THE NONPORTAL DISTRIBUTION OF THE TRABECULAE IN DIETARY CIRRHOSIS OF RATS AND IN CARBON TETRACHLORIDE CIRRHOSIS OF RATS AND GUINEA-PIGS *

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The relationship of choline, cystine, and methionine to necrosis and cirrhosis of the liver in rats has been investigated in a number of laboratories.[1-7] These two conditions now have been clearly identified and established as separate entities.[2,6] However, there still exist differences of opinion as to the architectural relationship of the connective tissue trabeculae in the cirrhotic livers produced by dietary deficiency of choline. Most workers have described the fibrous tissue as being formed in, or emanating from, the portal areas. This description corresponds to that of portal cirrhosis in man. In a previous report describing the histogenesis of dietary cirrhosis in rats,[8] we stated that the trabeculae of ceroid and connective tissue formed along the hepatic venules and only later abutted on portal areas. This interpretation does not permit the classification of the process as portal cirrhosis.

In order to test this interpretation, it was decided to continue the investigation of this subject using precise means of locating the position of the trabeculae. For this purpose, hepatic or portal veins were injected with a charcoal mass similar to that used by Mall [9] in his classic investigations on the hepatic lobule.

The centrolobular distribution of necrosis and fatty degeneration of the liver in CCl_4 intoxication is well recognized. However, the location of the subsequently formed connective tissue trabeculae is a controversial point. Therefore, the histogenesis of this process was investigated in the same manner as that of the dietary cirrhosis.

EXPERIMENTAL PROCEDURE

Forty-three albino rats at weaning were started on diet no. 545 which had the following composition: Leached casein, 4 per cent; cystine, 0.5 per cent; cod-liver oil, 2 per cent; Wesson oil, 3 per cent; Osborne and Mendel salt mixture,† 4 per cent; corn starch, 86.5 per cent. A supplement of 100 μg. of thiamin chloride, 50 μg. of riboflavin, 20 μg. of pyridoxine, 50 μg. of calcium pantothenate, and 1 mg. of

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†Osborne, T. B., and Mendel, L. B. The use of soy bean as food. *J. Biol. Chem.*, 1917, **32**, 369–387.

nicotinic acid was given to each rat daily. All rats received 20 per cent alcohol as a source of fluid. In order to obtain livers showing variable degrees of pathologic alteration, the rats were killed after having been under experimental conditions from 50 to 150 days. The livers were injected with charcoal gelatin mass through either the hepatic or portal veins.

Nineteen adult albino rats, weighing from 248 to 388 gm., were injected subcutaneously with carbon tetrachloride twice a week. The dose of carbon tetrachloride was 0.05 cc. per 100 gm. of initial body weight. The same dose was continued for the duration of the experiment regardless of the weight changes. These rats were fed an *ad libitum* diet of stock pellets and water. The rats were killed at intervals from 21 to 87 days and the livers were injected with charcoal gelatin mass.

In another experiment, guinea-pigs fed a stock vegetable diet received repeated subcutaneous injections of carbon tetrachloride at various dosage levels. Some of these animals developed cirrhosis. The livers of 9 were injected with the mass.

Preparation of Injection Mass. The injection mass is made just before use by dissolving 8 gm. of gelatin in 100 cc. of hot water and adding 20 gm. of animal charcoal. While the animal is being prepared for injection, the mass is kept in a conical glass in a warm water bath (about 55° C.). The charcoal is kept in suspension by use of a glass agitator rod of propeller type, powered by a small electric motor having an adjustable friction reduction gear.

Preparation of Animal and Injection of Liver. The animal is killed with chloroform and attached to a small wooden autopsy board. Anterior abdominal and thoracic walls are removed, and absorbent cotton is folded over the cut ends of the ribs to prevent injury to the liver. An obstructing tie is made around the inferior vena cava at any convenient point below the liver, usually just above or below the right renal vein. When it is desired to inject the hepatic veins, a glass cannula is inserted into the thoracic inferior vena cava through a slit made in the right auricle and tied in place. The cannula is attached by a 6 inch length of small rubber tubing to a 100 cc. syringe containing warm saline solution. After cutting the portal vein, gentle pressure on the syringe plunger is exerted to wash the blood from the liver. Most of the blood is quickly removed by this method. In some livers the blood remains in a few patches. The removal of this blood is effected by gently stroking the liver surface with the moist fingertips while continuing the irrigation. After the blood has been washed out, the charcoal gelatin mass is injected through the same tubing by replacing the syringe

containing saline solution with a 10 cc. syringe containing the mass. In the transfer of syringes, care should be taken to avoid the inclusion of air bubbles. The amount of pressure necessary to inject the hepatic tree is slight and is controlled by observation of the degree of filling of the subcapsular terminal branches of the hepatic veins. When the veins are filled, additional pressure may rupture the hepatic vein at the hilus. Injection of the intrahepatic portal veins is accomplished in a similar manner, the cannula being tied in the portal vein in any accessible location. The inferior vena cava is cut in the thorax to allow escape of the blood and saline solution. Since the terminal branches of the portal vein do not reach the surface of the liver, only a grayish color results from the injection. The injection is stopped when the system will not, with gentle pressure, receive more of the mass. Livers are injected either by hepatic or by portal veins, never by both. Although some of the gelatin passes through the hepatic lobules, the carbon does not, and is limited to the portal or hepatic systems, as the case may be. It should be noted here that occasionally some venous branches will not be filled by the mass.

After the injection is completed, the cannulized vessel is clamped and the entire liver removed and placed in the fixative, which in this study was either 10 per cent formalin or Zenker's fluid. The specimen is then placed in the refrigerator to accelerate the hardening of the mass. After 1 to 2 hours, the livers may be sliced at 3 to 4 mm. intervals, and fixation continued at room temperature.

For this study all tissue was embedded in paraffin. Sections were stained for connective tissue by Mallory's aniline blue and van Gieson's picrofuchsin methods. Lillie's modification of the di-ammine silver hydroxide method * was used for demonstrating reticulum.

Distribution of Trabeculae in Dietary Cirrhosis

In the dietary cirrhotic livers in which the charcoal gelatin mass was introduced through the hepatic vein, the injected veins were regularly included in, and connected by, the connective tissue trabeculae. In the livers injected through the portal veins, the injected veins were usually not in, or connected by, the trabeculae.

As previously reported,[8] the early deposition of fat was most marked about hepatic venules. Ceroid soon appeared in liver cells and in groups of phagocytes about hepatic veins and venules. These phagocytes became incorporated into loosely fibrous trabeculae. The trabeculae

* Lillie, R. D. A simplified method of preparation of di-ammine-silver hydroxide for reticulum impregnation; comments on the nature of so-called sensitization before impregnation. *Stain Technol.*, 1946, **21**, 69–72.

formed not only about the central vein but also along the sublobular and large branches of the hepatic veins. In fact, in some instances the first increase in connective tissue to be noted appeared around these larger vessels, more particularly near the hilar portion of the lobes.

In comparing portal versus hepatic distribution of fibrous tissue, one should compare similar levels of the two systems. This tends to lessen confusion in interpretation of the histologic picture. The point where the recognition of the terminal radicles of portal and hepatic veins is easiest is in the subcapsular layer of lobules. Here, where sections are made perpendicular to the surface, alternating central veins and portal veins of the same order can often be seen. It was apparent in the present study that the trabeculae coursed along these hepatic veins and reached and merged with the capsule, as is evident in Figure 2. It is significant that hepatic veins normally reach the capsule, whereas portal veins do not, a fact reported by Mall,[9] Figure 1. In deeper lobules the trabeculae are also seen to connect injected hepatic veins. Parts of three lobules with their constituent noninjected and uninvolved portal areas are shown in Figure 6. Part of the apparent disproportionate size of the portal and hepatic veins is due to distention of the latter by the injection mass.

Some trabeculae seen in livers injected through the hepatic vein did not contain the mass. This may be explained by the failure of the mass to reach the area due to narrowing of the vein or blockage at a lower level by an aggregate of charcoal particles. To determine how trabeculae were related to portal areas, it was necessary to study cirrhotic livers in which the charcoal mass was introduced through the portal vein. Figures 7 to 10 show injected portal veins in uninvolved portal areas. These lobules are surrounded by trabeculae containing no mass. Such relationship was found with regularity in those livers in which the cirrhotic process had not progressed to the point of extreme disarrangement of lobular architecture. Even in these, isolated nodules of hepatic parenchyma often showed uninvolved portal areas with injected veins (Fig. 11). In livers showing fairly advanced cirrhosis, portal areas were found at the margins of trabeculae and sometimes incorporated within them (Fig. 13). These portal areas contained vessels of relatively large size and obviously were not related directly to the functional liver unit. It was clear in some instances that a trabecula passed by, abutted on, or enclosed a portal area because destruction of one or more lobules had brought hepatic and portal vessels close together. Also the larger hepatic veins pursued an independent course to their exit from the liver,[10] a circumstance which in some instances brought the larger hepatic and portal vessels in close relationship at certain

points. Such a condition would account for the inclusion of an occasional portal area in a trabecula which was primarily related to the hepatic vein. In the extensively involved livers, some trabeculae extended to and incorporated the larger portal areas, and when such occurred the fibroblasts of these areas undoubtedly contributed to the new growth of connective tissue. In areas where almost complete replacement of parenchyma had taken place, usually near the hilus, injected portal veins of medium and large size were seen irregularly scattered throughout the ceroid and connective tissue mass.

Proliferation of small bile ducts is a feature of this cirrhosis which varies greatly in degree in different rats. It is usually prominent only in some of the animals showing moderately severe or severe damage. The presence of these ducts in the trabeculae served to confuse the picture and make interpretation difficult. That these bile ducts were present in the trabeculae, which in this study were shown to follow and connect hepatic veins, was evident; and the explanation for their presence was also evident in some preparations. These small ducts were seen extending from the portal area through the lobule between cell cords and reaching the trabeculae (Fig. 11). In most instances many more ducts were present in the trabeculae than were seen traversing the lobules. That these represented growth with tortuosity or budding in their abnormal location seems reasonable. In those livers where cirrhosis had proceeded to complete replacement of certain lobules, the reason for the presence of bile ducts in the proliferated connective tissue was obvious. This was seen particularly toward the base of the lobes and in diffuse or patchy subcapsular areas.

Distribution of Trabeculae in Carbon Tetrachloride Cirrhosis of the Liver

In hepatic cirrhosis induced by carbon tetrachloride the fatty degeneration and necrosis in both rats and guinea-pigs occurred around the hepatic veins, mainly the terminal branches (centrolobular) (Fig. 12). The trabeculae of fibrous tissue formed in the same location. These trabeculae and their relationship to the injected hepatic veins are shown in Figures 15 to 20. Their distribution was essentially the same as that already described in more detail for dietary cirrhosis.

The demonstration that the connective tissue trabeculae in cirrhosis due to CCl_4 follow and connect hepatic veins rather than the portal areas simplifies the understanding of the pathogenesis of this condition. The connective tissue proliferates in the same areas where damage to the liver takes place. Since this is true, it is unnecessary to assume, as has been suggested, the presence of an autolysate which travels in the

lymphatics from areas of necrosis to the portal areas, and there stimulates the production of connective tissue.

Discussion

The hepatic lobule is generally considered as having a terminal radicle of the hepatic vein at its center and being bounded peripherally by an imaginary line passing through the associated portal areas. This conception is based largely on the fact that in swine a connective tissue capsule is found in this location, thus enclosing a mass of liver cells—the hepatic lobule. In the seal, hepatic lobules are also well demarcated from one another, but in this animal it is a different lobule that is outlined.[11] Microscopically, it is seen that the hepatic veins are at the periphery, where, as lacunae or rectilinear venous sinuses, they form walls of separation of the portal lobule. Theile [12] demonstrated in the dog and rabbit the presence of cleavage planes between portal lobules. This was done by crushing, and washing the livers of these animals in a stream of water. When treated thus, the whole system of lobules became isolated and were clustered around the branches of the portal veins. The foregoing are cited to indicate that from a standpoint of architecture and comparative anatomy it should be possible to produce cirrhosis of the liver in which the trabeculae form at the periphery of either the hepatic or portal lobule, the type produced probably depending on the location of the injury.

The concept that the connective tissue proliferation occurs in the same location as the injury is supported by the fact that in carbon tetrachloride cirrhosis of rats and guinea-pigs, fatty degeneration and necrosis occur about the central veins and the fibrous tissue trabeculae form in and connect these areas. Further support for this concept is found in dietary cirrhosis. In the development of this condition, the deposition of fat usually occurs first, and is most pronounced, around the hepatic veins. This is followed by the accumulation of ceroid and the formation of connective tissue strands which follow and connect these hepatic veins.

Summary

Albino rats at weaning were placed on a cirrhosis-producing diet and killed after 50 to 150 days. The livers were injected through the portal or hepatic veins with charcoal gelatin mass to mark these structures effectively in the microscopic preparation. Histologic study showed that the fatty deposition, ceroid accumulation, and fibrous trabeculation primarily followed and connected hepatic veins. In livers showing marked alteration, the trabeculae sometimes coursed by, or abutted on,

large portal areas. However, even in these livers the portal areas comparable in level to the centrolobular veins were not primarily related to the trabeculae.

In other experiments, cirrhosis of the liver of rats and guinea-pigs was produced by the repeated subcutaneous administration of carbon tetrachloride. The livers were injected with the charcoal gelatin mass and studied histologically. The connective tissue trabeculae occurring in these livers were primarily related to hepatic veins and showed essentially the same distribution as that seen in the dietary cirrhosis.

REFERENCES

1. Blumberg, H., and McCollum, E. V. The prevention by choline of liver cirrhosis in rats on high fat, low protein diets. *Science,* 1941, **93,** 598–599.
2. Daft, F. S., Sebrell, W. H., and Lillie, R. D. Prevention by cystine or methionine of hemorrhage and necrosis of the liver in rats. *Proc. Soc. Exper. Biol. & Med.,* 1942, **50,** 1–5.
3. György, P., and Goldblatt, H. Observations on the conditions of dietary hepatic injury (necrosis, cirrhosis) in rats. *J. Exper. Med.,* 1942, **75,** 355–368.
4. Webster, G. T. Cirrhosis of the liver among rats receiving diets poor in protein and rich in fat. *J. Clin. Investigation,* 1942, **21,** 385–392.
5. Engel, R. W. Liver cirrhosis and choline. *Federation Proc.,* 1943, **2,** 62.
6. Himsworth, H. P., and Glynn, L. E. Massive hepatic necrosis and diffuse hepatic fibrosis (acute yellow atrophy and portal cirrhosis): Their production by means of diet. *Clin. Sc.,* 1944, **5,** 93–123.
7. Hock, A., and Fink, H. Über eine schwere ernährungsbedingte Stoffwechselstorüng und ihre Verhütung durch Cystin. *Ztschr. f. physiol. Chem.,* 1943, **278,** 136.
8. Lillie, R. D., Ashburn, L. L., Sebrell, W. H., Daft, F. S., and Lowry, J. V. Histogenesis and repair of the hepatic cirrhosis in rats produced on low protein diets and preventable with choline. *Pub. Health Rep.,* 1942, **57,** 502–508.
9. Mall, F. P. A study of the structural unit of the liver. *Am. J. Anat.,* 1906, **5,** 227–308.
10. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia & London, 1930.
11. Brissaud and Sabourin. Sur la constitution lobulaire du foie et les voies de la circulation sanguine intra-hépatique. *Compt. rend. Soc. de biol.,* 1888, **40,** 757–762.
12. Theile, F. W. T. (Cited by Mall.[9])

[*Illustrations follow*]

DESCRIPTION OF PLATES

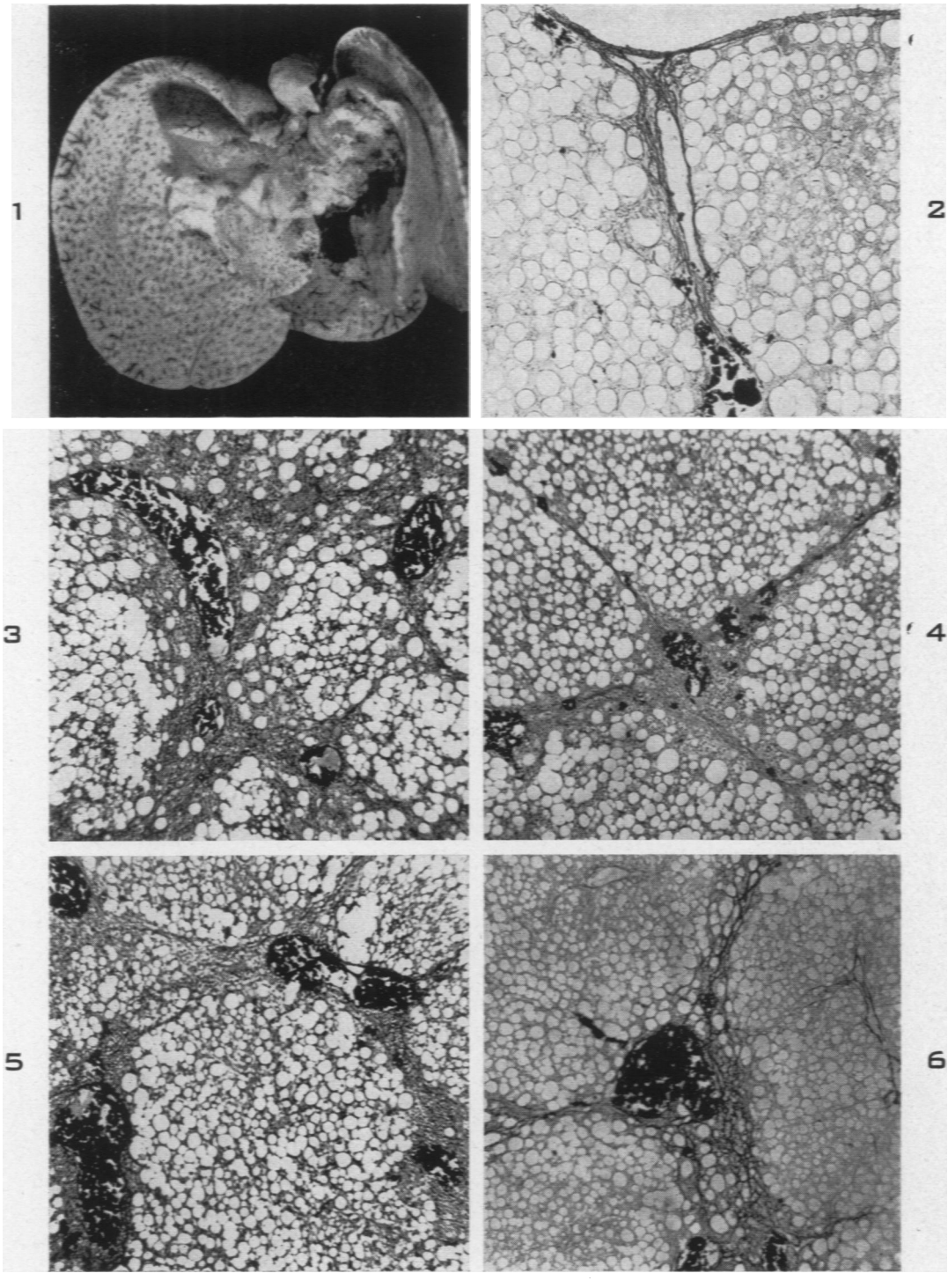
PLATE 26

FIG. 1. Dietary cirrhosis, rat. Gross photograph of liver injected with charcoal gelatin mass through hepatic vein. No gross evidence of cirrhosis.

FIG. 2. Dietary cirrhosis, rat. Liver showing increase in connective tissue around the injected hepatic vein. The connective tissue follows the vein to, and blends with, the capsule at the point of retraction. Reticulum stain. × 80.

FIGS. 3, 4, and 5. Dietary cirrhosis, rat. Relationship of trabeculae to the injected hepatic veins is shown. Ceroid appears in the trabeculae as small refractile globules. Van Gieson's stain. × 50.

FIG. 6. Dietary cirrhosis, rat. Injected hepatic veins incorporated in, and connected by, trabeculae. Parts of three lobules are shown; of note are the uninjected portal veins outlined by reticulum. Reticulum stain. × 50.



Ashburn, Endicott, Daft, and Lillie Nonportal Trabeculae in Experimental Cirrhosis

PLATE 27

FIGS. 7, 8, and 9. Dietary cirrhosis in a rat, with portal injection. Mallory's aniline blue stain. × 50.

FIG. 10. Dietary cirrhosis in a rat, with portal injection. Picrofuchsin stain. × 50.

FIG. 11. Dietary cirrhosis in a rat, with portal injection. Isolated nodule of liver. Hematoxylin and eosin stain. × 250.

FIG. 12. Carbon tetrachloride cirrhosis in a rat, with portal injection. Frozen section, stained with oil red O. × 50.

FIG. 13. Dietary cirrhosis in a rat, with portal injection. Narrow expansions from the thick trabeculae abut on the large portal area. The small portal area is unrelated to the proliferated connective tissue. Mallory's aniline blue stain. × 50.

FIG. 14. Carbon tetrachloride cirrhosis in a rat, with portal injection. Van Gieson's stain. × 50.

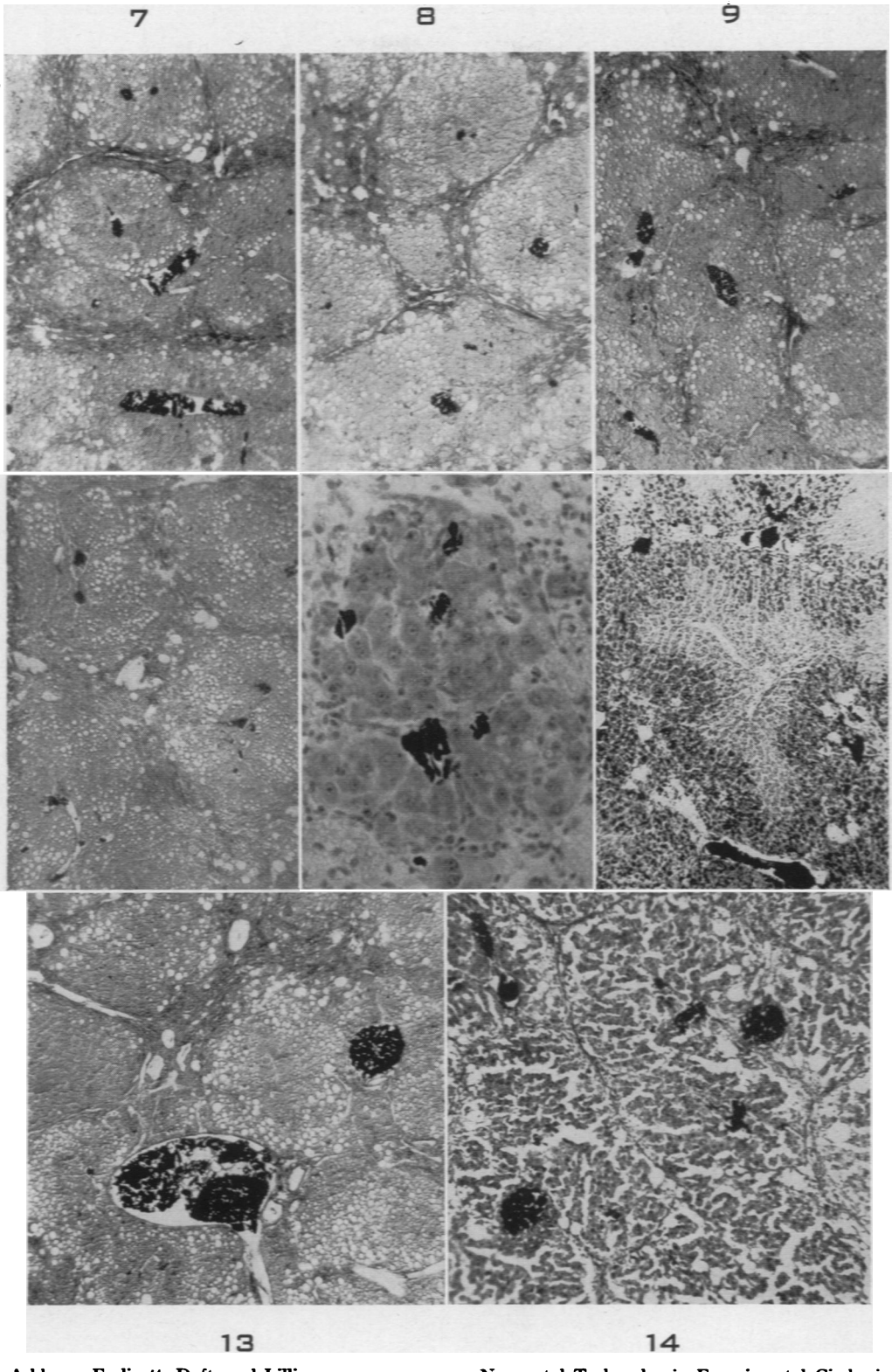


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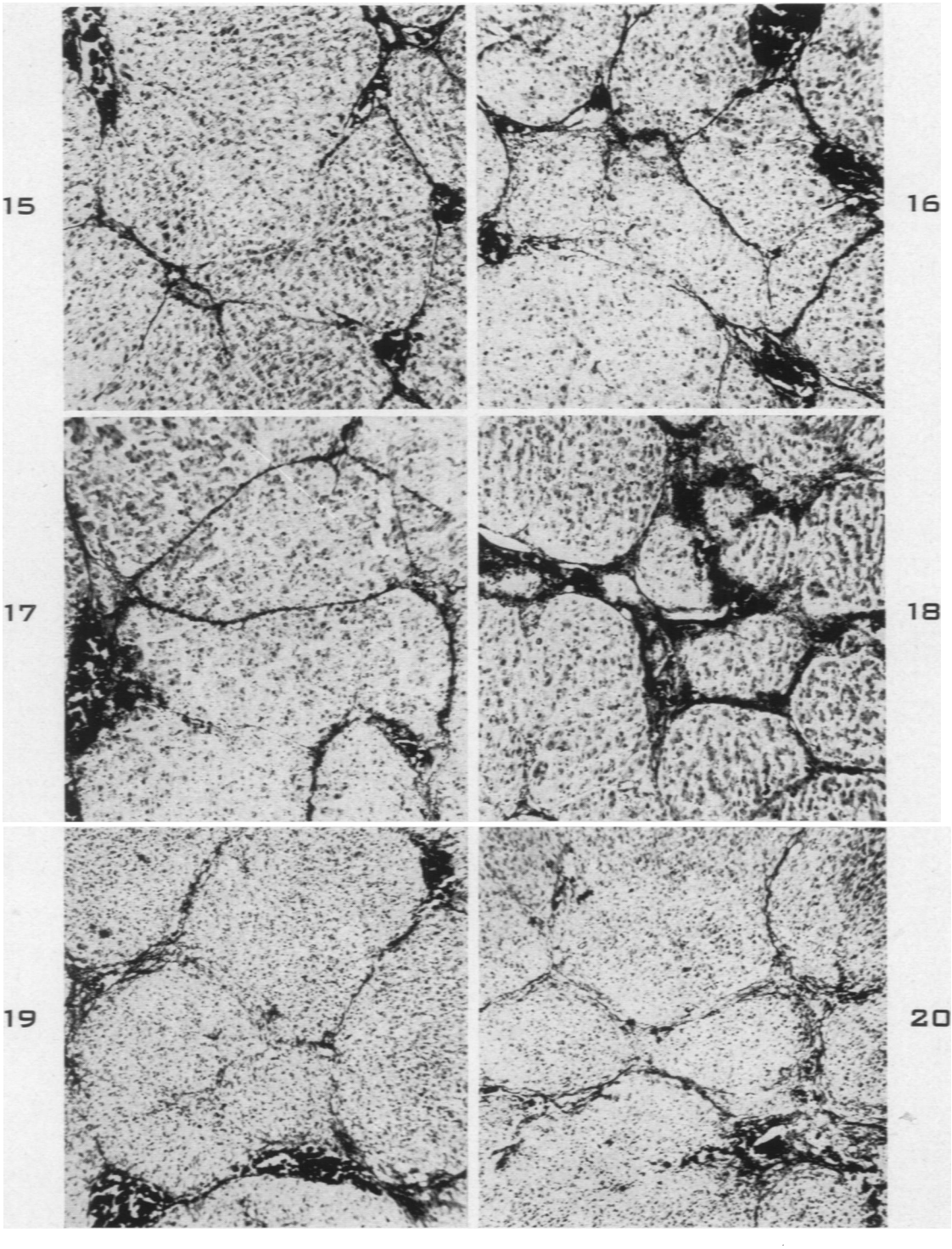
PLATE 28

FIGS. 15, 16, 17, and 18. Carbon tetrachloride cirrhosis in a rat; hepatic veins injected. There is considerable alteration of lobular architecture. Trabeculae follow and connect hepatic veins. Van Gieson's stain. × 50.

FIGS. 19 and 20. Carbon tetrachloride cirrhosis in a guinea-pig; hepatic veins injected. Injected veins are included in, and connected by, thin trabeculae. Van Gieson's stain. × 50.



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