Excretion of ^{3}H-Histamine in Urine of Rats with Portacaval Shunts[1] (35359)

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Gastric acid hypersecretion frequently associated with susceptibility to peptic ulceration has been found in animals subjected to portacaval shunts (1–7). It is possible that a potent humoral agent and mediator of the intestinal phase of gastric secretion escapes degradation by the liver in the presence of liver bypass. This humoral agent may enter the systemic circulation in sufficient quantity to stimulate the parietal cells of the stomach (7). The liver plays an important role in the metabolism and degradation of both endogenous and exogenous histamine, and it is possible that liver bypass may delay the catabolism of histamine. Several investigators proposed that histamine is the humoral mediator responsible for the gastric hyperacidity associated with liver bypass (4, 8–10). Other authors have provided evidence against the role of histamine in shunt-related gastric hypersecretion (5–7, 11, 12). More recently it has been suggested that the postshunt hypersecretion seen in rats may be due to increased synthesis of histamine by the gastric mucosa. It was found that the activity of histidine decarboxylase of gastric mucosa was elevated in rats with portacaval shunts and this was associated with an increase in urinary histamine (13, 14). This finding appears to be particularly important in view of the studies reporting that the stomach is a major source of urinary histamine in the rat (15, 16).

Isotopic *in vivo* measurement of urinary histamine is now considered to be the most specific and sensitive method for the study of the rate of histamine formation (15, 16). Rats with portacaval shunts and sham-operated rats were therefore injected with tritium-labeled histidine (17) and the urinary excretion of radioactive histamine was determined.

Materials and Methods. Virgin, female rats of Wistar strain (Woodlyn Farms, Guelph, Ontario), weighing 250–280 g, were operated under sodium pentobarbital anesthesia. End-to-side portacaval shunts were performed in 12 rats. Patency of the anastomoses has been proved by radiography (Fig. 1). In 11 control rats, sham operation, consisting of laparotomy, and closing of the abdominal wall was done. On the tenth day following surgery, the rats were placed individually in metabolic cages, and stayed there for 10 days prior to the study of urinary histamine. When in the metabolic cages, the animals were fed and received drinking water as desired. Purina laboratory chow as given to the rats was analyzed for the content of total protein, histidine, and histamine, and these were found to be 23.4, 0.44, and 0.061% of dry weight, respectively. Urine was collected from each animal for four consecutive 24-hr periods, following the injection of labeled histidine. Throughout the experiment, each rat received aminoguanidine sulfate (20 mg/kg of body wt of free base) subcutaneously once daily, to inhibit histaminase. One hr after the second injection of aminoguanidine, rats were given an intravenous injection (into a femoral vein) of ^{3}H-L-histidine monohydrochloride (sp act $= 30 \times 10^6$ dpm/mg, free base) (17). The dose was 1.6 mg/kg of body weight. One ml of 6 *N* HCl containing 17 mg of histamine dihydrochloride and 12 mg of histidine monohydrochloride, was added, as a carrier, to the 100-ml flask used for the collection of urine. Collected 24-hr samples were kept at 4° until analyzed (13).

Radioactivity in the urinary histamine was determined by a method following Schayer (18). Histamine was extracted from the urine

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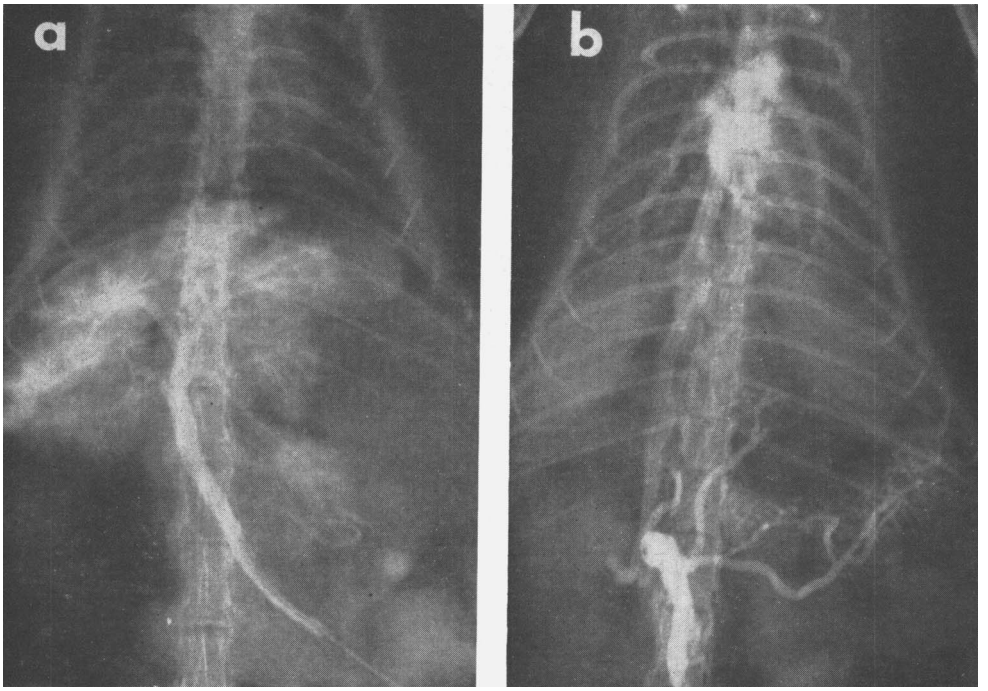

FIG. 1. Radiograms of control (a) and shunted rat (b): Contrast medium (75% Hypaque) injected into a mesenteric vein, bypasses the liver in a rat with portacaval shunt (b).

using a butanol–chloroform mixture, and purified by back extraction into a basic aqueous system. Bio-solv solubilizer (BBS-3, Beckman Instrument Co. Ltd.) (20) was added to the final extract together with PPO and POPOP in toluene. The samples were counted in the Picker Nuclear Liquimat 220 liquid scintillation system for 10 min. The efficiencies were determined using the channel ratio method and the results were expressed as disintegrations per minute (dpm) in each 24-hr specimen. Recovery of histamine ^3H was 78%. The radiochemical purity of the histidine ^3H used was checked by subjected samples to the histamine isolation procedure. Less than 0.05% of the radioactivity was recovered. Student's t test was used for analysis of the results.

Results. The amount of ^3H-histamine excreted by shunted rats was found to be lower than the amount excreted by the sham-operated group ($p<0.05$). This difference was apparent for the first two 24-hr periods following the injection of ^3H-L-histidine (Table I). The rate of excretion of ^3H-histamine in both groups of animals was high during the first 24 hr after the injection of the precursor. It was followed by a sharp decrease in the excretion during the remaining 24-hr periods (Table I). This agrees with previous observations, that most of the radioactive histamine appears in the urine in 24 hr following injection (15). Table II shows that shunted rats excreted more urine than control animals. The results of this study are summarized in Table II.

Discussion. According to Newman *et al.* (12) the three possible explanations for post-shunt gastric hypersecretion are (a) enhanced intestinal phase of gastric secretion, (b) altered responsiveness of the parietal cell, or (c) increased synthesis of histamine by the gastric mucosa. According to hypothesis (a), shunting of an intestinal gastric secretagogue away from the liver would decrease hepatic catabolism of this secretagogue. Histamine has been considered as such a secretagogue by several investigators (4, 8–10). More recent studies, based on measurement of blood and/or urinary histamine in shunted dogs, have failed to verify this hypothesis (5, 6, 12, 21). Regarding the

TABLE I. Urinary Excretion of ^{3}H-Histamine After Intravenous Injection of ^{3}H-L-Histidine (1.6 mg/kg of body wt) in Rats with Portacaval Shunt and Sham-Operated Controls.

Results are expressed as disintegration per minute (dpm) in 24-hr urine, during four 24-hr periods, following the injection; averages and SD.

No. of rats	Surgery	Urine collected (hr) 0–24	24–48	48–72	72–96
11	Sham	22867 ± 9287	3202 ± 1141	1569 ± 505	877 ± 461
12	Portacaval shunt	15913 ± 8172	2432 ± 874	1517 ± 608	1161 ± 927
p		<0.05	<0.05	>0.05	>0.05

possible increased responsiveness of gastric mucosa to all stimulants, in shunted animals (hypothesis b), histamine was not considered as a specific stimulant of physiological importance (22, 23).

The suggestion that histamine synthesis is increased in the stomachs of shunted animals (hypothesis c) was forwarded by Fischer and Snyder (13, 14). These authors found that enzymatic synthesis of histamine is increased in the gastric mucosa of rats with portacaval shunts. They suggested also, that an increase of circulating and gastric mucosal histamine in shunted rats, could not be the result of decreased hepatic catabolism of histamine, as stated by Day *et al.* (9), but represented increased synthesis of histamine. Their conclusions were based on finding an increase in the activity of histidine decarboxylase in the gastrointestinal tract of rats. No urinary determinations were done in their studies (13, 14). Newman *et al.* (12) attempted to verify hypothesis (c) in dogs. They were unable to demonstrate any change in enzymatic synthesis of histamine in animals with portacaval shunts (12). Reichle *et al.* (24) studied the metabolism of histidine after portacaval shunt in the rat. They did not use isotopic methods, but analyzed histidine metabolites in the livers and urine. They found that excretion of histidine and histamine was higher in portacaval shunted rats than in sham-operated rats, when both groups were challenged with a high histidine diet. Also enzymatic activity of histidase and histidine-pyruvate transaminase was increased in the liver of shunted rats (24). No explanation was given for these findings (24). It is unfortunate that in the presentation of their results, Fischer and Snyder (13, 14) and Reichle *et al.* (24) did not mention the sex of the rats used.

It is known that the urinary excretion of histamine is lower in male than in female rats (25) and this is due to the difference in the catabolism of histamine in the two sexes. The principal means of catabolism of histamine are oxidative deamination (by histaminase) and methylation. In male rats, methylhistamine is a major metabolite of histamine (16), as hormone-dependent capacity to methylate histamine is larger in male than in female rats (25). Histaminase appears on the other hand, to be the major catabolic enzyme in females. Histaminase inhibitor aminoguanidine, which increases free histamine excretion

TABLE II. Excretion of Urine (ml/24 hr) in Rats with Portacaval Shunt and Sham-Operated Controls.

Same animals as in Table I; averages and SD.

No. of rats	Surgery	Urine collected (hr) 0–24	24–48	48–72	72–96
11	Sham	10.6 ± 2.9	9.5 ± 3.9	8.3 ± 3.5	6.6 ± 2.7
12	Portacaval	29.0 ± 22.4	24.4 ± 18.7	21.2 ± 18.3	15.6 ± 9.6
p		<0.01	<0.01	<0.05	<0.05

in the urine of female rats had no effect in male rats (26). In our experiment, we used female rats. We also attempted to decrease the catabolic action of histaminase by aminoguanidine in order to stimulate the excretion of free histamine in the urine of these animals.

To determine the rate of whole-body histamine formation, radioactive histidine is injected into an animal and urinary excretion of radioactive histamine and methylhistamine is studied (16). We limited our study to the analysis of urinary ^{3}H-histamine, considering the importance of this substance in female rats. Determination of methylhistamine would be important in male rats (25, 26), but was not considered necessary when female animals were used. Consequently, we did not determine whole-body histamine formation, but we analyzed the rate of ^{3}H-histamine formation from an injected precursor, ^{3}H-L-histidine, during four 24-hr periods. It is apparent that our experiment cannot be compared directly with other studies on rats with portacaval shunts (13, 14, 24). The absence of information regarding the sex of the rats used and differences in the methods used by others (13, 14, 26) makes such a comparison difficult.

An increase in the rate of histamine synthesis should result in a comparable increase in urinary excretion, unless accompanied by a compensating increase in the rate of degradation. Such an increase is unlikely following portacaval shunt. The present results show a modest decrease in the short-term excretion of histamine which has previously been attributed to gastrointestinal histamine production (25). This decrease probably represents histidine metabolism showing a rapid turnover. This may, in part, explain the conflicting reports in the literature.

The finding of increased urine output in shunted rats, as compared with sham-operated rats, remains unexplained and deserves further experimental investigation.

Summary. It has been reported previously by two groups of investigators that portacaval shunt in rats is followed by the increased enzymatic synthesis of histamine in the gastrointestinal tract and by the elevated excretion of histamine in urine. The purpose of our experiment was to determine whether side-to-side portacaval shunt affects the urinary excretion of radioactive histamine following the injection of radioactive histidine. Female rats with shunts and sham-operated animals received ^{3}H-L-histidine intravenously. Urine was collected from these animals for four 24-hr periods and analyzed for ^{3}H-L-histamine. The expected elevation of urinary histamine in shunted animals was not found. The excretion of ^{3}H-histamine was lower in animals with portacaval shunts than in controls. Shunted animals excreted more urine than sham-operated rats. The results of this preliminary experiment do not support the hypothesis of an increased synthesis and excretion of histamine in animals with portacaval bypass.

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