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Microcirculatory Changes in the Liver of Choline-Deficient Rats.*† (23793)

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The promptness with which lesions occur in zone 3 of the "liver acinus" of a rat even after a short period of choline deficiency(8) made us think that a circulatory factor must be playing its part in this disorder. By "liver acinus" we mean the structural and functional hepatic unit(1-2), the concept of which is based on the dependence of a microscopic amount of hepatic parenchyma on the terminal afferent branches bringing in materials for metabolism and on the associated terminal bile ductule carrying away the secretory product, the bile. Zones can be outlined in the acinus, areas of distinct enzymatic patterns(3), close to, or remote from the source of supply of oxygen and nutrients, *i.e.*, the terminal afferent vascular branches. One can easily see (Fig. 1) that it is zone 3 of the acinus farthest from the terminal portal and hepatic arterial branches that shows the fatty change first. The following method was adopted to observe the early microcirculatory changes in choline-deficient livers.

Method. Fifteen weanling rats (male and female) weighing between 60-80 g were fed a severely choline-deficient diet for periods ranging from 12 hours to 7 days. The diet contained 20% mixed proteins, was low in methionine and supplemented with cystine to make the organic sulfur adequate. The intrahepatic circulation was studied *in vivo* in Dr. Knisely's laboratory using his transillumination technic with the fused quartz rod(4). A technic was developed to immobilize the liver and to observe its circulation under physiologic conditions in laparotomized rats. A polyethylene apron (Fig. 2) cut to the width and length of the abdominal cavity is slipped with its folded upper margin beneath the liver. This apron has a fine hole in the lower midline through which is passed a polyethylene tubing flanged at one end. The tubing is then connected with a container of warm Ringer solution. Inflow and pressure of the solution are adjusted by a micrometer screw. This device made it possible to control the pressure gradient between abdominal and thoracic cavity. The pressure in the latter was raised by the insufflation of O_2 under 40-50 mm Hg *via* a tracheal cannula in order to immobilize diaphragm and liver(5). The insufflation pressure was controlled by a needle valve. However, the increase in insufflation pressure required to immobilize these structures was 1/6

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to 1/10 of that needed when no abdominal apron was used to splint the diaphragm. In this preparation the sinusoidal circulation was continuous and swift, and it was possible to study the same field with the water immersion lens for hours.

Results. Six rats in choline deficiency from 24 to 36 hours showed definite changes in hepatic parenchyma and circulation. The liver edge was more transparent and contained tiny fat globules in the hepatic parenchymal

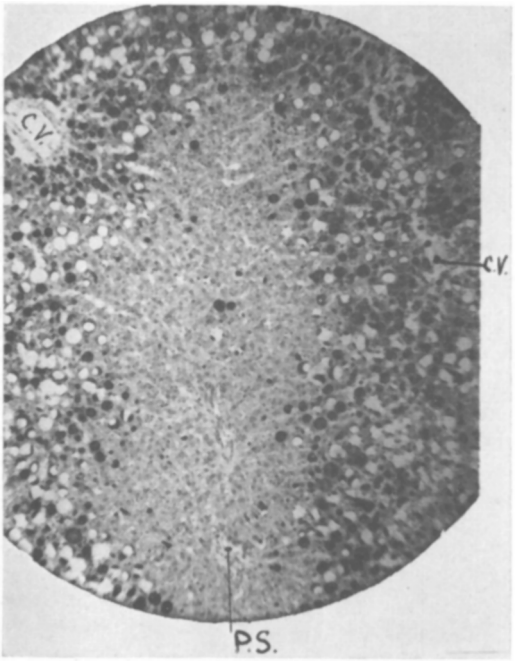

FIG. 1. Liver of a rat fed a low choline diet. One can recognize the fat droplets (black) in cells of zone 3 which lies concentrically around terminal afferent vessels branching out from a portal space (P.S.). The central veins (C.V.) at the periphery of the liver acinus are linked to each other by the band of fatty tissue arching around the tip of the acinus (90 × approx.).

cells, smaller and more regular than those seen in the mesentery. The sinusoidal pattern was smudged as if somebody had passed a greasy hand over the lenses. The central veins stood out in a field of less detail. Many of the outlet sphincters at the junction of sinusoids and hepatic veins were closed, and the liver seemed to be in a prolonged state of what Knisely termed "storage phase"(6-7). The tiny globules seen with the water immersion lens were single, or in rows, and adjoined

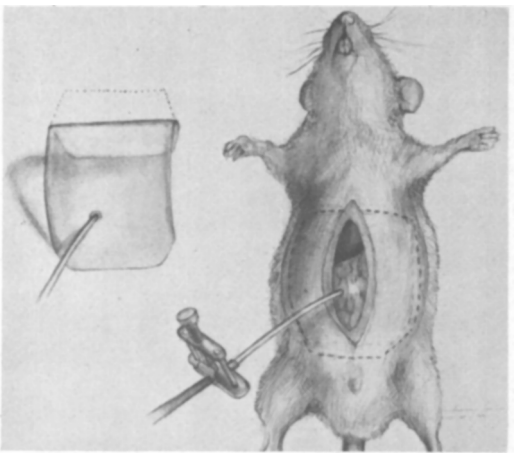

FIG. 2. Polyethylene apron covers viscera of a laparotomized rat. Ringer solution is introduced beneath the plastic sheet into peritoneal cavity raising intra-abdominal pressure.

the hepatic sinusoids (Fig. 3). Some overlay the sinusoids and swayed when the blood passed beneath them. When a coverglass was pressed slightly on the area, the tiny droplets produced pits in the tissue; on removal of the coverglass the pits disappeared and the droplets became prominent again. Thus we demonstrated that the fat droplets are less compressible than the parenchyma which yields to pressure which they exert. In one field such

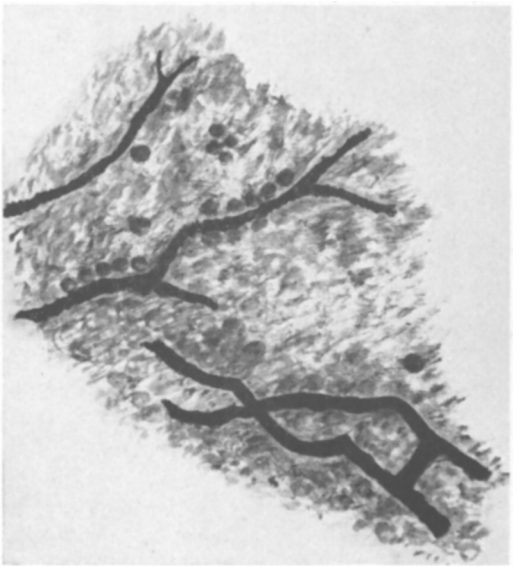

FIG. 3. Drawing of a rat liver transilluminated and observed *in vivo*. The animal was on a severely choline deficient diet for 36 hr. For description see text (× 225).

a globule overlay an arterialized sinusoid and pulsated back and forth with the onrushing and receding pulse wave beneath it. It was obvious at this magnification that the fat droplets definitely pressed on the sinusoids and impeded the passage of blood. When after hours of observation the continuous flow had slowed and become granular in character one could see red cells squeezing between opposing fat globules.

In 3 other rats studied after 48-96 hours of choline deficiency the changes were more accentuated. One had the impression that the tissue had been strewn with round transparent pellets that covered and distorted the sinusoids making the flow harder to follow. The central veins although slender still showed good flow coming from the deeper layers.

A group of 6 rats on a choline deficient diet for 5 to 7 days were studied by the same technic. Under low magnification (X 50) the sinusoidal pattern had almost faded. It could be made to reappear by raising the insufflation pressure of the O_2 to about 60 mm Hg. This pressure impeded the outflow of blood from the hepatic veins and the blood stagnated, dilating the hepatic sinusoids. Under medium magnification (X 100) the hepatic cells appeared fatty and some looked as if they were translucent bubbles. Rows of small globules were tightly packed against the sinusoidal walls. The red streaks representing the flowing blood in the sinusoids were narrow and had a conspicuously indented outline due to the compression of the sinusoidal walls by the fat globules. The smaller the globules the less compressible they were.

Summary. (1) A method has been developed by which intraabdominal pressure sufficient to immobilize the liver can be established in small animals laparotomized for the study of the intrahepatic circulation *in vivo*. (2) In choline-deficient rats there is an accumulation of tiny fat droplets along the sinusoidal walls or overlying them as early as 24 or 36 hours after the ingestion of the deficient diet. The small and barely compressible droplets exert a pressure on the pliable endothelial walls indenting them and impeding the sinusoidal flow. (3) These phenomena are conspicuous on the surface of the transilluminated liver edge which of course is formed by zones 3 of the acini under observation.

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The Penn Seroflocculation Reaction in Cancer Diagnosis.* (23794)

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A seroflocculation reaction was reported by Hall, Dowdy, Penn, and Bellamy(1) to have significant cancer selectivity, *i.e.*, in testing over 10,000 sera their correct positive results in the cancer patients ranged between 81 and 98%. Their false positive results in testing patients ranged between 9 and 33% while their false positive results in testing normal persons ranged between 0 and 4%. These results were obtained using 3 different "anti-

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