THE SIGNIFICANCE OF HORMONES, BILE SALTS, AND FEEDING IN THE REGULATION OF BILE AND OTHER DIGESTIVE SECRETIONS IN THE RAT

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Abstract

Conscious rats produced bile at $19 \pm 0 \cdot 6$ μl/kg/min during periods of bile deprivation. When sodium taurocholate was infused intravenously, the bile flow increased to $54 \pm 4 \cdot 8$ μl/kg/min—a value almost identical to that recorded in other rats during normal recirculation of endogenous bile salts.

The flow and bicarbonate content of pancreatic juice was stimulated by secretin, the protein content of pancreatic juice was stimulated by cholecystokinin, and pentagastrin stimulated the secretion of gastric acid. Feeding also stimulated secretion of pancreatic juice. All responses were much smaller than those reported in dogs and humans, and no changes occurred in the flow or composition of bile.

It is concluded that bile formation in the rat is not likely to be affected by hormones of the digestive tract, and that it is normally stimulated to a maximal or near-maximal extent by recirculated bile salts.

I. INTRODUCTION

The most important factors that may affect the secretion of hepatic bile in the dog have been shown to be associated with feeding (Fritz and Brooks 1963; Nahrwold and Grossman 1967; Jones and Grossman 1969*a*), and the presence in the blood of bile salts (Wheeler and Ramos 1960) and of the gastrointesinal hormones, secretin, cholecystokinin, and gastrin (Jones, Geist, and Hall 1971; Jones and Grossman 1969*b*, 1970). However, little information is available on the regulation of bile secretion in the rat, although it is known that the rat has an extremely high rate of bile production even when the enterohepatic circulation of bile salts is interrupted (Light, Witmar, and Vars 1959). It has been suggested that the mechanisms of bile formation and regulation in the rat differ from those in the dog (Klaasen 1971).

Various factors which could affect the secretion of bile in conscious rats were studied in the experiments described in this paper. Rats with bile and pancreatic fistulae were deprived of bile, and received infusions of either bile salts or hydrochloric acid into the duodenum, and of bile salts, secretin, cholecystokinin, or pentagastrin into the blood; the effects of these infusions were compared with control values and with those obtained after feeding. An effort was also made to assess the significance of these factors in relation to their effect on other digestive secretions, particularly pancreatic juice.

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II. Materials and Methods

Male albino rats, which had previously had free access to a diet of Rat and Mouse Kubes (Allied Feeds, Concord, N.S.W.) and which weighed 300–400 g, were fasted for 48 hr, then anaesthetized with intraperitoneal pentobarbital sodium (Sagatal, 60 mg/ml, May and Baker, West Footscray, Vic.) at the rate of 4 mg per 100 g rat. The abdomen was opened with a midline incision from the xiphoid process to the umbilicus. The common bile duct was cannulated with a piece of polyethylene tubing (1·00 mm o.d.) 15 cm in length, at a point about 5 mm below the hilus of the liver, and just above the point where pancreatic tissue no longer surrounds the duct. To collect pancreatic juice, a similar catheter was inserted into the common bile duct at the point where the duct enters the duodenal wall.

In some rats a polyethylene cannula was placed in the left femoral vein, and in others a polyethylene cannula was fixed with a purse-string suture into the first portion of the duodenum. In rats that were to receive pentagastrin, a polyvinyl chloride tube (3 mm o.d., Dural Plastics and Engineering, Pty. Ltd., Dural, N.S.W.) was fixed into the most dependent portion of the stomach and held in place with a purse-string suture. All cannulae were brought out through the lower end of the abdominal incision and this was then closed in two layers. The rats were kept in Bollman restraining cages and allowed access to 0·9% sodium chloride.

A Palmer infusion pump was used for intravenous infusions of pure, natural, porcine secretin, and cholecystokinin (Gastrointestinal Hormone Research Unit, Stockholm, Sweden), pentagastrin (Imperial Chemical Industries, Cheshire, England), and taurocholic acid (Sigma Chemical Co., St. Louis, Missouri), and intraduodenal infusions of taurocholic acid and of hydrochloric acid. During experiments on the effects of feeding, the rats were allowed access to a mixture of oats and proprietary cat food (Liver Dinner, K9 Pet Foods, Carnation Co. Pty. Ltd., Blacktown, N.S.W.). In all experiments, samples of bile and pancreatic juice were collected under paraffin oil in plastic vials and the volumes estimated from the net weight.

The concentration of bicarbonate in bile and pancreatic juice was measured by adding 0·2 ml of 0·1N HCl to 0·2-ml samples and swirling the mixture for 30 sec. This mixture was back-titrated after 5 min to pH 8·0 with 0·01N NaOH using an automatic titrator and pH-meter (Radiometer, Copenhagen). Total acidity in rat gastric juice was determined by titrating 0·2 ml of gastric juice with 0·01N NaOH to pH 8·3 with the automatic titrator and pH-meter. Total cholates in bile were estimated by the method of Irvin, Johnston, and Kopala (1944). Protein concentration of pancreatic juice was determined by the method of Lowry *et al.* (1951).

Student's *t*-tests, coupled with analyses of variance, were used to estimate whether significant differences existed between means obtained for the different parameters. The standard error of the mean (S.E.M.) for a single time interval or a single infusion rate, recorded at the right of Figures 1 and 3–6, was estimated from the error mean square (E.M.S.) and sample number (N): S.E.M. = $(\text{E.M.S.}/N)^{\frac{1}{2}}$.

III. Results

To measure bile flow rate with the enterohepatic circulation of bile salts intact, in four rats bile was rediverted into the duodenum until the rats had fully recovered from anaesthesia. Bile was then collected for three 10-min periods, and was found to flow at $17·9 \pm 0·8$ μl/min or $54 \pm 2·3$ μl/kg/min.

When bile was diverted to the exterior and the enterohepatic circulation of bile salts was interrupted, the flow of bile decreased to an average of $6·1 \pm 0·2$ μl/min ($19 \pm 0·6$ μl/kg/min). Diversion of pancreatic juice was associated with an increase in flow of pancreatic juice similar to that observed by Grossman (1958). The rate of flow of each secretion had reached a plateau after 18 hr and showed only minor fluctuations after this time. For this reason, all experiments were done on rats that had been deprived of bile and pancreatic juice for at least 18 hr. Each rat was also starved for a similar period before experiments.

(a) *Effect of Infusion of Various Substances on the Flow and Composition of Bile and Pancreatic Juice*

(i) *Sodium Taurocholate*

Infusions that provided 0·07, 0·15, 0·3, 0·45, 1·0, and 1·95 μ-equiv/min of sodium taurocholate were given intravenously in that order to each of five rats. Each infusion was given for 60 min and bile was collected during the last 20 min of this period; then the infusion rate was increased to the next value in the sequence. The flow of bile and its content of bile salts were compared with values obtained from control rats which were treated identically except that saline infusions were given.

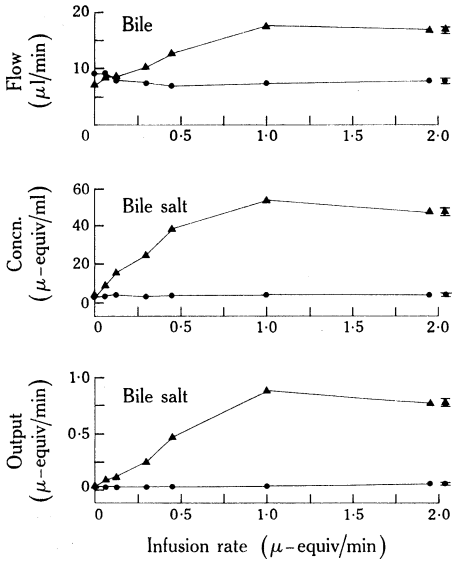

Fig. 1.—Effects of the rate of intravenous administration of sodium taurocholate (▲) on the bile flow and bile salt concentration and output. Control rats (●) were infused with saline. In all figures except Figure 2, bars at right represent ± standard error of the mean for a single infusion rate.

The flow of bile and the concentration and output of bile salts increased with each increase in the infusion rate of taurocholate until this reached 1 μ-equiv/min, but did not increase further when the infusion rate was increased to 1·95 μ-equiv/min

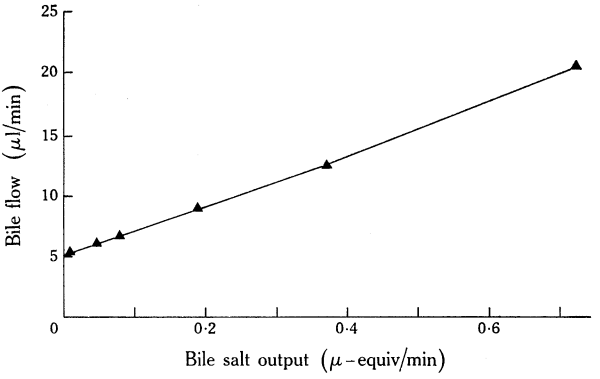

Fig. 2.—Relationship between bile salt output and bile flow in a single rat in which bile was diverted from the intestine, but sodium taurocholate was infused intravenously at various rates.

(Fig. 1). The maximum bile flow, which was an average of 17·6±1·6 μl/min (54±4·8 μl/kg/min), was more than twice the flow from control rats (Fig. 1). There was a linear relationship between the bile flow and bile salt output (Fig. 2), and, by

slight extrapolation, it could be estimated that the bile flow that was independent of bile salt secretion was about 5 μl/min (15 μl/kg/min).

(ii) *Hydrochloric Acid*

Hydrochloric acid in concentrations of 0·01, 0·025, 0·05, and 0·1N was infused into the duodenum of seven rats at 0·06 ml/min. Each infusion was given for 40 min, and bile and pancreatic juice was collected for the last 20 min, then the concentration of the infusate was increased to the next level. Rats in a control series were treated in an identical manner except that the acid was replaced by saline.

The infusions of acid were not associated with any significant stimulation of bile or pancreatic juice formation, as shown in the following tabulation:

	Before infusion	During infusion of 0·1N HCl
Bile flow (μl/min)	8·6±0·8	8·0±0·5
Bile bicarbonate (μ-equiv/min)	0·19±0·02	0·14±0·01
Pancreatic juice flow (μl/min)	6·3±1·0	7·3±0·7
Pancreatic juice bicarbonate (μ-equiv/min)	0·38±0·04	0·47±0·05

(iii) *Secretin*

Pure, natural secretin was infused intravenously at 2·3, 4·5, 9·0, 18, 45, 90, and 450 m-units/min into three rats for periods of 40 min. Bile and pancreatic juice were collected during the last 20 min of each period, then the infusion was increased to the next rate in the sequence. Control rats were infused with saline for the same periods of time.

No change in bile flow or bile constituents was observed with secretin but there occurred significant increases in the flow of pancreatic juice and in its concentration and output of bicarbonate (Fig. 3). The highest rate of infusion, equivalent to 1·4 units/kg/min, was associated with an increase in bicarbonate output in pancreatic juice of more than five times the pre-infusion rate, and the flow of pancreatic juice doubled (Fig. 3).

(iv) *Cholecystokinin*

Cholecystokinin was infused intravenously at 30, 150, and 600 m-units/min into four rats for periods of 40 min. Bile and pancreatic juice was collected during the last 20 min of this period, then infusions were raised to the next value. Again, control rats were infused with saline for the same periods. No changes in bile or pancreatic juice or bicarbonate were observed but the concentration and output of protein in pancreatic juice increased during infusions at 150 and 600 m-units/min ($P < 0·001$) (Fig. 4).

(v) *Pentagastrin*

Pentagastrin was infused at 0·15, 0·54, 1·5, and 3·0 μg/min for periods of 60 min, and samples of bile and pancreatic juice were collected for the last 20 min. Gastric juice was collected for the 60-min period but, as the pylorus was not ligated, it was not possible to estimate the volume of juice produced.

Pentagastrin stimulated secretion of acid from the stomach but, when it was given at rates of 0·54 μg/min (1·6 μg/kg/min) or higher, no additional increment in

Fig. 3

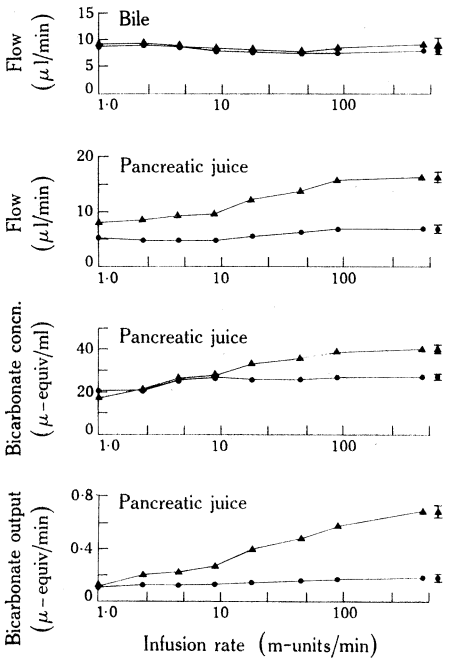

Fig. 4

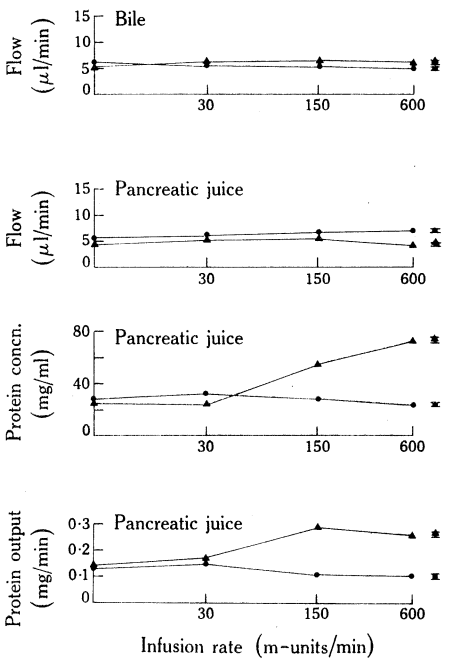

Fig. 5

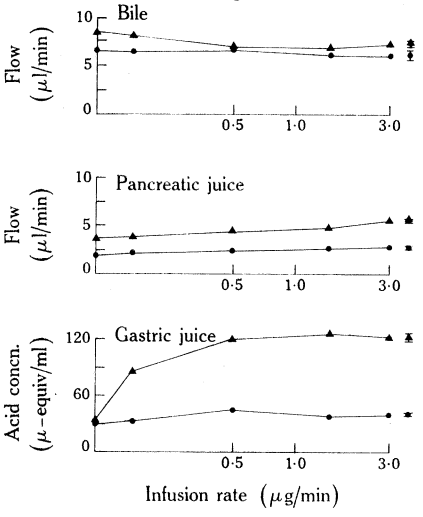

Fig. 3.—Effects of secretin (▲) on the flow of bile and pancreatic juice and the bicarbonate concentration and output of pancreatic juice. Control rats (●) were infused with saline. In this figure and also Figures 4 and 5 points on the vertical axis represent values before commencement of infusions.

Fig. 4.—Effects of cholecystokinin (▲) on the flow of bile and pancreatic juice and the protein concentration and output of pancreatic juice. Control rats (●) were infused with saline.

Fig. 5.—Effects of pentagastrin (▲) on the flow of bile and pancreatic juice and the acid concentration of the gastric juice. Control rats (●) were infused with saline.

the concentration of hydrogen ions occurred (Fig. 5). No change in bile or pancreatic flows or their respective bicarbonate contents could be demonstrated.

(*b*) *Effect of Food in the Absence of an Enterohepatic Circulation of Bile Salts*

Bile and pancreatic juice were collected for five 40-min control periods from four rats that had been deprived of bile salts and pancreatic juice for more than 18 hr. They were given access to oats and dried cat food and ate continuously and rapidly for 40 min, then the food was withdrawn. Samples of bile collected during feeding and during the next 160 min were similar to the control samples [Fig. 6(*a*)], but significant increases occurred in the flow of pancreatic juice, the output of protein, and the concentration and output of bicarbonate in the pancreatic juice ($P < 0{\cdot}001$) [Fig. 6(*a*)].

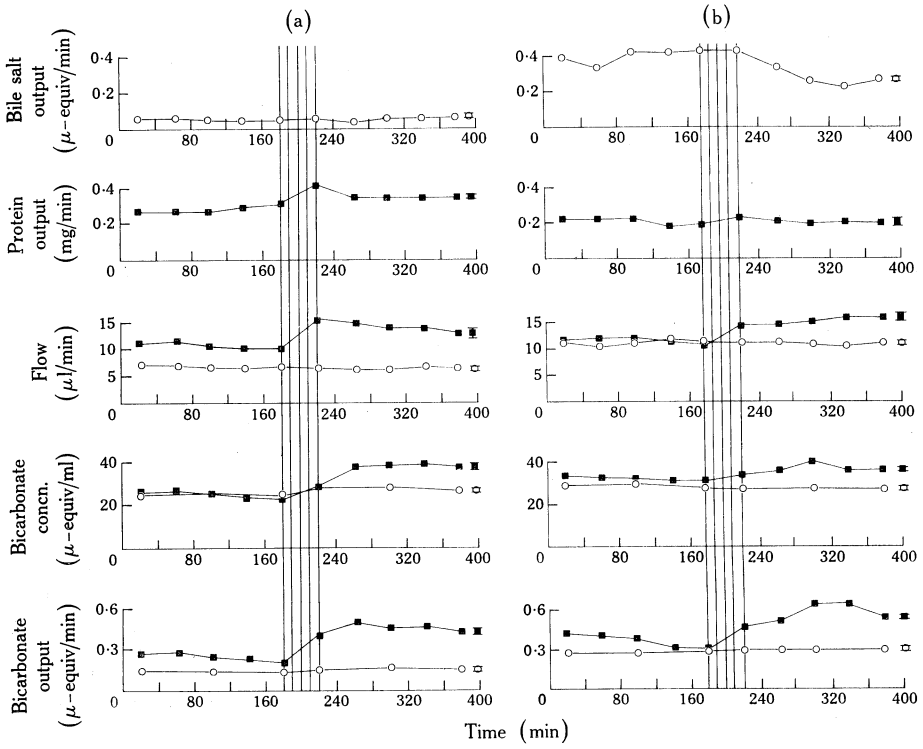

Fig. 6.—Effects of feeding on bile (○) and pancreatic juice (■) in rats without (*a*) and with (*b*) an intact enterohepatic circulation of bile salts. Food was offered for the 40-min period indicated by vertical lines.

(*c*) *Effect of Food in the Presence of an Enterohepatic Circulation of Bile Salts*

The effects of feeding were also studied in three rats which received an intraduodenal infusion of sodium taurocholate at 0·45 μ-equiv/min for the duration of the experiment [Fig. 6(*b*)]. No changes occurred in the flow of bile or its content of bicarbonate, but significant increases occurred in the flow of pancreatic juice and in its concentration and output of bicarbonate ($P < 0{\cdot}001$). No increase in the output of protein in pancreatic juice could be demonstrated in this group of rats [Fig. 6(*b*)].

IV. Discussion

The regulation of bile formation in the rat differs in a number of respects from that in other animals. The portion of bile flow that appears to be independent of bile salt excretion in the rat is about 15 μl/kg/min; this is high when compared with the value of about 5 μl/kg/min reported for the dog (Wheeler, Ross, and Bradley 1968). However, infusions of bile salts will stimulate bile formation in the rat, and the maximum rate of bile flow recorded during infusion of taurocholate is similar to that recorded in conscious rats with an intact enterohepatic circulation of bile salts. The maximum increment in bile flow that resulted from the infusions of taurocholate to conscious rats (about 30 μl/kg/min) is of the same order as that (about 20–30 μl/kg/min) estimated from published data obtained from conscious sheep and dogs during infusions of large amounts of bile salts (Wheeler, Ross, and Bradley 1968; Heath, Caple, and Redding 1970; Caple and Heath 1972). In the dog and sheep, however, a number of factors in addition to bile salts will stimulate bile formation (Fritz and Brooks 1963; Caple and Heath 1972); in the dog, these include feeding, duodenal administration of acid, and intravenous administration of secretin, cholecystokinin, or gastrin (Fritz and Brooks 1963; Zaterka and Grossman 1966; Jones and Grossman 1970). These agents have no demonstrable effect on bile formation in the rat.

Infusions of secretin and cholecystokinin stimulate secretion into pancreatic juice in the rat, but the doses required are many times higher than those necessary to produce similar responses in the dog (see Lin and Alphin 1962). Similarly, infusions of pentagastrin stimulate secretion of gastric acid, but the rat stomach seems very much less sensitive to pentagastrin than the human or dog stomach (see Konturek *et al.* 1969; Chey, Lee, and Lorber 1970). It is possible that the small response of the rat stomach and pancreas, and the absence of a biliary response, may result from important structural differences between the infused preparations and the natural hormones of the rat. However, the entry of acid into the rat duodenum had no apparent effect on secretion of either bile or pancreatic juice (see also Grossman 1958), despite the reported presence of secretin in the intestinal mucosa of the rat (Dorchester and Haist 1952).

When rats were fed, the flow of pancreatic juice did increase by about 50%, but this is very much less than the fivefold increase that occurs in the dog after feeding (Cooke, Nahrwold, and Grossman 1967). However, in the rat, the initial flow is relatively much higher than that reported for the dog (Cooke, Nahrwold, and Grossman 1967), and the maximum flow after feeding (about 50 μl/kg/min) is similar in the two species.

In dogs that had been starved for 48 hr, a choleresis occurs after the ingestion of proprietary food similar to that ingested by the rats used in these experiments. This choleresis is similar to that produced by infusions of secretin, but the response is greater if bile is present in the intestine than if it is absent (Nahrwold and Grossman 1967). After feeding, the flow of bile and its content of bicarbonate remained constant in the rat, even when taurocholate, which was transported into bile with considerable efficiency, was infused into the intestine.

The rat does not possess a gall bladder, and it seems likely that bile enters the duodenum more or less continuously under normal conditions. The reabsorbed bile

salts thus provide a relatively constant stimulus to bile formation, and it seems unlikely that feeding would exert any important influence on this enterohepatic circulation of bile salts. Thus the absence of a choleretic response to feeding in the rat provides support for the view that hormonal factors are of negligible importance in the regulation of bile formation in rats.

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