Primary Regulation of Histamine Metabolism by the Liver *

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CONSIDERABLE EXPERIMENTAL evidence exists at the present time that the liver plays a primary role in the metabolism and degradation of endogenous histamine and that hepatocellular damage or diversion of portal blood may alter this metabolism producing excessive systemic histamine effects such as gastric hypersecretion. Anrep in 1953 [1] repeatedly demonstrated higher blood histamine levels in portal blood compared to either hepatic or systemic venous blood. Clark [3] and Silen and Eiseman [14, 15] and their associates conclusively demonstrated that portal venous bypass in animals routinely produces hypersecretion from denervated Heidenhain pouches. Anrep,[2] Menguy,[9] Silen [16] and Skillman [18, 19] noted that liver injury produced by a variety of causes, including biliary obstruction or diversion and the prolonged oral administration of carbon tetrachloride and alcohol, similarly produces gastric hypersecretion.

The bulk of the evidence at the present time lends strong support to the hypothesis advocated by Irvine [6] and by Silen and associates [17] that endogenous histamine is the agent involved and that liver damage or liver bypass decreases inactivation of this potent secretagogue resulting in direct and excessive stimulation of gastric parietal cells.

The present series of experiments were undertaken to study further the quantitative capacity of the liver to remove histamine from the portal circulation and to evaluate the possible role of the lymphatic system in the visceral transport of histamine.

Methods

Twenty-three adult mongrel dogs of varying sizes were studied in four experimental groups (Table 1).

Portal Infusion Group. Ten dogs were anesthetized with 30 mg. pentobarbital sodium/Kg. body weight administered intravenously. Through a short midline abdominal incision three fine polyethylene catheters were placed for blood sampling and histamine infusion (Fig. 1). One catheter was threaded through the splenic vein to the bifurcation of the portal vein at the porta hepatis. Another catheter was introduced into the free segment of the left interlobar hepatic vein and carefully secured with a fine purse-string suture. The end of this catheter was passed into the hepatic vein 1 to 2 cm. and directed toward the liver away from the vena cava to avoid both an hepatic wedge sample and a sample mixed with blood from the vena cava. The position of this catheter was judged critical and was verified at the conclusion of each experiment. It had been previously shown that this vein drains approximately 40 per

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TABLE 1. *Experimental Groups*

Sub-Group	Experiments No.
I. Portal Histamine Infusion	10
II. Hepatic Artery Ligation	4
III. Thoracic Duct Lymph Study	6
IV. Isolated Liver Perfusion	3
Total	23

cent of the entire liver mass in the dog. A third catheter used for the histamine infusion was placed into a distal ileal vein. The abdominal catheters were carried out through stab wounds and secured to the skin. All catheters were intermittently flushed with dilute heparinized saline solution to ensure continued patency and to permit free flow of blood during subsequent studies. Although the majority of the experiments were performed as acute studies, a few dogs were allowed to recover for subsequent study a few days later without any anesthesia or sedation. A solution of histamine dihydrochloride containing 2.5 or 5.0 mg. of free histamine in 100 ml. of normal saline was then administered via ileal vein catheter at a constant rate of 8 ml./min. with a calibrated constant infusion pump. It had been established in preliminary experiments that this concentration and rate of infusion of histamine gave the desired range of portal histamine levels. Duplicate samples of whole blood using sodium oxalate as an anticoagulant were slowly drawn simultaneously from the portal and hepatic vein catheters at 3 to 5 min. intervals before, during and after the infusion of histamine.

Hematocrits were performed before and at the termination of each experiment. Usually only a single experiment was performed on each animal. In a few dogs, arterial blood samples were also simultaneously drawn from the femoral artery to check systemic histamine levels during the infusion study.

Hepatic Artery Ligation Group. In four additional dogs prepared with catheters in a fashion similar to the previous group, acute ligation of the main hepatic artery and its major gastroduodenal branch was performed with heavy silk ligatures. It was the purpose of this phase of the study to determine the effect of acute arterial hepatic anoxia on the clearance of histamine. Studies in these dogs were all performed from 1 to 3 hours after the interruption of the hepatic artery supply.

Lymph Study Group. In six dogs, following insertion of the abdominal catheters for sampling and infusion, a thoracotomy was performed through the left fourth intercostal space. The thoracic duct was dissected free and ligated at the point where it crosses the left subclavian artery in the left thorax. A small polyethylene catheter was then introduced into the thoracic duct distally for a distance of 1 to 2 cm. and anchored in position with a few sutures. The catheter was then placed on gravity drainage for continuous sampling of tho-

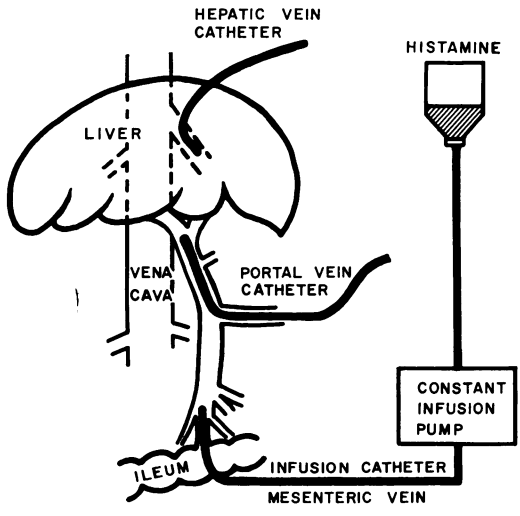

FIG. 1. Technic of portal infusion and blood sampling in the first 3 groups of experiments.

racic duct lymph. Samples were collected at successive 10-min. periods for histamine analysis and the volume collected was carefully recorded for each period before, during and after the histamine infusion. All lymph was collected in clean graduated tubes containing oxalate to prevent clotting.

Isolated Liver Perfusion Group. In three dogs, total isolation of the liver was performed *in situ* by a technic modified from that described by Kestens and associates.[7] Briefly this technic involved isolation and cannulation of the segment of abdominal vena cava draining the hepatic veins and continuous perfusion of the liver through the isolated hepatic artery and portal vein by means of a series of pumps and a bubble oxygenator (Fig. 2). By this method continuous physiologic perfusion of the liver was performed by an external system utilizing only the hepatic venous drainage. Only minimal heparinization of the blood in the perfusate was utilized in order to avoid altering the clearance or release of histamine by the liver.[10] Careful monitoring of the oxygen saturation of the hepatic venous, hepatic arterial and portal venous blood was performed. Similarly pH, temperature and total hepatic blood flow were monitored and adjusted to maintain optimum physiologic conditions. External bypass of the portal blood draining the abdominal viscera and of the inferior vena cava was simultaneously performed in the intact dog through a separate system of catheters and a pump in order to maintain normal perfusion of extrahepatic structures during the period of liver isolation and perfusion. Previous studies using tagged red cells or T-1824 dye had demonstrated that no detectable leak was present into either of the perfusion systems during a 2 to 3 hour perfusion period. Histamine loads of 2.5 or 5.0 mg. were added as a single dose to the 1.0 L. perfusion system via the portal reservoir after baseline histamine determinations had been obtained. Blood samples for histamine determinations were

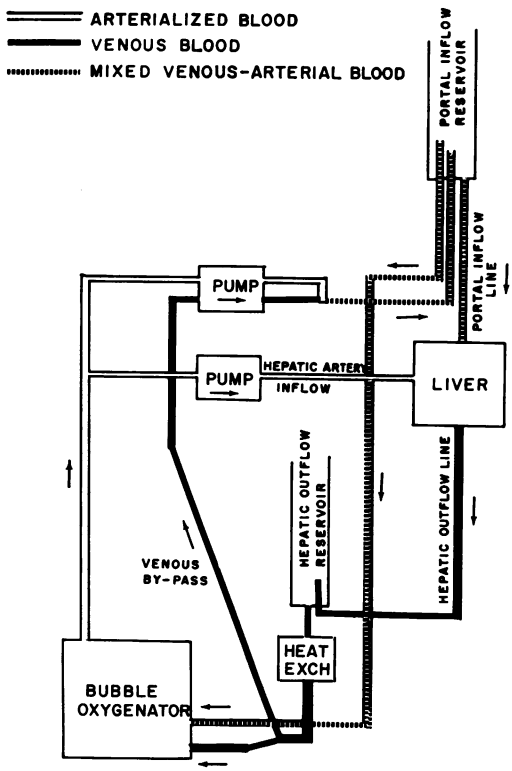

FIG. 2. Diagram of technic of *in situ* perfusion of isolated liver. Hepatic venous outflow is collected by gravity into a reservoir, then passed through a heat exchanger to maintain normal body temperatures (36°–38° C.). The blood then passes through a bubble oxygenator and is either pumped directly into isolated hepatic artery or into a portal inflow reservoir at a level of 15 to 25 cm. above the liver. Blood in portal reservoir is mixed with unoxygenated venous blood and maintained at normal portal venous saturation of 65 to 85%. Total hepatic blood flow is maintained at normal rate of 35 to 45 ml./Kg. body weight/min.

obtained from the liver perfusion circuit at 2, 4, 10, 15, 20 and 25 min. after loading. Control experiments without a liver in the system revealed no disappearance of the histamine load as measured in the whole blood within the perfusion system.

Whole blood histamine concentration was measured in duplicate by the fluorometric assay of Shore, Burkhalter and Cohn[13] utilizing an Aminco-Bowman spectrophotofluorometer with an excitation at 360 millimicrons and the fluorescence at 450 millimicrons. This method gave a linear curve of results for known standards in the range

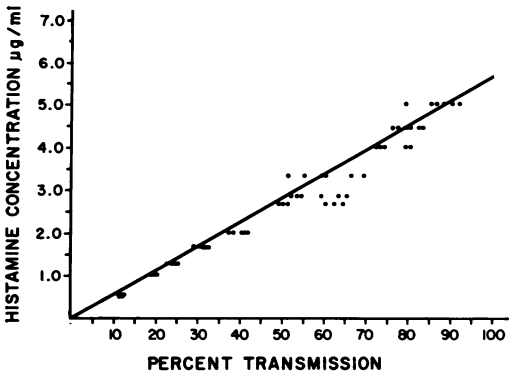

FIG. 3. Linearity of histamine recovery from known standards is shown utilizing fluorometric technic described. Considerable accuracy and reproducibility of technic is apparent. In each histamine determination from all experiments duplicate values were compared and reported as their mean as a further assurance of accuracy.

of histamine levels encountered in this study and was felt to be extremely sensitive and accurate (Fig. 3).

Results

The mean fasting pre-infusion histamine concentration in the portal vein was 0.16 μGm./ml. of whole blood (S.D. = 0.03). The simultaneous mean hepatic vein concentration was 0.14 μGm./ml. (S.D. = 0.04). When the histamine solution was infused into the portal bed through a mesenteric branch, the portal histamine levels showed a progressive rise during the 12 to 15 minute infusion in all instances. In Figure 4 a typical experiment is depicted showing the concentrations of histamine in the portal and hepatic veins and in the femoral artery before, during and after infusion.

The large rise in portal histamine during infusion, approaching 50 times that of the pre-infusion level, was accompanied by a smaller increase in hepatic vein histamine. Systemic arterial (femoral) samples showed only minimal elevation of histamine from control values. In this study, elevation of portal histamine by the infusion was felt to exceed markedly any physiologic or pathologic conditions ever encountered. Despite these values, however, marked clearance of histamine was produced by the liver. When a smaller dose (2.5 mg.) of histamine was infused in other experiments, essentially all of the portal histamine was cleared by the liver and the hepatic venous and systemic arterial histamine levels did not change significantly from the pre-infusion values.

Ligation of the hepatic artery and its major collateral branches prior to the infusion of histamine into the portal system did not appear to alter significantly the clearance of histamine by the liver. In Figure 5 a typical experiment is depicted from one of the dogs in this group. Despite a 30-fold rise in portal histamine, hepatic vein histamine remained essentially unchanged. Thus the liver was able to clear histamine even during periods of acute hepatic arterial inflow occlusion (1 to 2 hours). Similar results were encountered in the other three dogs in this group.

To correlate all of the experiments in the 14 dogs in the previous two groups, a scattergram comparing portal and hepatic venous histamine levels is presented (Fig. 6). Simultaneous portal and hepatic vein

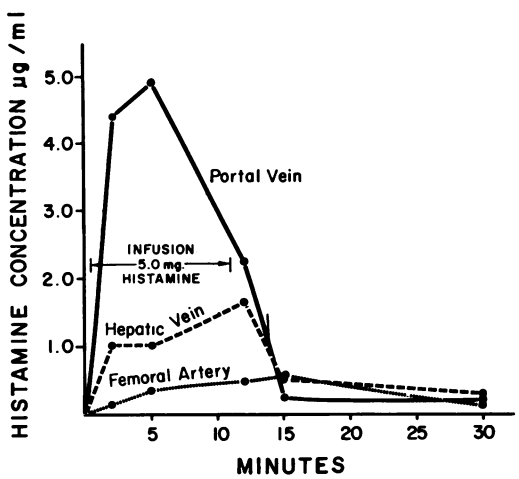

FIG. 4. Results of a single experimental infusion of histamine solution into portal system through a mesenteric vein. Total of 5.0 mg. of histamine was administered over a 12-min. period in this dog.

samples during these experiments are included showing the effect of increasing portal histamine on hepatic vein histamine levels. It is apparent in these dogs that the liver was able to remove, in a single passage of portal blood, substantial quantities of histamine even at infusion rates which produced portal histamine elevations up to 50 times the control values for a 15-minute period.

In the six dogs prepared for study with a thoracic duct lymph fistula, a prompt and marked increase in thoracic duct flow occurred during the intraportal infusion of histamine. In Figure 7 are depicted the results from a single experiment in this group. Thoracic duct lymph flow increased from an average control of 60 ml./hr. to a peak of 160 ml./hr. 10 minutes after the start of the histamine infusion. Lymph flow did not return to normal until approximately 45 minutes after the infusion was terminated. Marked clearance of portal vein histamine was noted and was similar to the clearance in the dogs studied in the previous groups. Of interest, however, was the observation that lymph histamine values remained unaltered from normal. It was apparent, therefore, that free histamine was not entering the circulation through the lymphatic system in this study. Similar results were noted in the remaining dogs in this group. The average peak increase in the thoracic duct lymph flow from all six experiments was 225 per cent of control.

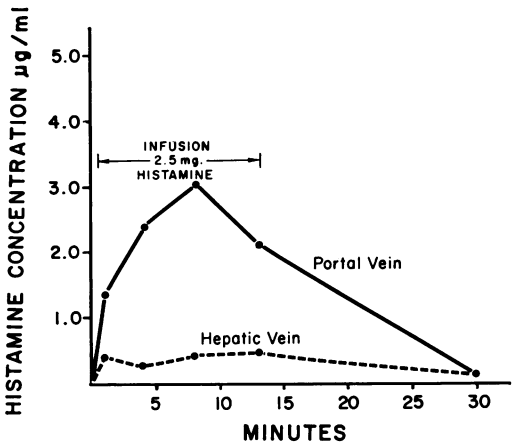

FIG. 5. Effect of ligation of hepatic artery on hepatic histamine clearance from a single experiment. Marked elevation of portal histamine does not produce any significant change in hepatic vein histamine, indicating essentially normal hepatic clearance despite acute hepatic arterial anoxia.

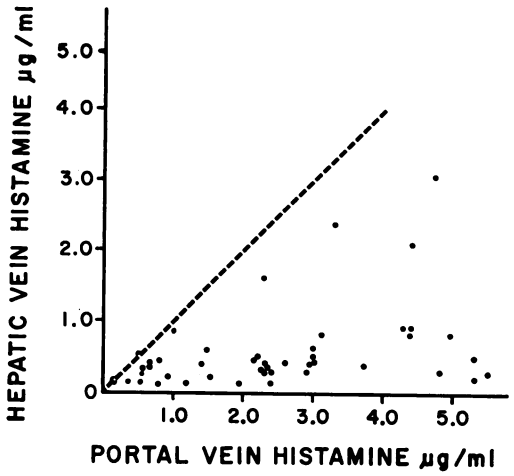

FIG. 6. Scattergram of simultaneous portal and hepatic vein, histamine determinations from 14 dogs during intraportal infusions of histamine in amounts from 2.5 to 5.0 mg. given in 12 to 15 min. period. Pre-infusion control values omitted. Broken line indicates theoretic line around which all points would be expected to group if liver were not clearing histamine (portal vein histamine = hepatic vein histamine). Almost all points fall considerably below this line reflecting a lowered hepatic vein histamine and rapid hepatic clearance. Values obtained from dogs with hepatic arterial ligation are scattered within entire group and cannot be distinguished from values obtained in normal dogs in first group.

Isolated perfusion of the liver, as performed in the fourth group of dogs, gave strong confirmation that the liver *per se* was rapidly clearing a large single histamine load within 20 to 30 minutes of addition of the load to the perfusion system (Fig. 8). In a few instances where smaller amounts of histamine were added to the portal reservoir, almost total clearance occurred within the estimated 5-minute single circulation time of the perfusion circuit. Some degree of *outflow block,* i.e., an increase in hepatic resistance, was noted in every experiment and this lasted for a variable period after

the histamine load. This was evidenced by an immediate decrease in hepatic blood flow, an increasing portal pressure and a turgid, congested liver. Usually, however, the block cleared soon after return of histamine to control levels. Table 2 presents the whole blood histamine levels from this group of experiments before and after a histamine load.

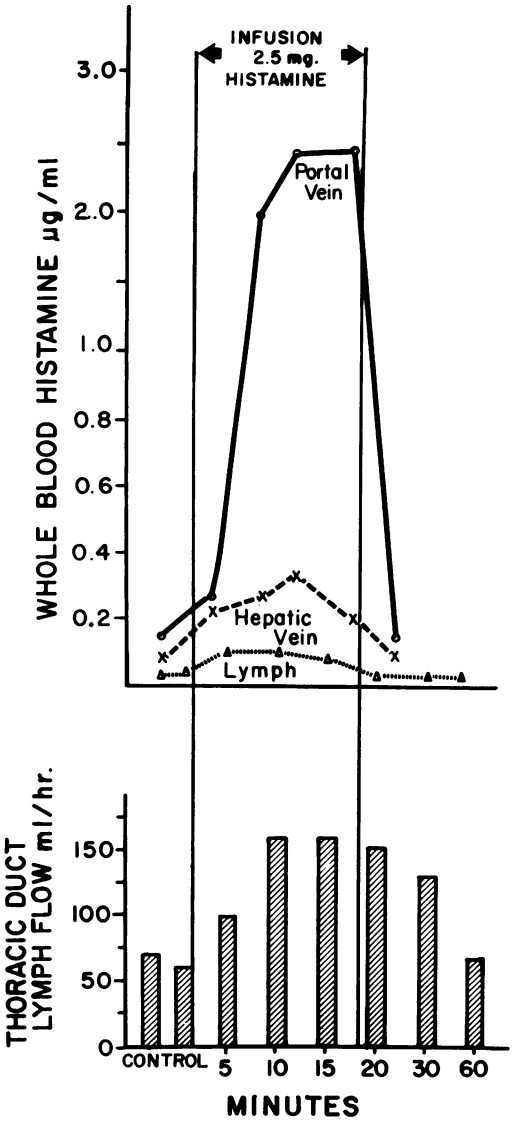

FIG. 7. Effect of single experimental intraportal infusion of histamine solution on portal, hepatic vein and thoracic duct lymph histamine and thoracic duct lymph flow.

Discussion

Eiseman, Moore and Normell, utilizing a technic of isolated perfusion of the pig liver [5] and canine liver [11] in isolated chambers, were able to determine some of the factors which affect the degradation of histamine by the liver. They reported that the major portion of a histamine load was cleared from the blood during its first passage through such a liver preparation. Our results, using a similar preparation but with a liver perfused *in situ* in an intact animal, confirm the fact that this clearance, even of a grossly unphysiologic load, occurs with great rapidity. Further support of the rapidity of hepatic clearance of histamine was gained in these studies by the demonstration of little or no increase of hepatic vein histamine, despite elevation of portal levels up to 50 times normal, produced by continuous infusion of histamine into the portal bed.

The exact mechanism of degradation of histamine by the liver remains unclear but the bulk of evidence at the present time indicates that methylation is the primary metabolic pathway. Lindahl [8] in her extensive studies of histamine degradation has shown that the enzyme histamine methyltransferase is present in the hepatic cellular cytoplasm of man and a variety of other species including the pig, dog, mouse, hamster, rabbit and cat. This enzyme catalyzes the conjugation of histamine to methyl histamine, the reaction probably utilizing the methyl group of methionine as the methyl donor. The methyl histamine is then further broken down by oxidation in successive steps, the metabolic end products being finally excreted in the urine.

Eiseman and associates [8] further indicated that alkalosis improves and acidosis diminishes such histamine degradation. This is probably related to the ionization constants of histamine which would make it a poorer substrate for histamine methyltransferase inactivation in the acid range.

Although for many years diamine oxidase (histaminase) was considered the only primary enzyme attacking histamine, degradation by this metabolic pathway is probably only of lesser importance in the liver. This would be in keeping with the observation by Eiseman and coworkers that amine oxidase inhibitors, when added to their perfusion system, had little effect upon the rapid rate of clearance of histamine loads by the pig liver.

Of extreme interest in these experiments was the observation that hepatic clearance of histamine continued at a rapid rate despite ligation of all arterial inflow to the canine liver. It was assumed from this observation that sufficient oxygen was available to hepatic cells via portal blood supply to maintain this enzymatic function. Eiseman and coworkers were similarly able to demonstrate practically normal histamine clearance despite 30-minute periods of total hepatic anoxia during perfusion of the pig liver. They concluded that these enzymes were extremely durable to short periods of anoxia. To what extent anoxia may affect subsequent oxidation of methyl histamine to methyl imidazole acetic acid is unknown but it is a fair assumption that prolonged anoxia would eventually impair histamine degradation both by this pathway and by the alternate but lesser route of amine oxidation.

The syndrome of hepatic "outflow block" characterized in the canine liver by a tense swollen liver and increased hepatic vascular resistance [4] was well demonstrated in these experiments and occurred not only in the livers perfused *in situ* but also in normal dogs following infusion of histamine into the portal vein. This was accompanied by a marked decrease in hepatic blood flow. However, despite varying degrees of outflow block, histamine clearance did not appear to be materially affected.

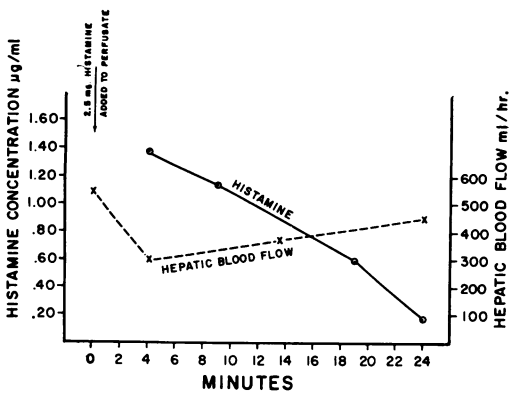

FIG. 8. Clearance of a 2.5 mg. load of histamine from whole blood during isolated liver perfusion *in situ* from a single experiment. Entire histamine load was removed by perfused liver in 24 min. Note marked decrease in hepatic blood flow produced by histamine load. This was accompanied with a moderate but temporary "outflow block" as evidenced by a dusky congested liver.

The possibility was raised during these experiments, particularly following the observation of the "outflow block," that free histamine could conceivably be rapidly drained from portal blood through the hepatic sinusoid into the hepatic lymphatics and thereby enter the systemic circulation through the thoracic duct. Furthermore it had been demonstrated from previous experiments in our laboratory [12] and later confirmed by Zeppa and Womack [20] that hista-

TABLE 2. *Clearance of Histamine from Perfusion System*

Perfusion No.	Histamine Load mg.	Whole Blood Histamine (µGm./ml.)						
		Control	2 min.	4 min.	10 min.	15 min.	20 min.	25 min.
7	2.5	0.07	—	1.39	1.15	—	0.59	0.19
14	2.5	0.21	—	0.51	0.36	0.12	0.14	0.14
16	5.0	0.04	0.07	1.97	0.94	0.32	0.17	—

mine produces a marked increase in hepatic lymph flow. In as much as the contribution of the liver to the volume of thoracic duct lymph generally measures one fourth to one half of the total, it appeared reasonable, therefore, to postulate that any significant increase in the histamine content of hepatic lymph would be reflected in the histamine levels of thoracic duct lymph. Indeed, Zeppa and Womack have shown an increase in thoracic duct lymph flow following infusion of histamine solely into the portal bed and have postulated the liver was the primary source of the added increment to thoracic duct lymph flow under these conditions. The absence of any significant increases in the histamine content of thoracic duct lymph during and after intraportal infusion of histamine in our experiments appears to rule out, therefore, transport of unconjugated histamine from the portal bed via hepatic lymph. Whether or not rapid conjugation of free histamine to methyl histamine can occur in the liver followed by entry of this conjugated histamine into the lymph cannot be answered by these experiments. The spectrofluorometric method used in these studies was not capable of picking up conjugated or methyl histamine. It is probable, however, that histamine removal and transport through the lymph is not an important mechanism because of the demonstrated ability of the isolated liver to clear portal histamine even after interruption of all lymphatics. Finally, these experiments strongly support the mounting evidence that the liver occupies a primary role in the metabolism and degradation of histamine and particularly in the regulation of its visceral histamine entering through the portal circulation. This would be consistent with the major role of the liver in the metabolism, storage or conjugation of a large variety of metabolites entering the portal circulation including glucose, amino acids, ammonia and other amines such as serotonin.

The demonstration *per se* of this effective histamine removal by the liver lends indirect but strong support to the concept that in event of portal venous bypass into the systemic circulation by shunts, both natural or surgical, or by hepatic cellular damage, histamine may be the agent responsible for gastric hypersecretion. This would also explain the high incidence of peptic ulceration known to occur in these patients.

Summary and Conclusions

Hepatic clearance of exogenously administered free histamine was evaluated by a series of experiments which included direct measurement of portal venous, hepatic venous systemic arterial histamine levels.

The normal portal whole blood histamine in fasting dogs was 0.16 μGm./ml. whole blood (S.D. = 0.03), and the hepatic venous concentration was 0.14 μGm./ml. (S.D. = 0.04).

Infusions of histamine into the portal vein produced elevations in portal histamine up to 50 times that of pre-infusion levels. Simultaneous hepatic venous and systemic arterial blood samples showed little or no rise in histamine, indicating marked histamine clearance in a single passage of portal blood across the liver bed. Ligation of hepatic arterial blood supply did not significantly alter this clearance.

Clearance of a histamine load from a circuit used for isolated *in situ* perfusion of liver occurred within 20 to 30 minutes after addition.

Varying degrees of temporary hepatic "outflow block" were noted following infusion of histamine into the portal vein. This was accompanied by a decrease in hepatic blood flow and increased hepatic vascular resistance.

Histamine produced a marked increase in thoracic duct lymph flow, averaging 225 per cent of control, which lasted up to 45 minutes after cessation of histamine infusion.

Increase in thoracic duct lymph histamine did not occur during portal histamine infusion. It was concluded that lymph transport of histamine from the liver was not responsible for the lowering of hepatic venous histamine levels.

These experiments indicate that the liver is capable of degrading large quantities of histamine entering via the portal blood. The liver therefore occupies an important position in the regulation of histamine metabolism. Strong indirect support thus indicates that diversion of portal blood around the liver may be manifest by altered histamine metabolism resulting in gastric hypersecretion and peptic ulceration.

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