and attached to the topmost arm of the clamp. The attachment is accomplished by means of a small wire retainer made by bending both the small loop and the long end of the large loop of an ordinary paper clip down in the same direction until they are parallel to each other and perpendicular to the unbent, horizontal portion of wire. The wire of the small loop is then cut just above its lower curve. The ends of the two straight parallel wires of the cover slip retainer fit into small holes drilled through each end of the uppermost arm of the clamp. The cover slip is held between the clamp and the horizontal portion of the retainer.

The following is an enumeration of all the succeeding steps of the entire autoradiographic process:

1. Punch several small holes in one end of the cover slip. 2. Mount the bone sections on the other end of the cover slip. 3. Cut a rectangular section out of the center of the plastic cover slip. 4. Clamp the cover slip to the nuclear track plate and cement the end containing the small holes to the nuclear track plate with the special wax. 5. Remove the clamp. 6. Expose the track plate with a lead weight resting on that end of the cover slip on which the bone sections are mounted. 7. After the exposure, remove the lead weights and place the undeveloped autoradiographs in the plate carrier. 8. Fill the 3 compartments of the processing tank with developer, short stop, and acid fixer solutions respectively. 9. Process the plates. 10. Remove the processed autoradiographs from the plate carrier. 11. Prepare for washing by attaching the clamp and holding the cover slip perpendicular to the track plate with the cover slip retainer. 12. Wash the plates. 13. Remove clamp and cover slip retainer when the plates are dry.

The 3 requirements of a technique suitable for the production of autoradiographs to be used in the study of the distribution and concentration of a radioactive substance metabolically deposited in bone are fulfilled by this technique. (1) The attachment of the flexible cover slip containing the bone sections at one end with the special wax permits the bone sections to be removed from the portion of the nuclear track plate being processed. The bone sections are protected from contact with the processing solutions by the plate carrier. (2) The secure attachment of the cover slip to the nuclear track plate at one end also permits the bone sections to be removed from the nuclear track emulsion during the making of photomicrographs. (3) Since the cover slip is constantly attached to the nuclear track plate at one end, the bone sections mounted on the other end always return to exactly their original positions when desired.

The plate carrier, with a capacity for eight plates, and the motor-driven agitator prove to be valuable time-saving devices, for it is now possible to produce twice as many autoradiographs in a certain period of time as was formerly possible. This may be attributed to the capacity of the plate carriers and the actual reduction of the processing time, evidently due to superior agitation. Moreover, the quality of the autoradiographs is greatly improved, for no discoloration of the nuclear track plates is encountered.

Received October 23, 1950. P.S.E.B.M., 1950, v75.

Histopathology of the Liver Following Histamine Infusion.* (18330)

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One of the important aspects of hepatic physiology is the study of effects wrought by changes in the circulatory conditions in the liver. The present report is part of a series of such studies in this laboratory and is con-

* This work was supported in part by a grant (to R.W.B.) from the Research Grants Division, U. S. Public Health Service.

cerned with histopathological changes during the continuous infusion of histamine into the portal vein of dogs. A more extensive discussion of physiological variables will be presented elsewhere(1).

Methods. Healthy mongrel dogs of either sex, kept 2 or more weeks in the laboratory on standard kennel ration, were used. Sodium pentobarbital anesthesia (33 mg/kg intravenously at the start and 5 mg/kg every 90 to 120 minutes if needed) was employed. Mean arterial blood pressures were recorded from the left carotid artery. For the continuous infusions a catheter was inserted at laparotomy into a mesenteric vein, and passed thence into the portal vein; the wound was closed by clips, and the free end of the catheter was connected to an infusion set consisting of Marioøtte flask, drop counter, and tunnel clamp. The solutions infused were 0.9% NaCl in the controls and 2.0 x 10^{-4} M histamine diphosphate in 0.9% NaCl in the experimental animals. Collections of blood and liver lymph were carried out as described elsewhere(2). Bromsulphthalein concentrations in the blood were determined by the method described elsewhere(3). Twenty mg of dye were used and the values reported as mean percent of dye removed from the plasma in 5 minute periods. Tissue blocks of the liver, for histological study, were removed prior to sacrificing the animals and were fixed immediately in 10% neutral formalin. In several of the experiments, tissues were taken from other organs for histological examination. Paraffin embedding, and hematoxylin and eosin staining were used.

Results. Infusion rates were gauged to maintain arterial blood pressures 45 to 55% of the preinfusion level. This required initial infusion rates from one to three cc/min. in various dogs; during the course of most experiments the rate of infusion had to be gradually accelerated up to 3 to 6 cc/min.

TABLE I. Circulatory Changes During Histamine or Saline Infusions Into the Portal Vein of Dogs.

No. of dogs*	Material infused	Blood pressure during infusion —% of initial	% hematocrit		% BSP—5 min.	
			Before infusion	During infusion	Before infusion	During infusion
8	Histamine sol.	60.9 ±6.4	49.1 ±4.2	48.4 ±4.0	59.6 ±3.5	47.8 ±4.2
7	Saline	92.2 ±7.8	42.8 ±4.8	42.9 ±4.4	65.3 ±3.8	56.4 ±6.5

* These dogs were selected at random for BSP studies before conducting an experiment.

TABLE II. Effects of Intraportal Histamine Infusion Upon Relative Liver Lymph Flow and Blood Pressure.

(1) Dog No.	(2) Arterial blood pressure—% of initial during hist. infus.	(3) Lymph flow g/min. during saline infusion	(4) Lymph flow g/min. during hist. infusion	(5) Ratio of 4 to 3
47-49	85	.037	1.069	35.0
48-13	53	.098	.349	23.6
49-3	85	.047	.594	12.6
49-4	33	.354	1.30	3.6
49-6	72	.020	.185	9.3
49-9	83	.065	.303	5.5
49-10	46	.051	.636	12.3
49-37	91	.067	.127	1.9

Even after 4 or 5 hours of such infusion interruption of histamine flow invariably resulted in recovery of arterial blood pressures to preinfusion values within 15 to 25 minutes. Saline infused at rates comparable to the histamine infusions caused no significant changes in arterial blood pressure.

Table II shows the increase of liver lymph flow during histamine as compared to saline infusion. Liver lymph flow is considerably more sensitive to histamine infusion than the arterial blood pressure.

BSP extraction is slightly reduced by saline as well as by histamine infusions. A significant effect of histamine on this function was not observed. (Table I).

In the control group of 12 animals, which

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2. Brauer, R. W., and Hardenbergh, E., *Am. J. Physiol.*, 1947, v150, 746.

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were subjected to 480 to 600 minutes of saline infusion, the only noteworthy gross change in the liver was prominence of the portal areas. Microscopically the only demonstrable change was dilatation and congestion of the portal lymphatics.

In the 19 experimental animals the livers became enlarged, boggy and mottled red and red-brown in color 3 to 5 minutes after beginning the histamine infusion, and remained so throughout the experiments. There were dilated subcapsular lymphatics in addition to prominent portal areas.

Histologically the livers from these animals may be divided into 2 groups. In Group I, 4 animals which died in shock or were sacrificed after 45 to 165 minutes of histamine infusion, changes were minimal and consisted only of constriction of the central veins, congestion of sinusoids and dilatation of portal lymphatics. In Group II, 15 animals infused for 220 to 540 minutes, the portal veins (Fig. 1) were dilated, congested and their walls were infiltrated with neutrophils and lymphocytes. The fibrous tissue stroma of the portal areas was edematous and infiltrated with variable numbers of neutrophils, lymphocytes, and eosinophils. An occasional portal area was diffusely infiltrated (Fig. 2) with leukocytes or contained a hemorrhage. The portal lymphatics were dilated and congested with lymph. The sinusoids were dilated and congested, and an occasional neutrophil was marginated. Hyaline droplets were occasionally present within the liver cells. The central veins (Fig. 3) were markedly constricted and bloodless. The surrounding stroma was congested, edematous and infiltrated with neutrophils, lymphocytes, and macrophages. Eosinophils were sparse.

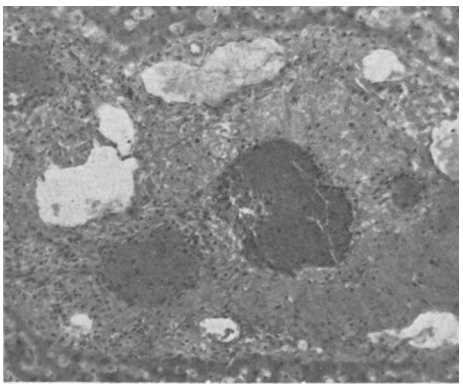

FIG. 1.
(Dog 47-70) portal area. Note the congested portal vein with the cellular reaction in the vessel wall which extends into the surrounding stroma. The dilated lymph channels are partly filled by coagulated lymph, and the edematous stroma contains scattered inflammatory cells. Hematoxylin and eosin ×60.

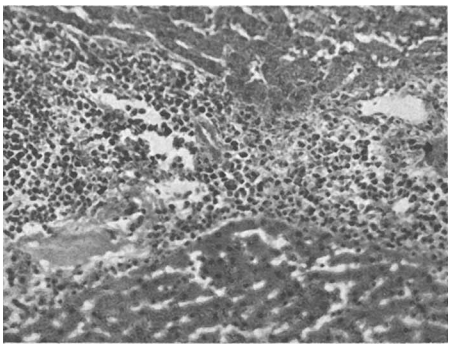

FIG. 2.
(Dog 49-15) portal area with diffuse inflammatory cell infiltration. Hematoxylin and eosin ×60.

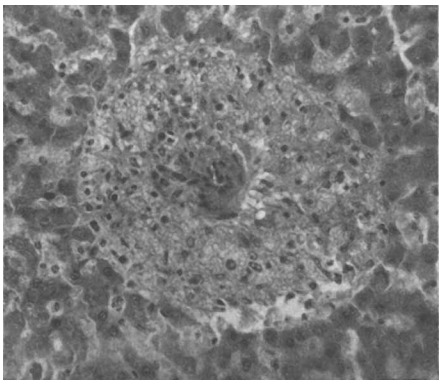

FIG. 3.
(Dog 47-70). The central vein is constricted and the surrounding stroma is edematous. Scattered inflammatory cells are present and the sinusoids are dilated and congested with red blood cells. Hematoxylin and eosin ×125.

Heart, lungs, spleen, pancreas, stomach, ileum, adrenals, and kidneys in several histamine treated animals were studied, in addition to the livers. However, no gross pathological or noteworthy microscopic changes were present in any of these specimens.

Discussion. Under the experimental conditions chosen irreversible shock does not set in even after 4 to 5 hours of histamine infusion; thus, low blood pressures must be

maintained by increasing doses of histamine as each experiment progresses, and prompt recovery follows interruption of the infusion. Hematocrits remained constant throughout as a result of the infusion.

Barring marked changes in the arteriohepatic venous gradient of BSP (which were not found)(1) the failure of histamine to significantly affect BSP clearance in these experiments indicates that total hepatic blood flow was little altered by the drug. Since there are indications of reduced portal venous blood flow (due to reflex vasoconstriction of splanchnic bed among other things) this observation suggests that the proportion of arterial blood supplied to the liver is increased under histamine infusion.

Hepatic lymph flow increases within a few minutes of the start of histamine infusion; it is preceded by visible engorgement of the liver. These observations suggest an increase of intrahepatic blood pressure, in accord with older reports(5) of a histamine activated sluice mechanism in the hepatic venules of the dog. To what extent increased permeability of the sinusoidal lining (similar to the "second permeability phase" described by Knisely)(6) also contributes to the increased lymph flow is uncertain at present. The cell content of hepatic lymph does not increase greatly unless arterial blood pressures fall below 20 to 25% of initial. These, in brief, are the circulatory conditions underlying the anatomical changes.

Histological evidence of liver cell damage is minimal (hyaline droplets in the cytoplasm of liver cells), and the early changes observed are confined almost exclusively to the vascular system. The late stage is one of exudation of leukocytes into portal and central vein regions in addition to the vascular changes. The leukocytic reaction is confined almost entirely to portal and central vein areas. The absence of central necrosis of the lobules in the presence of a leukocytic reaction differentiates the lesion described from that seen in pure chronic passive congestion(7). The histological picture described here differs also from that reported as a result of single large doses of histamine(8); the lesion under those conditions consists primarily of extensive extravasation of erythrocytes, and reflects conditions of irreversible shock with blood pressures of 20 to 30 mm Hg.

Summary. 1. The continuous infusion at sub-shock rates of histamine into the portal veins of dogs for periods exceeding 220 minutes results in the production of a definite liver lesion, distinguishable from the congestion observed after periods of histamine infusion less than 90 minutes, from the lesion of prolonged profound histamine shock, and from the cirrhosis of chronic histamine exposure. 2. The animals are not in shock and recover following discontinuance of the histamine infusion. 2. Liver lymph flow is greatly increased by histamine infusion, while bromsulphthalein extraction is reduced only slightly. Circulatory conditions in the histamine treated liver have been discussed briefly in terms of these findings. 4. In a series of control animals receiving prolonged saline infusions into their portal veins definite histological changes were not observed in the liver.

The authors are indebted to Mr. Antol Herskovitz, Montefiore Hospital, New York for the photomicrography.

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Received November 14, 1950. P.S.E.B.M., 1950, v75.