreas. Hence he speaks of the distant parts as being perhaps "reached by gradual diffusion through continuous layers of connective tissue." With this supposition I can hardly agree. In the first place, the

[17] *Contributions to the Science of Medicine by Pupils of William H. Welch*, 1900, p. 859; also *Johns Hopkins Hospital Reports*, Vol. IX (1900), p. 859.

intra-thoracic and subcutaneous foci, however numerous, are separated by considerable amounts of fat tissue that is intact. Again, I have often observed small foci deep in the omental fat, entirely separated from the surface by fat microscopically normal. Neither is any great length of time required to produce extra-abdominal foci, as is shown by the experiments of the protocols of which the following are brief abstracts:

Protocol 50.—Dog, received 50 c.c. of an emulsion of fresh hog's pancreas in 0.2 per cent. acetic acid intraperitoneally. Death occurred about twelve hours later. Foci of fat necrosis abundant throughout the abdominal fat, and in the lower part of the pericardial fat were a few small areas.

Protocol 56.—A cat, which received intraperitoneally 10 c.c. of emulsion of fresh hog's pancreas in 1 per cent. Na_2CO_3, died after about eighteen hours. Moderate amount of fat necrosis in the abdominal fat, and in the lower part of the pericardium a few foci were found, but there were none above the heart.

Protocol 60.—A cat received intraperitoneally 20 c.c. of an emulsion of fresh hog's pancreas in 0.4 per cent. acetic acid. Killed after eighteen hours. Fat necrosis was distributed throughout the abdominal fat in moderate amount, and a few foci were found in the lower part of the pericardium.

Protocol 101.—A small cat received intraperitoneally 10 c.c. of an emulsion containing 0.5 c.c. of pancreatin (Truax, Greene & Co.) in water. Killed twenty-four hours later, and an enormous amount of fat necrosis was found in the abdomen, in addition to which abundant foci were found all over the pericardium and in the fat above it as high as the clavicles, along which were a few spots. There were no foci along the ribs, but there were a few in the posterior mediastinal fat.

Protocol 92.—Injected into a dog 10 c.c. of water containing 0.5 gm. pancreatin. Killed after two days. In addition to a very extensive fat necrosis throughout the abdominal cavity, there were a few foci in the pericardium, but none elsewhere.

Protocol 77.—A large cat received 5 c.c. of water containing 0.25 gm. of pancreatin. Killed after nine days. Most extensive fat necrosis throughout the abdomen, many typical foci in the subcutaneous fat from the costal arch to the umbilicus, several large foci on the pericardium as high as the base of the heart, but none above that point. Extending along the ribs, beneath the pleura on each side, are rows of small foci, most abundant about the middle of each thoracic wall, although there are many as far back as the costo-vertebral junction.

If one considers the distribution of these lesions, especially as seen in 77 and 101, and in the human case described by Chiari, and in the experiments of Opie himself, the probability that the path of dissemination is the lymphatics is strong. In what other way could such a localization occur without involving all the intervening tissue? As will be noticed, all the foci are found along the course of the lymphatic channels, particularly the beadings between the ribs, along the course of the intercostal vessels. In just such a way is miliary tuberculosis spread, with the bacilli localizing their action at various points along the lymph vessels, favored by local stagnation or obstruction. In favor of this view is the following observation: In the fat below the surface of the omentum in the experiments performed to show the sequence of changes, there were frequently observed minute foci of fat necrosis appearing about five hours after the process had been started, involving at first but one or two cells, but later spreading so that they sometimes formed foci of a size readily visible to the naked eye. While in their earliest stages it was possible to determine that the starting-point was a cell in contact

on one side with a lymph space, which was readily seen, as all the lymphatic channels were greatly distended because of the inflammatory process. As there could be found in serial sections no other way for the necrotizing agent to pass from the surface to these areas, it seemed probable that the lymph had brought it from the eroded surface.

Vascular transportation is improbable because of the location of the secondary foci. One experiment only was made with intravascular injection, with entirely negative results. Five c.c. of water containing 0.25 gr. of pancreatin was injected into the femoral vein of a medium-sized dog. On the following day the dog appeared somewhat weak, but not in bad condition, and after twenty-four hours it was killed. Autopsy disclosed no lesions in the body, except inflammation at the point of injection.

The peculiar character of the lesions themselves, their formation in minute foci like miliary tubercles, is another characteristic of the process. This seems to be due entirely to local conditions. From the way the omentum and intestines lie in the abdominal cavity it is evident that any injected or extravasated fluid must lie in small nooks and crannies, and thus come in contact only with small areas of fat which alone suffer. In proof of this is the fact that when the amount of injected material is large and active, so that a large surface of omental fat comes in contact, the entire surface necroses *en masse* and bands of friable white fat are seen that bear no resemblance to the more common focal lesions except in color and consistence. In fat necrosis produced by placing wads of cotton saturated with pancreatin in contact with fat tissue, the necrosis occurs chiefly in lines and masses on the surface in contact. When fat necrosis arises from pancreatic lesions, the omentum and peripancreatic fat nearest the gland often form one necrotic mass, while farther away, where the fluid is present in smaller quantity and thus not affecting large surfaces, the focal character is seen. In experiments upon dogs, in which a feeble solution was used so that the resulting foci were few in number, they were usually almost entirely limited to the fold of fat that lies along the anterior surface of the peritoneum, where the injected fluid naturally gravitates in these quadrupeds.

The earlier writers found that in human fat necrosis the lesions tended to be limited by the normal septa of the fat, confining it to individual lobules, and they considered this lobular character of some importance. It was found in these experiments that when spreading fat necrosis reached connective tissue septa it stopped there, although the connective tissue was often somewhat involved in the necrosis. However, the necrosis bears no relation to the septa further than this, and the septa only form the boundary when the necrosis spreads sufficiently to reach them; small foci are bounded by intact fat cells or by leucocytes.

SUMMARY

1. Fat necrosis seems to be merely a special form of necrosis of fat tissue, differing from simple necrosis chiefly in the sharp limitation of the affected area, usually by a wall of leucocytes and later by connective tissue; and the filling of the necrosed cells with products of fat-splitting. Each of these features can be produced experi-

mentally in various ways, but the complete picture has never yet been produced except by products of the pancreas.

2. Fat necrosis can be produced with constancy in cats, dogs, and rabbits by intraperitoneal injections of emulsion of fresh hog's pancreas.

3. Equally constant results can be obtained with ordinary commercial "pancreatins."

4. The results are the same with solutions in weak alkalies (Na_2CO_3), weak acids (CH_3COOH), or in water.

5. This property of the pancreatin to produce fat necrosis survives heating for five minutes at a temperature as high as some point between 65° and 71° C.; above this point the property is entirely lost. The amount of fat necrosis produced decreases steadily after exposures of 55° and upward. These observations point to enzyme action as the source of the condition.

6. Fat necrosis produced in this way is the same in appearance as human fat necrosis, both macro- and microscopically.

7. Dissemination outside the abdominal cavity has been observed as early as twelve hours after intraperitoneal injection. The route by which the spreading is accomplished is probably the lymphatic system.

8. The forms of the foci produced depend upon the areas exposed to the action of the pancreatin.

9. The earliest change in fat necrosis is a simple necrosis of the surface tissue, which extends gradually into the deeper fat cells.

10. Fat-splitting is subsequent to the necrosis, and not its cause. At first the products are non-crystalline, but become so later.

11. Calcification occurs probably by diffusion of the calcium salts from the fluids of adjacent intact areas.

12. The process progresses for but a few hours at any one point, the extension seeming to be limited by surrounding leucocytes.

13. Absorption of the area is accomplished by leucocytes, and healing is by proliferation of connective tissue from the margins. Adhesions are seldom formed.

14. The foci become visible to the naked eye in three to five hours. They may disappear within eleven days, or persist for a much longer time, depending chiefly upon their size.

15. Fat necrosis by itself is not dangerous to the affected animal, and may cause no observable symptoms.

16. The lipase of the fat tissue does not destroy the fat when the tissue has been made necrotic and preserved from outside influences of absorption, etc.

17. Simple alkaline solutions of the strength of pancreatic juice, or slightly stronger [$NaOH$, $Ca(OH)_2$, Na_2CO_3], do not produce fat necrosis.

18. Many of the features of fat necrosis may be produced after death in animals, and also *in vitro*, with pancreatin, but the resulting condition does not resemble fat necrosis at all closely.