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Liver Cell Damage Produced by Portacaval Shunts.* (29807)

EMANUEL RUBIN, NORMAN R. GEVIRTZ, PERRY COHAN, FUSAYOSHI TOMITA AND JULIUS H. JACOBSON II (Introduced by Hans Popper)

Departments of Pathology, Hematology and Surgery, The Mount Sinai Hospital, New York City

Increasing evidence has accumulated that portacaval shunts for relief of portal hypertension may produce undesirable side effects such as portasystemic encephalopathy(1), demyelinating disease of the spinal cord(2), hyperbilirubinemia, possibly owing to hemolytic anemia(3) and diabetes mellitus(4). Hepatic siderosis and even generalized hemochromatosis(5) have followed construction of portacaval shunts. Cirrhosis is not necessary for the accumulation of hepatic iron, since increased hepatic hemosiderin has been produced in normal dogs(6) and in rats(7) by end-to-side portacaval shunts alone. Untoward side effects are said to result simply from the diversion of the portal flow from the liver. To investigate further the integrity of the liver cells after shunts, portacaval anastamoses were constructed in rats by microsurgical techniques after which autoradiographic studies of DNA synthesis and fine structural studies were performed.

Materials and methods. The experimental animals consisted of 24 Sprague-Dawley rats, with an average initial body weight of 400 g. All were fed Rockland Farms complete rat diet. They were divided into groups of 8 rats each: Group I. End-to-side portacaval shunts. Group II. Side-to-side portacaval shunts. Group III. Sham operations, with mobilization of the portal vein and vena cava, but without construction of a shunt.

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Two rats from each group were sacrificed 30, 60, 90 and 120 days later. Two hours before sacrifice each animal was given 0.5 microcurie of tritiated thymidine intraperitoneally. A small sample of liver tissue was immediately fixed in buffered osmic acid, and embedded in epon. One micron and 0.1 micron thick sections were cut with a Porter-Blum microtome. The former were stained with toluidine blue for examination under the light microscope, while the latter were stained with lead hydroxide, and examined with a Hitachi HS-7 electron microscope. Portions of the liver were also fixed in 10% neutral buffered formalin after which paraffin sections were stained with hematoxylin and eosin, and subjected to Perl's reaction for iron. Paraffin sections were also coated with Kodak NTB-3 nuclear track emulsion for autoradiography. These were exposed for 16 days, developed in Kodak D-19 solution and stained with Harris hematoxylin.

Results. After end-to-side shunts, the animals lost up to 20% of their initial weight by the end of experiment. The liver weight/body weight ratio remained about the same in all animals. Rats subjected to side-to-side shunts and to sham operations gained up to 25% of initial weight by the end of experiment. The livers after both types of shunts and after sham operations appeared normal in sections stained with hematoxylin and eosin. Small amounts of iron accumulated in the centrolobular zones of animals with end-to-side shunts after 90 days. Autoradiographs had about 3 to 4 times as many labeled hepatocytes in rats subjected to end-to-side shunts

TABLE I. Number of Labeled Hepatocytes Per 10,000 Hepatocytes in Livers of Rats Subjected to Portacaval Shunts and Sham Operations.

Operation	No. of days after operation			
	30	60	90	120
Sham	14	18	16	16
Side-to-side shunt	17	12	11	14
End-to- " "	48	61	70	57

TABLE II. Number of Labeled Mesenchymal Cells Per 10,000 Mesenchymal in Livers of Rats Subjected to Portacaval Shunts and Sham Operations.

Operation	No. of days after operation			
	30	60	90	120
Sham	37	33	34	46
Side-to-side shunt	32	47	36	49
End-to- " "	37	36	40	32

as in sham operated animals (Table I). No significant differences were observed between specimens taken at various times. The number of labeled hepatocytes after side-to-side shunt was about the same as in the sham operated controls. The number of labeled mesenchymal cells was in the same range in all groups (Table II).

In one-micron thick sections of animals with end-to-side shunts variation in staining qualities of liver cells, so-called "light" and "dark" cells, was pronounced. Numerous small, round, intracytoplasmic vacuoles were frequent in hepatocytes. Electron microscopic examination disclosed the following aberrations in animals with end-to-side shunts: 1) The electron density of the hyaloplasm of many hepatocytes was decreased, resulting in pronounced variation in density between neighboring cells ("light" and "dark" cells) (Fig. 1). 2) The cytoplasmic vacuoles previously noted were 0.5 to 2 μ in diameter, and contained clear centers and a dense osmiophilic rim (Fig. 2). 3) In some cells, mitochondria appeared swollen and increased in number. 4) Occasionally the rough profiled endoplasmic reticulum was focally dilated to form vesicles. The livers of animals with side-to-side shunts and those of sham operated animals showed no unusual features.

Discussion. End-to-side portacaval shunts, in which the entire portal flow is diverted from the liver, result in increased DNA synthesis in hepatocytes. This confirms the claim that portal blood is not essential for hepatic regeneration(8). After side-to-side shunts, no differences from sham operated controls were observed. This contrasts with the clinical

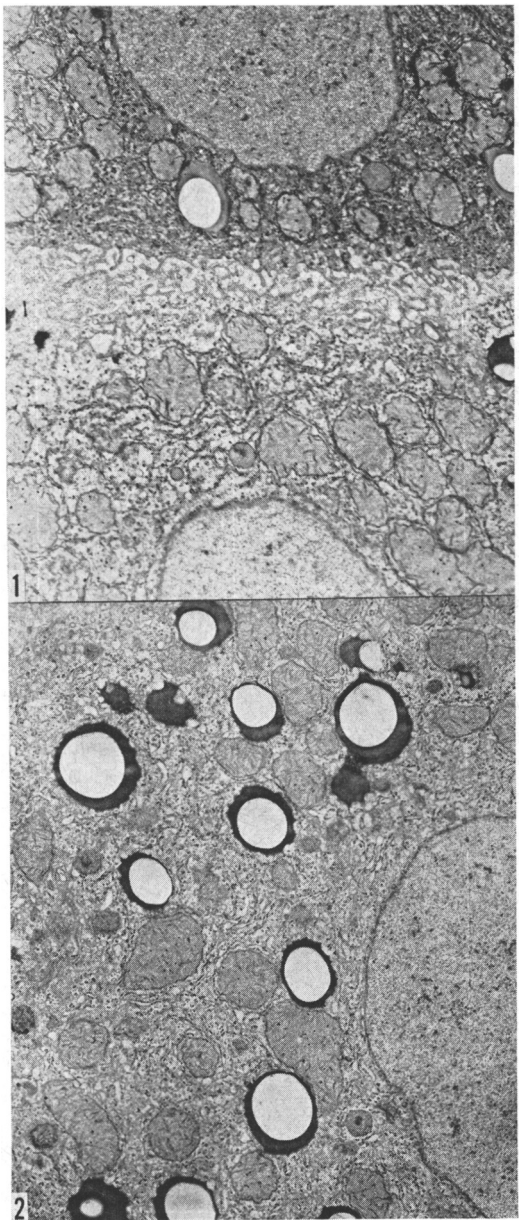

FIG. 1. Electron micrograph of liver of rat 60 days following end-to-side portacaval shunt showing "dark cell" (top) and "light cell" (bottom) (× 8,800).

FIG. 2. Electron micrograph of liver of rat 60 days following end-to-side protacaval shunt. Numerous cytoplasmic vacuoles with clear centers and osmiophilic borders are seen. (× 11,200).

situation in cirrhosis with portal hypertension where a side-to-side shunt may be as effective in the diversion of hepatic flow as an end-to-side shunt(9). A direct stimulation to cell proliferation by the end-to-side shunt is unlikely since the livers were decreased in weight. Consequently the increased DNA synthesis is a response to loss of liver cells, a process which is not appreciated in routine histologic sections. The almost equal thymidine uptake at all times suggests that liver cells are lost persistently and at the same rate.

In other experimental models with severe liver cell damage such as carbon tetrachloride (10) or ethionine intoxication(11), a hepatitis results, with the increase in mesenchymal cell proliferation more prominent than that in hepatocytes. Diversion of the portal flow from the liver, which also produces liver cell damage, does not produce such a hepatitis.

Fine structural changes of liver cells include an increase in "light" and "dark" cells, cytoplasmic vacuoles, mitochondrial alterations and vesiculation of endoplasmic reticulum. The variation in staining intensity and electron density, reflected in "light" and "dark" cells, may be due to differences in hydration of cells, and is not a specific change, being found in various types of hepatic injury (12). The cytoplasmic vacuoles appear to be composed of an outer lipid layer and an apparently hydropic center, although the clear centers may be an artifact. They may represent an hypoxic change since pinocytic vacuoles, lacking lipid borders, have been produced by anoxia(13). Further evidence for hypoxic damage is the centrolobular distribution of iron after portacaval shunts(1), since this zone is most susceptible to anoxia. The alterations of mitochondria are non-specific indications of cell damage while the changes of the endoplasmic reticulum apparently reflect poor nutrition in animals with end-to-side portacaval shunts(14).

The liver cell damage after end-to-side portacaval shunts, demonstrated electron microscopically and autoradiographically, may produce some of the undesirable clinical side effects.

Summary. Diversion of the portal blood flow from the liver in rats by construction of end-to-side portacaval shunts increased DNA synthesis in hepatocytes, presumably as a response to liver cell damage. Electron microscopically increased variability in staining and electron density, cytoplasmic vacuoles and mitochondrial changes were noted. The liver cell damage associated with diversion of the portal flow may account in part for some of the undesirable side effects associated with portacaval shunts in man.

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