FATTY INFILTRATION AND CIRRHOSIS OF THE LIVER IN DEPANCREATIZED DOGS MAINTAINED WITH INSULIN *

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It has been shown in this laboratory that completely depancreatized dogs treated with insulin survive for long periods when maintained on a diet adequate in calories, proteins, salts and vitamins, but lacking pancreas.[1] Under such conditions a number of pathological changes appear in the tissues of these animals. Bilateral cataracts have been found as early as 1 year following pancreatectomy.[2] A disturbance has also been found in the blood lipids: all constituents, in particular cholesterol esters, have markedly fallen soon after excision of the gland.[3] The most striking change occurs in the liver, in which large amounts of fat are deposited. These fat changes in blood and liver appear in the absence of pancreas in the diet, for by the addition of pancreas the fall in blood lipids, as well as the accumulation of fat in the liver, can be prevented.[4]

For the present report, the anatomical changes associated with fatty livers have been examined at various intervals following pancreatectomy. Although fatty livers may appear early and remain for long periods following excision of the pancreas, a regression in the fat content of the liver finally occurs. In 3 dogs that survived between 4 and 5.5 years the fat content of the liver returned to levels close to normal. During this entire period of observation the livers of the 49 dogs examined showed two types of lesions. The first of these occurred in association with the early infiltration of fat, while the second or final stage appeared most characteristically in those livers in which the regression of fat had taken place. Such livers showed an extensive periportal fibrosis with irregular lobulation indicative of cirrhosis. The depancreatized dog thus provides a new method for the production of

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experimental cirrhosis, this occurring as a final stage in response to a fatty infiltration of long standing in the liver.

Experimental

The preparation and treatment of the depancreatized dogs employed in this study have been described elsewhere.[1] After pancreatectomy each dog received twice daily a mixture containing meat, sucrose and bone ash. Vitamin supplements (A and D as cod liver oil *; the B complex in the form of a concentrate obtained from rice bran **) were added to the diet mixture twice a week. Each dog received 16 units of insulin daily, 8 units at each time of feeding.

A careful search for pancreatic tissue was made in all depancreatized dogs at autopsy. The completeness of pancreatectomy in all animals recorded in this study was verified.

The autopsies were performed immediately after the animals were sacrificed or within a few hours after death. A complete autopsy was performed in every case, and blocks of tissue were removed from all organs for histological study. After the blocks were removed from the liver for histological examination, the rest of the organ was immediately ground and thoroughly mixed, and samples were taken for determinations of total fatty acids, cholesterol and phospholipids. The methods employed for lipid analyses have been described elsewhere.[4]

I. The Fatty Changes in the Liver after Pancreatectomy

Although, as already pointed out, large amounts of fat may appear in the liver soon after pancreatectomy, extremely fatty livers are not of constant occurrence in the early periods. Not only the degree of lipid infiltration, but also the time of onset of such changes show considerable variation. Thus, mixed samples of the entire livers obtained from 2 dogs that received the same treatment contained 32 and 11 per cent of fatty acids at an interval of 3.5 weeks after pancreatectomy. Equally significant variations were found at other intervals. In a series of 30 dogs, however, it

* The standardized cod liver oil used in this study was kindly furnished in part by Mead Johnson and Company, Evansville, Indiana.

** The rice bran concentrate was kindly furnished by Vitab Products, Inc., Emeryville, California.

was found that fatty acids in excess of 14 per cent were present at an interval of 20 to 30 weeks after excision of the gland.

In the normal dog's liver the lobules are not outlined by limiting strands of connective tissue and there is an extremely scanty amount of connective tissue associated with the portal triads. The finer biliary radicles are usually difficult to demonstrate. The central veins can be distinguished: they are situated at regular distances from the portal triads. The cords of hepatic cells are evenly arranged, and the sinusoids radiate uniformly from the portal triads to the central veins. The hepatic cells occasionally contain a small vacuole which is shown by a scharlach R stain to be fat. By chemical analyses such livers have been shown to contain approximately 3 to 4 per cent of fatty acids.

Microscopic Appearance of Livers Containing Various Amounts of Fat Obtained from Depancreatized Dogs in Good Condition

The livers were removed at various intervals after pancreatectomy. In livers containing relatively small amounts of lipids (about 10 per cent), the fat was deposited in large globules replacing the cytoplasm of cells in scattered areas within a lobule (Fig. 1). In some cases the fat globules were irregularly distributed toward the mid portion of a lobule. No change other than the usual displacement of the nucleus was observed in the cells of these livers. In general, the size of the fat globules was uniform.

In livers showing a greater lipid change (approximately 18 per cent), fat was found in almost every cell of the lobule (Fig. 2). The largest globules of fat were generally found in the cells around the centers of the lobules, while the remainder of the cells showed small droplets scattered throughout the cytoplasm. In many of the cells around the portal triads the cytoplasm had become granular and somewhat hyaline in appearance. There were no nuclear changes. The sinusoids were not easily distinguished. The cells of the large bile ducts occasionally contained fat in their cytoplasm.

When about 30 per cent of fat had accumulated in the liver, almost every hepatic cell was completely replaced by fat (Fig. 3). A rare cell, however, retained some cytoplasm which frequently had a hyaline, granular appearance. The sinusoids were completely obliterated and their compressed cells varied in size and

shape. Nuclei may not be present in every cell. There were no changes in the structure of the portal triads. Nearly all the lining epithelial cells of the bile ducts contained fat.

Distribution of Fat in the Liver

The distribution of lipids was studied in the livers of depancreatized dogs in which various degrees of fat infiltration were produced experimentally. Blocks of tissue, varying from 4 to 6 sq. cm. in area and from 3 to 4 mm. in thickness, were removed from each of the lobes for histological examination. Three consecutive slices were removed from each block of tissue. The middle section was used for histological study while the two end slices were combined for chemical analysis.

In livers containing little fat this was distributed in a fairly regular and equal manner throughout the various lobes. Moreover, the sections showed that the fat was evenly distributed within the lobules (Fig. 1). With increasing amounts of fat, however, the various lobes at times showed extreme variations in their fat content (Fig. 2). This was confirmed by chemical analyses. The lobular distribution of lipids likewise varied in these cases; some showed nearly all of the cells filled with fat-stained globules, while others had fat only in the peripheral cells or around the central vein. In a liver containing about 50 per cent of fat the lobes showed very little variation in lipid distribution and the lobules showed all cells uniformly filled with fat (Fig. 3). Thus, while livers that contain either very small or very large amounts of lipids have the fat uniformly distributed, livers in which a moderate degree of lipid infiltration has occurred may show an uneven distribution of fat. The storage and liberation of fat apparently do not occur in a constant or regular manner in the various parts of the liver.

II. Early Fibrotic Changes

A second group of livers developed, in addition to the fatty changes noted above, a prominence of the portal spaces caused by a fibrous tissue proliferation that is never seen in the normal liver. The lobulation, however, is not complete, nor is the extensive distortion of the parenchyma so marked as in the cirrhotic livers to be described below. There is some proliferation of the bile ducts. The hepatic cells near the portal fibrous tissue septums generally

show a hyaline granular alteration of the cytoplasm, and occasional cells have undergone marked fatty changes. All these livers show a variable degree of lymphocytic and plasma cell infiltration in association with the growing connective tissue.

1. Dog G8: Female, depancreatized April 2, 1931. This animal survived for 3.3 years after pancreatectomy, at the end of which time it was sacrificed for examination of the liver.

Microscopic Appearance of Liver: The portal spaces were prominent, as there was definite fibroblastic proliferation around them, which gave the liver a definite lobular pattern. The hepatic cells showed extreme variation in fat content, large groups of cells being completely filled whereas adjacent groups had a granular hyaline cytoplasm with no visible fat. The central veins were not easily distinguished (Fig. 4).

2. Dog DJ: Female, depancreatized June 29, 1932. This animal was in good condition until 3 days before Feb. 4, 1934, when it died. The period of survival was 1.6 years. At autopsy an extensive retroperitoneal hemorrhage and cellulitis, which also involved the heart and aorta, were found. Focal hemorrhage and leukocytic infiltration of the right auricular muscle were present.

Microscopic Appearance of Liver: The histological appearance of this liver was similar in all details to that of Dog G8. The terminal acute infectious process had not altered the previous changes in the liver.

3. Dog DG: Female, depancreatized Aug. 1, 1932, died Feb. 14, 1934. Period of survival 1.5 years. This animal refused food for 2 weeks before death occurred. Autopsy revealed an acute urinary tract infection. A mixed sample of the liver contained 29.2 per cent of total lipids.

Microscopic Appearance of Liver: There was a diffuse fatty infiltration in all parts of the liver. The periportal fibrous tissue was arranged in rather thin bands and appeared condensed. Occasional leukocytes, lymphocytes and plasma cells were present in the fibrous tissue. Groups of cells lying against the fibrous tissue septums showed hyaline changes.

4. Dog K: Male, depancreatized Dec. 25, 1930, died Sept. 12, 1933. Period of survival 2.7 years. For several weeks before death this animal lost its appetite and finally refused all food. It was

emaciated at the time of death. At autopsy bronchopneumonia and an acute pyelitis were found. The liver weighed 920 gm. (*i.e.*, 14.2 per cent of the final body weight). A mixed sample of the whole liver contained 33.8 per cent of total lipids.

Microscopic Appearance of Liver: The tendency toward the formation of lobules by a slight periportal fibrosis was seen in all sections. All hepatic cells were well filled with fat, so that the peripherally situated cells rarely showed the hyaline alteration of the cytoplasm. Proliferation of the bile ducts was not distinct. The bile canaliculi occasionally contained plugs of inspissated bile. In the periportal fibrous tissue lymphocytes and plasma cells were rare.

III. Cirrhosis of the Liver in Depancreatized Dogs

Extensive cirrhosis was found in 4 dogs. In 2 of these (dogs DA and DB) the livers presented the characteristic hob-nailed appearance. The surface was reddish brown in color and was covered by nodules of varying size, the largest of which measured 10 mm. in diameter. The liver on cut section felt sclerotic, and the cut surface showed the parenchyma to be composed of irregular lobules. In the remainder of the animals the surfaces of the livers appeared normal. All livers were enlarged. In all cases the gall-bladder and the extrahepatic bile ducts were normal on gross examination. Bile could be expressed through the papilla by pressure on the gall-bladder. The animals were not jaundiced. All the organs were examined at autopsy and routine sections taken from each one. The protocols of these animals are as follows:

1. Dog DA: Female, depancreatized March 11, 1931. This animal was in good condition at the end of the period of maintenance, Sept. 25, 1936. Its period of survival was 5.5 years. At autopsy the liver was found greatly enlarged and hob-nailed in appearance. It weighed 565 gm., or 7.5 per cent of the body weight. A mixed sample of the liver contained 5.6 per cent of total lipids.

Microscopic Appearance of Liver: A striking amount of fibrous tissue was found around the portal spaces (Fig. 5). These spaces were irregularly distributed, and the fibrous tissue branched from one to another. In this manner the liver parenchyma had been divided into sharply circumscribed lobules that varied extremely in size and shape. Glisson's capsule was greatly thickened. The

central veins were not easily distinguishable and were eccentrically placed. Within the lobules the hepatic cells were arranged in distorted cords compressing the sinusoids. These cells contained a variable amount of fat; in some lobules many cells might contain a large globule of fat, whereas in the adjacent lobule the cells might have very little. Some cells had a markedly vacuolated cytoplasm, whereas others contained a cytoplasm of a granular or hyaline nature. There was a great variation in the thickness of the fibrous septums. Usually there was an associated lymphocytic and plasma cell infiltration, together with a prominent proliferation of the small bile ducts. In the fibrous septums a group of hepatic cells would often be enclosed, and these cells generally showed a condensation of the cytoplasm into hyaline granular masses (Fig. 6).

2. Dog DB: Male, depancreatized July 27, 1932. This animal was in good condition at the end of the period of maintenance, Sept. 25, 1936. Its period of survival after pancreatectomy was 4.2 years. At autopsy the liver was found greatly enlarged and its surface hob-nailed in appearance. It weighed 400 gm., or 5.7 per cent of the body weight. A mixed sample of the entire liver contained 3.5 per cent of total lipids.

Microscopic Appearance of Liver: This was similar in all details to that observed in Dog DA described above.

3. Dog DC: Female, depancreatized Sept. 1, 1931. This animal was in good condition at the end of the period of maintenance, Sept. 29, 1936. Its period of survival was 5.1 years. At autopsy the liver was found enlarged, weighing 480 gm., or 4.5 per cent of the total body weight. A mixed sample of the whole liver contained 3.9 per cent of total lipids.

Microscopic Appearance of Liver: Connective tissue was present in fine radiating strands usually extending outwards from the portal triads in irregular fashion. The lobular pattern could be readily distinguished, but the fibrous tissue septums were not so prominent as in dogs DA and DB. The hyaline alteration of the cytoplasm of the cells at the periphery of the lobule was, however, more striking in this animal than in those described above. Some cells were also undergoing marked shrinkage or showed complete replacement of the cytoplasm by fat.

4. Dog DE: Depancreatized Sept. 5, 1932. This dog was maintained for 2.3 years on the stock diet recorded above, which contained no raw pancreas. For the next 15 weeks it received 250 gm. of raw pancreas daily in addition to the regular stock diet, and at the end of this time it was sacrificed for study of the liver. The total period of survival was 2.6 years, during which the animal was in good condition. The liver was large and weighed 640 gm., or 7.4 per cent of the body weight. A mixed sample of the entire liver contained 4.6 per cent of total lipids.

Microscopic Appearance of Liver: The histological structure was similar to that observed in Dog DC.

In 6 dogs that survived for periods longer than 1 year no cirrhosis or abnormal degree of fibrosis was found in the liver. One of these (Dog DF) was examined 3.1 years after removal of the pancreas, whereas in the 5 other animals (dogs G1, A1, A3, G3 and G2) the livers were removed for study at intervals between 1.3 and 1.8 years after pancreatectomy.

Discussion

The results of the present investigation demonstrate the occurrence of cirrhosis of the liver under conditions not hitherto described. Sixteen completely depancreatized dogs were maintained for periods longer than 1 year. The tissues in 14 of these animals were subjected to a careful histological study. Extensive cirrhosis was found in 4 dogs, while in 4 others an abnormal degree of fibrosis was present. Infection seems to play no part in the production of this scarring, for the 4 dogs in which the most marked cirrhosis was found showed no other pathological changes when sacrificed. Incidental acute infections were present in 3 of the second group of 4 dogs. From the appearance of the livers, however, it is obvious that these terminal infections are in no way related to the fibrotic changes that occurred. In all dogs recorded in this study there was no evidence of obstruction in the extrahepatic bile passages at autopsy. The type of lesion produced does not resemble in any particular the changes associated with infection or extrahepatic biliary obstruction.

A constant finding in all these dogs is an early increase in the amount of fat in the liver. This usually takes place in the first few

months after pancreatectomy and remains for long periods. It was shown by chemical analyses that a fatty liver may be present as late as 3 years after pancreatectomy. The fat first appears in scattered cells within a lobule and slowly extends outward from the central veins. The peripheral cells, however, are the first to show cytoplasmic alterations and this is followed by hyaline atrophy of the whole. The process is apparently slow and stimulates avascular fibroblastic proliferation. A few lymphocytes and plasma cells accompany this reaction. Strands of fibrous tissue then intertwine around other peripherally located cells, thus isolating them and making the process a progressive one.

In all livers showing fibrosis some cells can always be found which show extreme hyalinization and granularity of the cytoplasm. These granules may be pushed off to one side if a fat globule is present in the cell. Usually the cells containing this marked cytoplasmic change are arranged in small groups.

The time of onset of these fibrous changes in the liver remains to be considered. No direct relation between the interval after pancreatectomy and the degree of fibrous proliferation was found in the 8 dogs studied. Thus, while all the animals that survived between 4.2 and 5.5 years showed marked cirrhosis, a greater degree of fibrosis was found in 1 dog 2.6 years after pancreatectomy than in another 3.3 years after. No evidence of fibrosis was found in the first few months after pancreatectomy; the earliest signs of such changes occurred after an interval of 1.5 years.

Summary and Conclusions

Depancreatized dogs constantly develop fatty livers at variable periods after the operation has been performed. In those kept for from 2.6 to 5.5 years upon an adequate diet and insulin, 8 of 16 developed more or less interlobular fibrosis of the liver associated with a hyaline or colloid degeneration of many cells and hyaline atrophy of peripheral cells. In 4 animals this was so pronounced, both grossly and microscopically, that the picture of a well advanced portal cirrhosis of the liver was present. By the time this severe cirrhosis had occurred, the fat content of the livers had returned to normal and there was little histological evidence that a markedly fatty liver had preceded the fibrosis. The sequence of events appears to be fatty infiltration, hyaline degeneration and

atrophy of cells at the periphery of lobules, and fibroblastic proliferation in orderly fashion, ending with the typical hob-nail appearance and fibrotic structure of cirrhosis. Necrotizing agents introduced from the outside, infection, and extra-hepatic biliary obstruction were excluded as causative factors.

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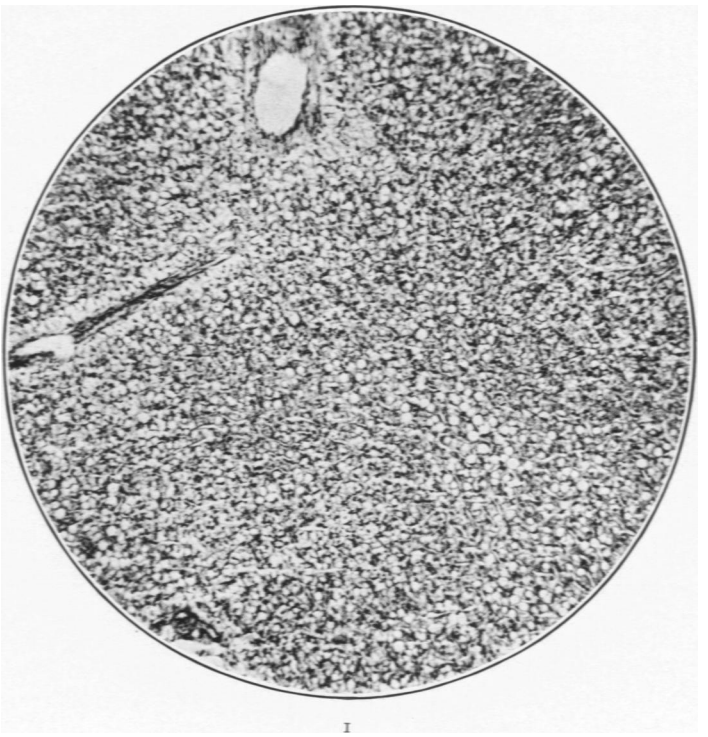
DESCRIPTION OF PLATES

PLATE 20

FIG. 1. Liver of Dog D100A showing early stage of fatty infiltration 3.5 weeks after pancreatectomy. Fatty acid content 10.5 per cent. Hematoxylin-eosin stain. × 73.

FIG. 2. Liver of Dog D94D showing fatty infiltration of intermediate degree in liver 14.5 weeks after pancreatectomy. The liver immediately adjacent to this area contained 17.5 per cent fatty acids. Hematoxylin-eosin stain. × 73.



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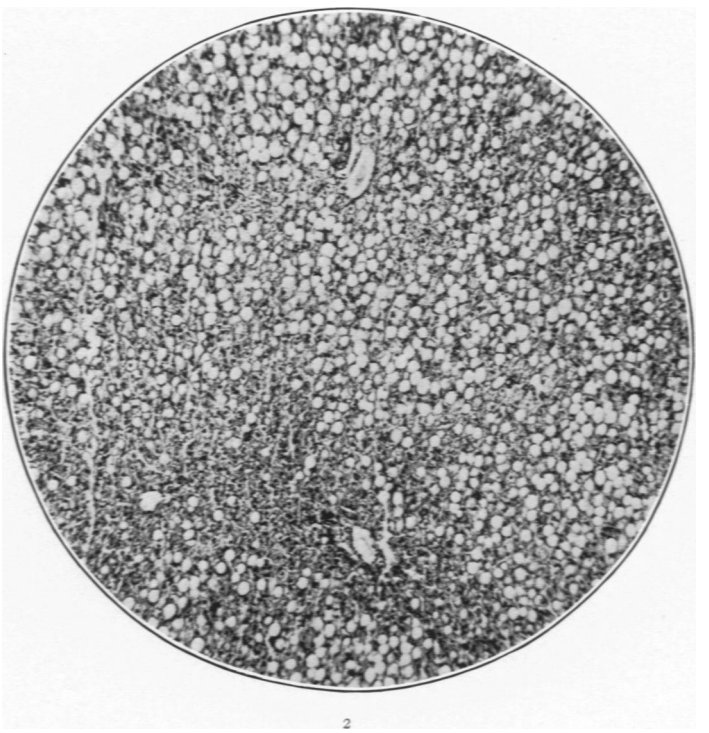

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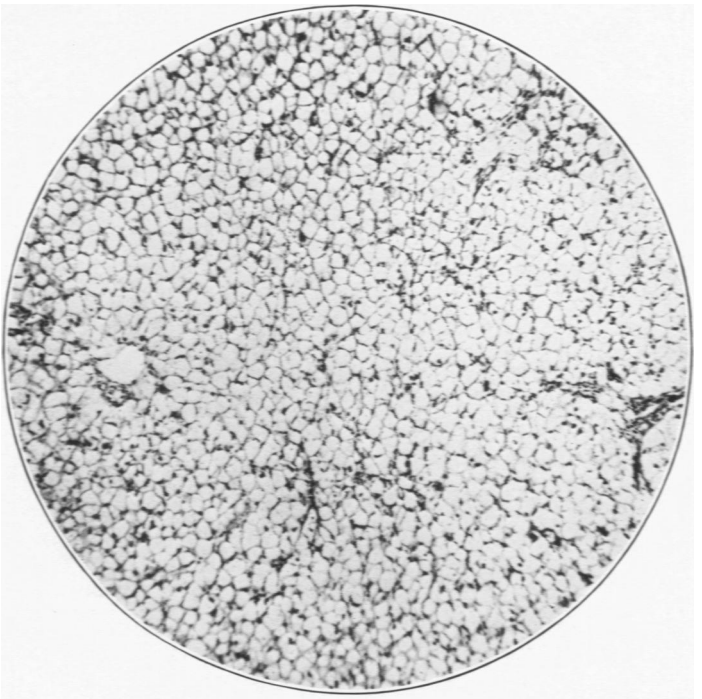
PLATE 21

FIG. 3. Liver of Dog D1 showing advanced degree of fatty infiltration 34 weeks after pancreatectomy. A mixed sample of the remaining liver contained 26.3 per cent fatty acids. Hematoxylin-eosin stain. × 73.

FIG. 4. Liver of Dog G8 showing fibroblastic proliferation with decrease in amount of fat present 3.3 years after pancreatectomy. Phosphotungstic acid hematoxylin stain. × 73.



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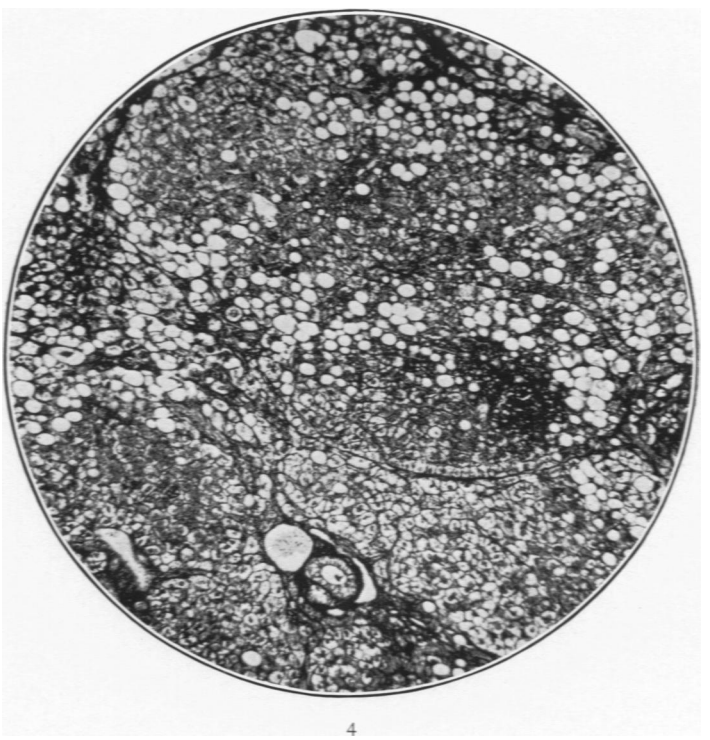

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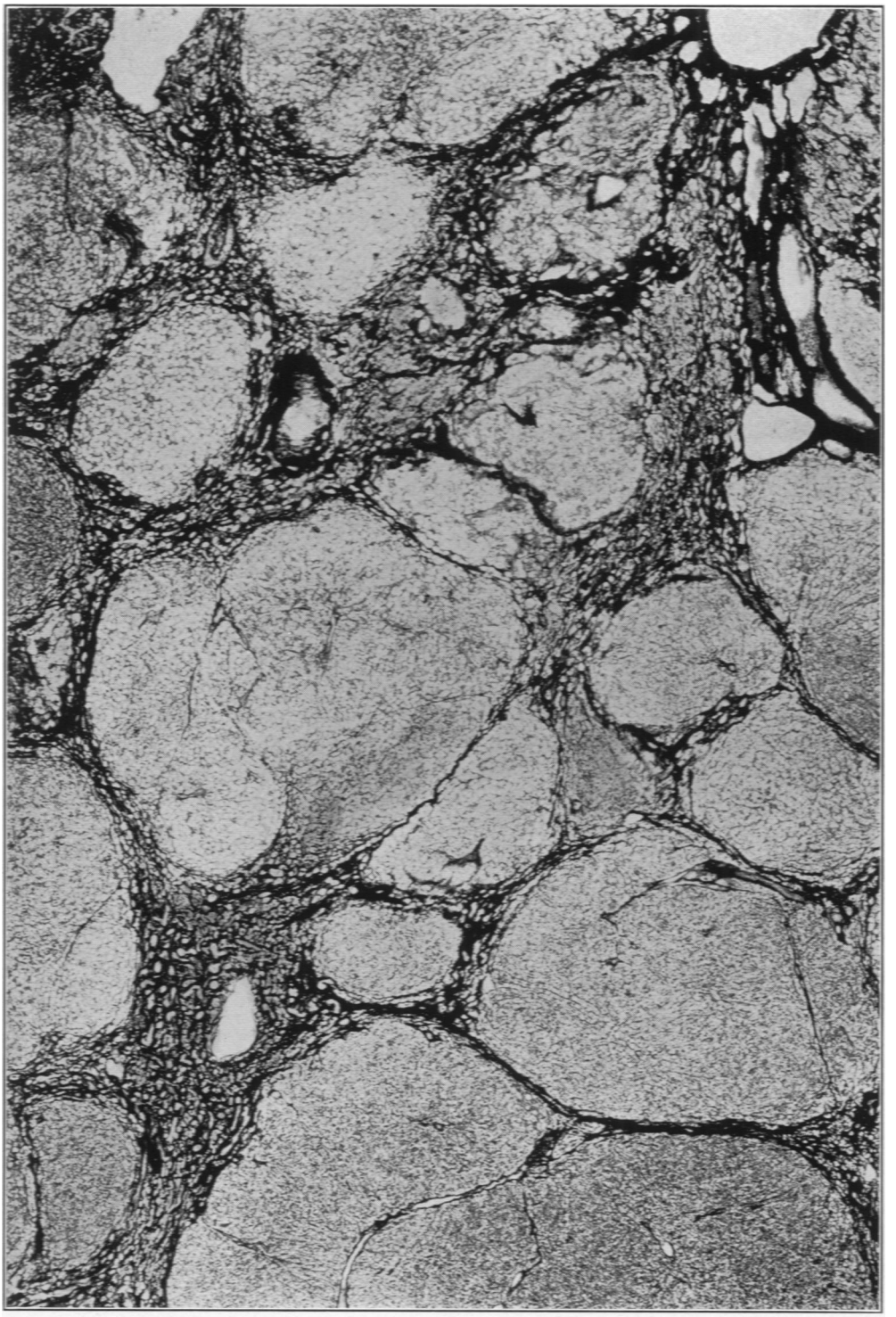
PLATE 22

FIG. 5. Liver of Dog DA showing well advanced cirrhosis of the liver 5.5 years after pancreatectomy. There is very little fat present now. Total lipids 5.6 per cent. Phosphotungstic acid hematoxylin stain. × 38.



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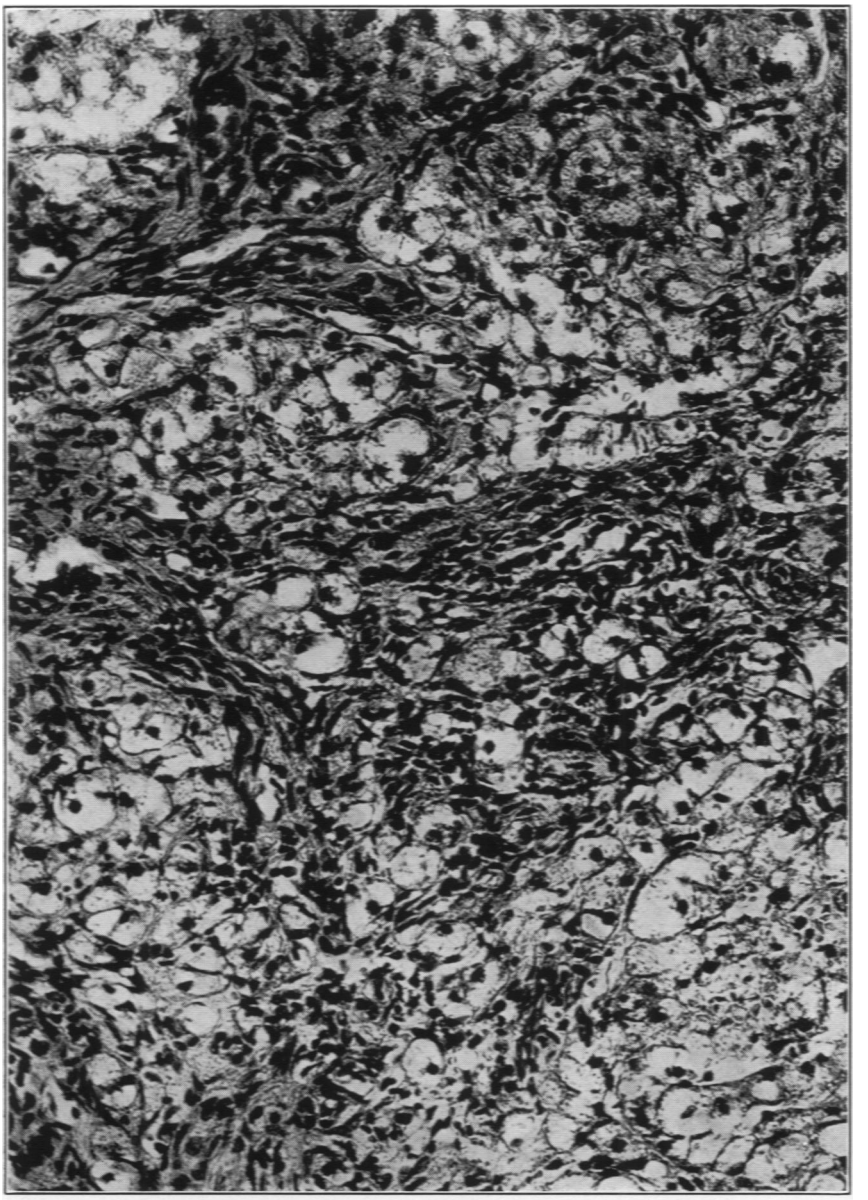
Fatty Infiltration and Cirrhosis of Liver

PLATE 23

FIG. 6. Liver of Dog DA showing details of fibroblastic proliferation in liver shown also in Fig. 5. Fat not prominent in cells, but the cytoplasm in many cells is lumpy and hyaline in appearance. Some rounded masses resemble "colloid" bodies. Dark, irregular homogeneous masses between strands of connective tissue are atrophied hyaline liver cells. Hematoxylin-eosin stain. × 300.



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