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Received February 25, 1953. P.S.E.B.M., 1953, v82.

Hepatic Circulation in Hemorrhagic Shock in the Rat.* (20201)

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Intrahepatic vasoconstriction during hemorrhagic shock has been demonstrated in the dog by measurements of portal-caval pressure gradients(1,2) and by x-ray visualization of the intrahepatic veins delineated by thorotrast injection(2). This communication deals with experimental hemorrhagic shock in rats in which the reactions of the sinusoids and the pre-sinusoidal and post-sinusoidal venules were observed by direct microscopy of the transilluminated liver during a prolonged period of hypotension and after transfusion. Data are included on portal venous pressures.

Methods. Pressure in the portal venous bed was measured by a saline manometer connected to a cannula in a mesanteric vein. The ligature on the cannula also secured the accompanying artery to prevent blood loss into the area of obstructed venous return. A segment of intestine about an inch long was thus deprived of blood flow, but the remainder of the intestinal circulation was not disturbed. The incision was closed about the catheter and the rat was allowed to recover from ether anesthesia. After control readings were taken, irreversible hemorrhagic shock was produced as previously described(3). In a number of these rats, *thorotrast* was injected through the catheter to permit x-ray demonstration of the portal vein and its branches. For *direct microscopy,* the liver edge was exposed through a transverse subcostal incision, transilluminated with the aid of a curved quartz rod conducting light from a 200-watt tungsten filament lamp, and examined at a magnification of 150 x. The tip of the rod and the exposed liver were bathed in flowing gelatin-Ringers solution at 37°C. A metal bracket, curved to fit the dome of the liver and notched to avoid vena cava and aorta, was inserted between liver and diaphragm and clamped securely to minimize respiratory motion. Control observations showed that the presence of the bracket did not change vessel size or blood flow. Estimation of change in vessel size was aided by a micrometer scale in the ocular lens (each division representing 7.7 micra in the field). To make these observations, it was necessary to avoid initial heparinization. Hence these rats were bled in accordance with the criteria described(3) but they were not cannulated for continuous measurement of arterial pressure until ready for transfusion.

TABLE I. Portal Venous Pressures in Rats before and after Bleeding and Blood Replacement. 11 experiments.

Portal venous pressure (cm H_2O)			
Before bleeding	During hypotension	After blood replacement	After additional transfusions
17	8.5	15.5	—
15	9	—	—
10.5	7.5	10.4	—
12	7	9	11.5
15	10	16	26
16	10	16	16
17.5	10	18	17.5
14.5	10	15	18
17	11	15	17.2
14.5	9.5	15	22.0
15.1	9.0	15.2	15.9
Mean 15	9.0	14.5	—

Results. Portal venous pressure (Table I). Portal pressures fell from the mean control level of 15 cm H_2O(4) to a mean of 9 cm H_2O

* The investigation was aided in part by a research grant from the U. S. Public Health Service.

during hemorrhagic hypotension and returned to the prebleeding level after transfusion. Eight rats were given additional transfusions in volumes equal to 25% to 100% of their estimated initial blood volume; in only 2 did this procedure elevate the portal venous pressure above 18 cm H_2O.

X-ray visualization of the intrahepatic portal bed. The portal vessels were observed to constrict to 50-70% of the control cross-sectional area. Blood replacement after several hours of hypotension promptly reestablished normal vessel caliber.

In vivo microscopy. Examination of the liver edge in the normal rat revealed a) portal venules approximately 30 to 40 micra in diameter, b) sinusoids 6 to 10 micra in diameter, and c) central and sub-lobular veins 30 to 80 micra in diameter. No hepatic arterioles were recognized. The portal venules were deep within the liver parenchyma, and flow within them was rapid without interruption or intermittency. No sphincteric action could be recognized at the tips of the smallest branches of the portal venules. Branching and intercommunicating sinusoids were observed clearly, though only infrequently could a single sinusoid be traced from origin to termination. Minute constrictions were noted frequently where sinusoids emptied into central and sublobular veins. Two or more sinusoids often joined to form a common channel before entering a collecting vein. Individual sinusoids at different moments contained flowing blood, stationary cells or else appeared empty. This intermittency of flow was associated with changes in sinusoid caliber and with what seemed to be sphincteric action at the junction of sinusoid and collecting vein. The caliber of the larger efferent vessels remained nearly constant, as did the velocity of flow within them. No arterio-venous or veno-venous anastomoses were seen.

Twenty-four rats were bled. Withdrawal of less than 0.7 cc/100 cm^2 of body surface produced no change in intrahepatic vessel caliber or blood flow. Slightly larger hemorrhage was quickly followed by blanching of the sinusoid bed. More than half of the sinusoids became bloodless and the remaining columns of cells moved slowly. Many of the open sinusoids were markedly narrowed at their junctions with the central veins. Central and sublobular veins were detectably narrowed. Sinusoid caliber and flow slowly returned toward normal if blood withdrawal stopped, constriction persisting longest in the central portion of the lobule.

Restoration of the original blood volume (15 rats) produced immediate congestion. All vessels except portal venules dilated beyond their original diameters. For some five minutes, the rate of flow remained slow, even though no site of persisting constriction was observed. Thereafter, flow gradually increased throughout the liver vasculature; the sinusoids resumed their normal size, first in the periportal areas where relative paling of the sinusoid bed could be discerned, later in the centrilobular zone. Finally, the central and sublobular veins narrowed to their resting diameter and flow again became brisk. Within 30 minutes the field regained a normal appearance in animals which remained at a stable normal arterial pressure and in those which subsequently relapsed into "irreversible" shock. The livers were not grossly congested at post-mortem examination in rats dying of irreversible hemorrhagic shock.

Discussion. There is reason to believe that, in the dog, the injury sustained by the liver during a long period of severe hemorrhagic hypotension is related to the failure of recovery after replacement transfusion(5). Intrahepatic vasoconstriction persists in the post-transfusion period and obstructs venous return from the splanchnic bed(1,2), but splanchnic obstruction is not the critical effect for an Eck-fistula does not protect the dog in hemorrhagic shock(6). Pharmacologic efforts have failed to release intrahepatic vasoconstriction so as to improve blood flow through the liver in "irreversible" shock(10).

In the rat, good flow within the hepatic sinusoids is restored by transfusion even if there is subsequent relapse into irreversibility. In most instances, the portal pressure is not elevated after blood replacement or after additional transfusions. The post-transfusion congestion which persists in the dog is transient in the rat. In addition, the rat's liver may be less vulnerable to anoxia than the dog's

liver, for, unlike the dog, the rat tolerates hepatic artery ligation without antibiotic protection(7,8) and, again unlike the dog, the normal rat's liver does not harbor bacteria(9).

Summary. 1. The average portal venous pressure of rats was 15 cm H_2O in the control period, 9 cm H_2O after bleeding to an arterial pressure of 45 mm Hg, and 14.5 cm H_2O immediatly after blood replacement. 2. Direct microscopy disclosed reduction in blood flow and narrowing of sinusoids, central venules and sublobular venules after bleeding from 0.7 to 1.0 cc/100 cm^2 of body surface. These changes became extreme after a hemorrhage of 2.1-2.4 cc/100 cm^2. Replacement transfusion after 3 hours of shock restored normal vessel caliber and blood flow, whether recovery followed or not.

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Received February 25, 1953. P.S.E.B.M., 1953, v82.

Embryonic Development in Turkey Eggs Laid 60-224 Days Following Removal of Males. (20202)

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Turkey spermatozoa remain viable within the body of the female for a far longer time than those of chickens. Fertile turkey eggs have been obtained and even poults hatched from eggs laid more than 50 days after removal of the male or following a single artificial insemination(1-3). An occasional fertile egg was encountered as long as 70 days after a single insemination(4).

Routine fertility checks of segregated turkey females were made by breaking incubated eggs laid by these hens prior to the time the birds were to be used in later matings. During the course of these fertility checks, eggs were encountered showing what appeared to be a delayed and retarded type of embryonic development. Furthermore, eggs showing these forms continued to be laid beyond the expected time limit recorded previously for the duration of fertility in turkeys. These observations made it desirable to study in greater detail the factor or factors responsible for eggs showing development of this type.

Materials and Methods. A group of 30 Beltsville Small White turkey hens, 32 to 38 weeks of age were segregated from males, on Jan. 21, 1952. Prior to the time of housing these 30 hens had been with sexually mature males of their own age but none of the females had produced any eggs. Every precaution was taken to assure that no male came in contact with these females after January 21. The first egg was laid by this group of 30 hens on March 18, 1952, 54 days after the removal of the males. All eggs produced thereafter were saved for incubation and were stored in a refrigerated room maintained at a temperature of about 55°F. The relative humidity averaged about 80%. The eggs were placed in an incubator each week and each setting of eggs was incubated for a period of 7-11 days. Upon removal of the eggs from the incubator they were broken and examined macroscopically for evidences of development. The