REGULATION OF OUTPUT OF ELECTROLYTES IN BILE AND PANCREATIC JUICE IN SHEEP

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Abstract

Bile and pancreatic juice were collected from conscious, standing sheep with fistulae of the common bile duct, before and during infusions of secretin to the portal vein, and during infusion of acid to the duodenum. The output of volume and electrolytes, particularly bicarbonate, in bile and in pancreatic juice increased during infusion of secretin. However, the output of volume and of bicarbonate was three to five times higher in bile than in pancreatic juice. When acid was infused into the duodenum a similar result was obtained, and the increment in total bicarbonate output was similar to the amount of acid infused.

Infusions of sodium taurocholate were associated with further increases in the output of volume, bile salts, chloride, sodium, potassium, and bicarbonate in bile, but there were no changes in the volume or composition of pancreatic juice.

It is suggested that in normal sheep the entry of acid into the duodenum results in release of secretin, and that this stimulates the output of water, bicarbonate, and other electrolytes, particularly from the liver.

I. Introduction

Important amounts of bile and pancreatic juice enter the intestine during digestion, and in animals with simple stomachs the contribution of water and electrolytes, particularly bicarbonate, from the pancreas is much greater than that from the liver (Harper 1967; Wheeler 1968). In these animals, the rate of secretion of water and of bicarbonate and other ions from the pancreas and to a lesser extent from the liver may be increased by the hormone secretin, which is released from the intestinal wall mainly in response to acid chyme in the intestine (Wheeler 1968).

No detailed information has been published on the regulation of the output of electrolytes in bile and pancreatic juice in sheep. Although secretin may stimulate secretion of bile (Heath 1970) and pancreatic juice (Magee 1961), the effects of pure natural secretin on the entry of water and electrolytes into the sheep gut have not been recorded. In the experiments reported here, pure natural secretin was infused into the portal vein or the jugular vein of conscious, standing sheep, and measurements were made of the flow and electrolyte content of bile and of pancreatic juice. In addition, other experiments were designed to assess the role of acid in the duodenum, and of the enterohepatic circulation of bile salts, in regulating the output of water and electrolytes from the liver and pancreas in sheep.

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II. Materials and Methods

(a) *Animals*

Merino wethers that weighed 28–34 kg were fed on 400 g/day of lucerne chaff; cubes (Allied Feeds, Rhodes, N.S.W.) were always available until the time of the operation.

Each sheep was operated on under aseptic conditions, then allowed to recover before being used for experiments. Anaesthesia was induced with pentobarbitone sodium and maintained with halothane and oxygen in a closed circuit. An incision was made parallel to and just behind the last rib on the right side, then the gall bladder was removed. Two polyvinylchloride cannulae (Dural Plastics and Engineering Pty. Ltd., Dural, N.S.W.) were placed in the common bile duct so that bile and pancreatic juice could be collected separately (Fig. 1, and Heath and Morris 1963).

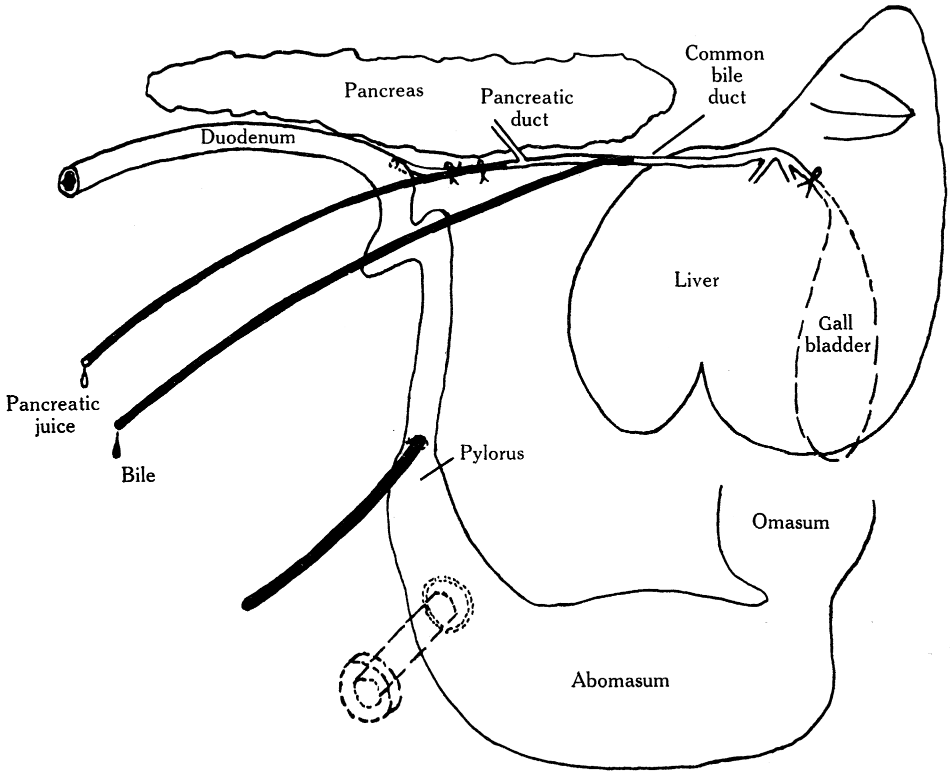

Fig. 1.—Schematic diagram showing locations of cannulae used in experiments.

Polyvinylchloride cannulae for the infusion of bile salts or of secretin were introduced into the portal vein through a mesenteric tributary, and cannulae for the infusion of bile and pancreatic juice, or of acid, were placed in the duodenum. In some sheep an abomasal fistula was established just proximal to the pylorus, and this was used to divert abomasal contents to the exterior.

Bile and pancreatic juice were returned to the duodenum between experiments on three sheep that received infusions of acid into the duodenum. The other sheep were deprived of bile and pancreatic juice but electrolyte depletion was prevented by the infusion of 750 ml/day of a solution of sodium chloride (120 mM), sodium bicarbonate (18 mM), and potassium chloride (7 mM) into the duodenum.

(b) *Experiments*

In the experiments which are described in detail in the Section III, infusions of secretin (Pure Natural Secretin, Karolinska Institutet, Stockholm) and of sodium taurocholate (Sigma Chemical Company, St. Louis, Missouri), were given into the portal vein with a Palmer pump, and a Harvard pump was used to infuse diluted A.R. hydrochloric acid into the duodenum.

Bile and pancreatic juice were collected under paraffin oil in graduated centrifuge tubes, and the bicarbonate concentration was estimated immediately. This was done by a method similar to that described by Segal (1955) for plasma: 0·5 ml bile or pancreatic juice was added to 0·5 ml 0·1N hydrochloric acid, then the mixture was boiled briefly and cooled. Titration to the original pH of the bile or pancreatic juice was performed with previously-standardized sodium hydroxide of about 0·01N. Sodium and potassium concentrations were estimated with a Baird–Atomic flame-photometer, and chloride by potentiometric titration with silver nitrate using a silver electrode (Radiometer A/S, Copenhagen). The method of Irvin, Johnston, and Kopola (1944) was used to estimate the concentration of bile salts in bile.

Analyses of variance and Student's t-tests were used to evaluate the statistical significance of changes that occurred in the various parameters during the experiments (see Heath 1970).

III. Results

(a) *Basal Secretion of Bile and Pancreatic Juice*

Bile and pancreatic juice were collected for five periods of 10 min from each of four sheep that had been deprived of food and of bile and pancreatic juice for 48 hr. No significant differences existed between bile and pancreatic juice in the concentration of individual electrolytes, but the outputs of volume, sodium, potassium, chloride, and bicarbonate in bile were each three to five times higher than the corresponding outputs in pancreatic juice (Table 1).

Table 1

BASAL SECRETION OF BILE AND PANCREATIC JUICE

Bile and pancreatic juice were collected from four sheep that had been deprived of bile and pancreatic juice and of food for 48 hr, but received daily infusions of an electrolyte solution. Bile flow was $0\cdot27 \pm 0\cdot66$ ml/min; pancreatic juice flow was $0\cdot06 \pm 0\cdot01$ ml/min

	Concn. (μmoles/ml)		Output (μmoles/min)	
	Bile	Pancreatic juice	Bile	Pancreatic juice
Bile salt	$10\cdot9 \pm 1\cdot7$		$3\cdot1 \pm 1\cdot1$	
Bicarbonate	$23 \pm 1\cdot7$	$28 \pm 6\cdot0$	$6\cdot5 \pm 1\cdot9$	$1\cdot8 \pm 0\cdot7$
Chloride	$118 \pm 9\cdot1$	$123 \pm 6\cdot4$	$33 \pm 7\cdot1$	$7\cdot2 \pm 1\cdot6$
Sodium	$150 \pm 2\cdot4$	$147 \pm 4\cdot3$	$40 \pm 9\cdot7$	$8\cdot8 \pm 2\cdot0$
Potassium	$4\cdot4 \pm 0\cdot9$	$4\cdot6 \pm 0\cdot4$	$1\cdot1 \pm 0\cdot2$	$0\cdot3 \pm 0\cdot1$

(b) *Effects of Portal Infusions of Secretin and of Sodium Taurocholate on Bile and Pancreatic Juice*

The four sheep referred to under (*a*) were each deprived of bile and pancreatic juice, but received infusions of sodium taurocholate during some experiments. Each sheep received a daily duodenal infusion of electrolytes as described in Section II(*a*).

(i) *Effect of Secretin Alone*

Bile and pancreatic juice were collected for two 10-min periods, then an infusion of secretin that provided either 0·44, 0·88, 1·76, or 3·52 units/min was given into the portal vein of each sheep. The infusions, which were arranged in random order, were each given for 20 min, and bile and pancreatic juice were collected for the last two 5-min periods. After one infusion was finished, another was not commenced until the flow of bile and of pancreatic juice had returned to the rates observed in the control period.

The infusions of secretin were each associated with increases in the flow, and in the concentration and output of bicarbonate in both secretions (Fig. 2). The increases

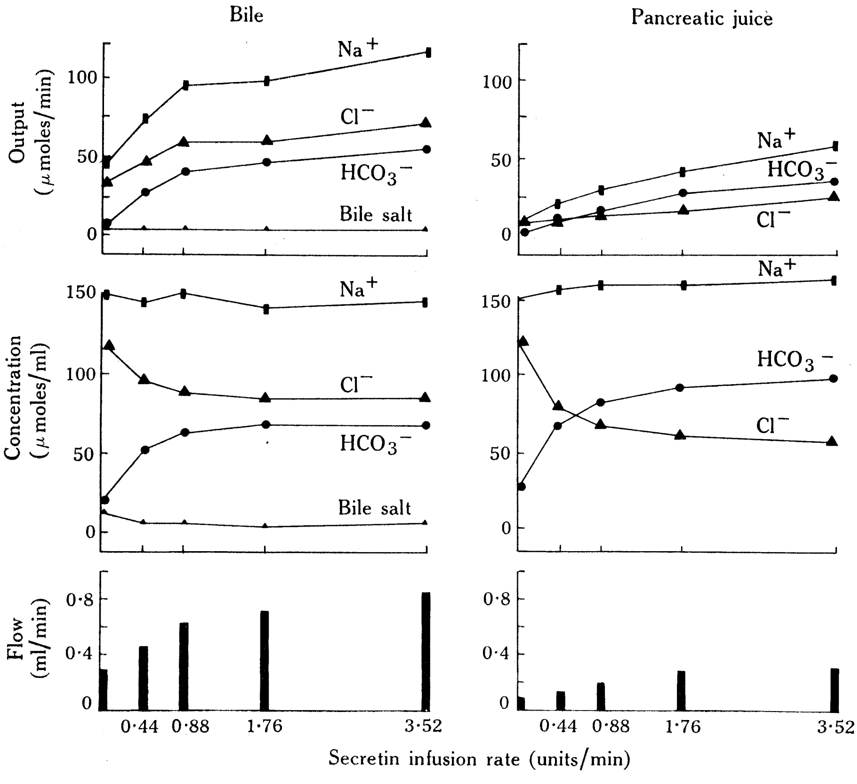

Fig. 2.—Effect of the rate of administration of secretin into the portal vein on the flow and composition of bile and pancreatic juice collected simultaneously in each of four sheep that had been deprived of bile salts.

in flow and in bicarbonate output were related significantly to the logarithm of the rate of secretin infusion ($P < 0 \cdot 005$; Fig. 3), and each twofold increase in secretin infusion was associated with the following average increments:

	Bile	Pancreatic juice
Flow (ml/min)	0·12	0·06
Bicarbonate output (μmoles/min)	9·7	7·8

The concentration of chloride decreased progressively as the rate of secretin infusion increased, but the decreases in chloride concentration were less than the simultaneous increases in flow, and the output of chloride in both secretions increased ($P<0 \cdot 01$ for comparison between control output and that during infusion of $3 \cdot 24$ units/min of secretin). The concentration of bile salts in the bile decreased during each infusion of secretin, but the output of bile salts did not change. No changes occurred in the concentration of sodium or of potassium, and the output of these cations increased progressively with each increase in secretin infusion (Fig. 2).

Although qualitatively similar changes occurred in bile and in pancreatic juice during the infusions of secretin, the output of each of the electrolytes in the bile was always higher than that in pancreatic juice (Fig. 2).

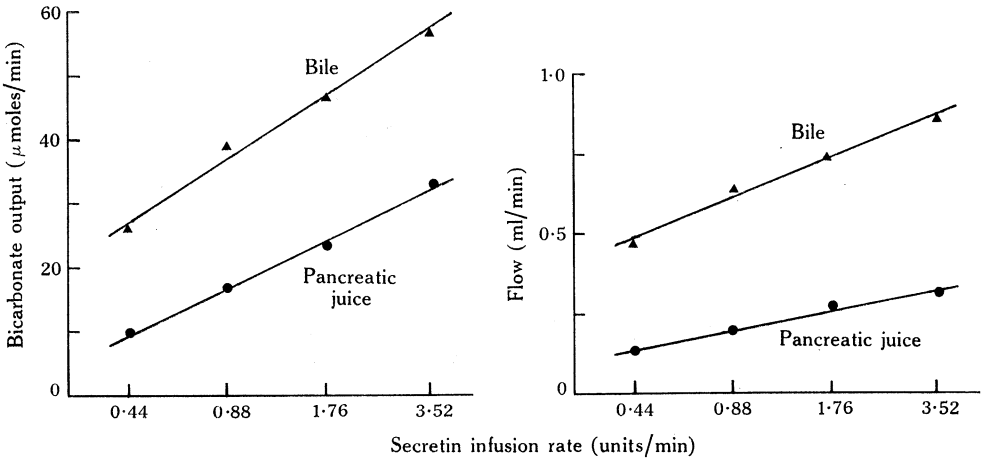

Fig. 3.—Relationship between the rate of infusion of secretin and the flow and bicarbonate output of bile and pancreatic juice collected simultaneously in each of four sheep that were deprived of bile salts.

(ii) *Effect of Sodium Taurocholate*

Control samples of bile and pancreatic juice were collected for two periods, each of 10 min, then sodium taurocholate solutions were infused into the portal vein at $0 \cdot 32$ ml/min to provide either 0, 22, 37, 74, or 112 μmoles/min for an equilibration period of 60 min. The infusions, which were given in random order, were continued during the collection of two consecutive 10-min samples, and during the experiments described under the next subheading.

The flow of bile increased during each of the infusions of taurocholate, and reached a mean of $1 \cdot 28 \pm 0 \cdot 12$ ml/min when the infusion rate was 112 μmoles/min. This was about four times the flow recorded when no taurocholate was given (Fig. 4). The infusions of taurocholate did not cause any significant changes in the concentration of bicarbonate in bile, but the concentration of chloride did decrease ($P<0 \cdot 01$) to a value (85–95 μmoles/min) that was similar for each rate of infusion. The concentration of bile salts increased progressively with each increase in the rate of taurocholate infusion, and reached a value that was about six times that recorded in the absence of taurocholate infusion (Fig. 4). The mean concentration of sodium, which was 150 μmoles/ml when no taurocholate was given, increased to 167 μmoles/ml when the infusion rate was 112 μmoles/min ($P<0\cdot05$), but no significant changes occurred in the concentration of potassium in the bile.

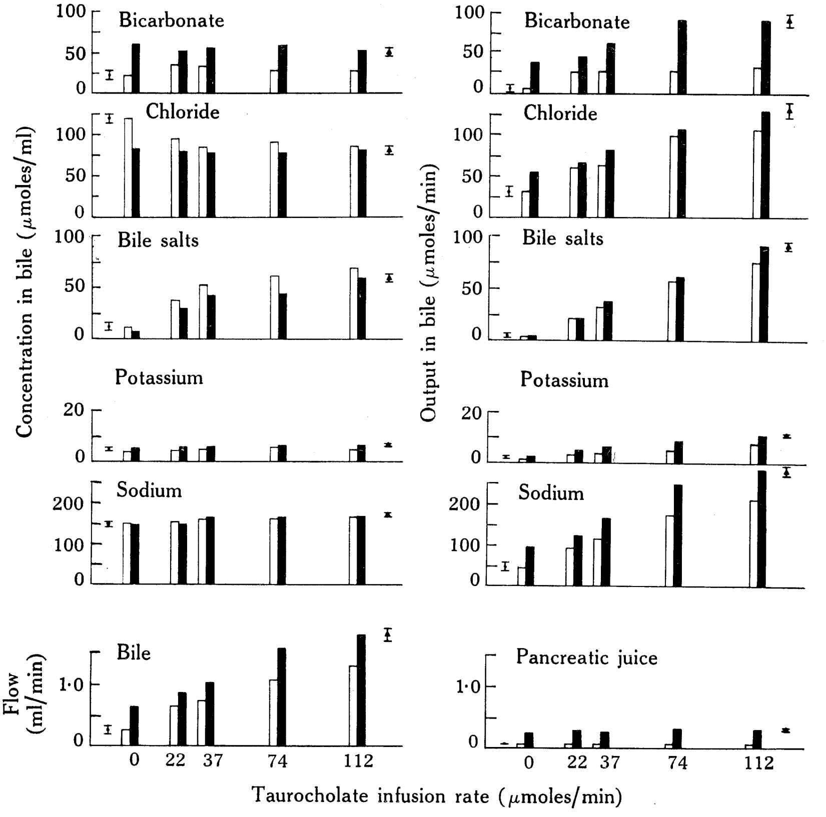

Fig. 4.—Effect of secretin on the response of the liver and pancreas to sodium taurocholate. Each block of histogram represents the mean value for four sheep that received infusions into the portal vein of taurocholate with (closed blocks) and without (open blocks) $0\cdot88$ units/min of secretin. The taurocholate infusions were given for an equilibration period of 1 hr, and those of secretin for 20 min before bile and pancreatic juice samples were collected. Symbols at right and left of each histogram represent $\pm$ S.E. for a single infusion rate of taurocholate, and of the combined infusion of taurocholate and secretin, respectively.

The output of sodium, potassium, bile salt, and chloride increased during the infusions of taurocholate, and the extent of the increase appeared to be directly related to the rate of infusion. When 112 μmoles of taurocholate was given each minute, the output of sodium, potassium, and chloride was 3–5 times, and that of bile salts 25 times, that recorded in the absence of infused taurocholate. The output of bicarbonate increased during infusion of taurocholate at 22 μmoles/min ($P<0\cdot05$) but there were no further significant increases at the higher infusion rates (Fig. 4).

The taurocholate had no demonstrable effect on the secretion of pancreatic juice; neither the flow of pancreatic juice (Fig. 4) nor its concentration or output of electrolytes altered during any of the infusions.

(iii) *Effect of Secretin During the Simultaneous Infusion of Taurocholate*

The portal infusions of taurocholate used in the experiments described above were continued, but in addition an infusion of secretin (4 units/ml) at 0·88 units/min was commenced through a second portal cannula immediately after the collection of the second sample. After 10 min of the dual infusion, bile and pancreatic juice were each collected for the next two 5-min periods, then the infusions were stopped. The next experiment was not commenced until the flow and composition of bile and pancreatic juice had returned to control levels.

The infusions of secretin did not cause any significant change in the concentration of sodium or potassium in bile, but in each experiment secretin was associated with a decrease in the concentration of bile salts and chloride, and an increase in the concentration of bicarbonate in the bile (Fig. 4). The flow of bile, and the output of sodium, potassium, chloride, and bicarbonate increased during the infusion of secretin ($P < 0 \cdot 05–0 \cdot 001$), but when the results were examined by analysis of variance (see Heath 1970), it was not possible to demonstrate any significant interactions between the responses to secretin and those to taurocholate.

Table 2

EFFECTS OF ALTERING THE ROUTE ADMINISTRATION ON THE RESPONSES OF INFUSIONS OF SECRETIN

Three sheep each received a continuous infusion of sodium taurocholate to the portal vein at 22 μmoles/min, and infusions of secretin at 0·88 units/min were given alternately into the portal vein and the jugular vein

	Flow (ml/min)		Bicarbonate output (μmoles/min)	
	Bile	Pancreatic juice	Bile	Pancreatic juice
No secretin	0·49±0·01	0·05±0·01	10±0·9	1·7±0·3
Secretin to portal vein	1·01±0·05	0·21±0·05	57±10·4	20 ±7·9
Secretin to jugular vein	1·03±0·04	0·18±0·02	50±3·9	14 ±2·9

The flow and electrolyte content of pancreatic juice increased during infusions of secretin, but this response was not altered by the simultaneous infusion of taurocholate.

(iv) *Comparison of Pancreatic and Biliary Response to Portal and Jugular Administration of Secretin*

To determine the effect of route of administration of secretin on the flow and bicarbonate output of bile and of pancreatic juice, secretin was infused alternately into the jugular vein and the portal vein of two sheep. A simultaneous infusion of 22 μmoles/min of sodium taurocholate was given to ensure a stable basal rate of bile flow.

The flow and bicarbonate output of each secretion did not appear to be affected by changing the route of administration, and in each case the flow and bicarbonate output of bile was greater than that of pancreatic juice (Table 2).

(c) *Effect of Introduction of Acid into the Duodenum*

In three sheep, abomasal contents were diverted to the exterior through an abomasal fistula, and sodium taurocholate was infused into the portal vein at 22 μmoles/min for the duration of the experiment. Control samples of bile and pancreatic juice were collected for six consecutive 10-min periods during the second hour of the taurocholate infusion, then either 0, 0·5, 1·0, or 2·0 mmoles of hydrochloric acid in 40 ml saline was infused into the duodenum during a 15-min period. Bile and pancreatic juice were each collected for consecutive 10-min periods until the flow returned to the control rate.

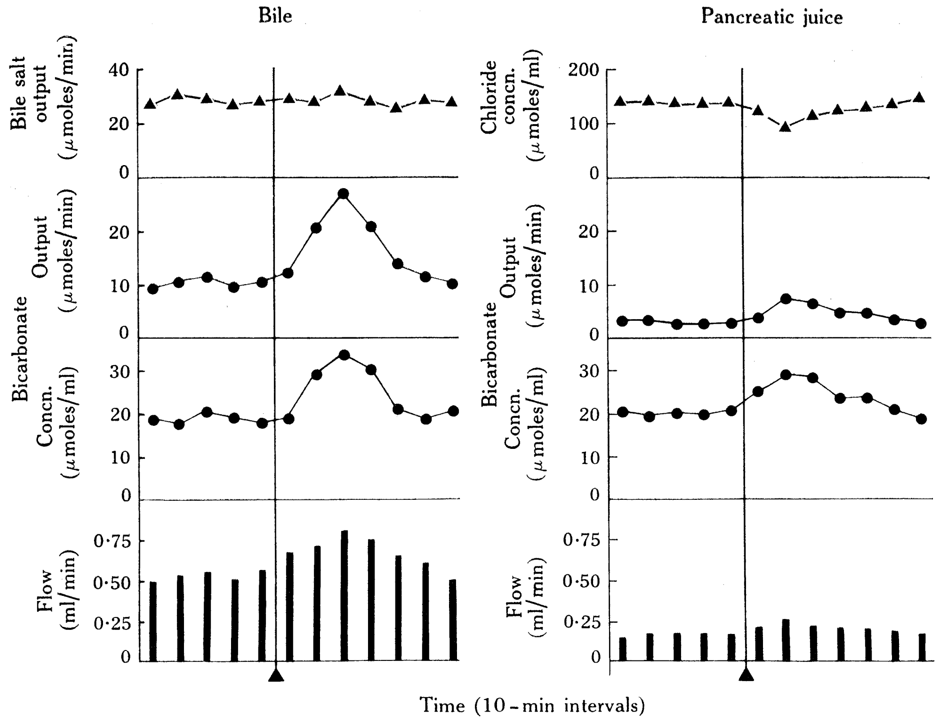

Fig. 5.—Effect of infusion of 1 mmole of hydrochloric acid in 40 ml of saline to the duodenum of a 35-kg sheep that was receiving an infusion of 22 μmoles/min of taurocholate into the portal vein. The flow and composition of bile and pancreatic juice were measured during 10-min periods before and after acid was given.

Increases occurred in the flow, bicarbonate concentration, and bicarbonate and chloride output of bile and pancreatic juice, and these became evident within 10 min after the beginning of the acid infusion. Although the concentration of bile salts in the bile decreased, the output of bile salts remained constant (Fig. 5). When the increment in bicarbonate output in response to the acid was estimated from the output of bicarbonate before and after the infusion, it was found to be equal to or a little less than the amount of acid infused (Fig. 6). However, the contribution from the bile was greater than that from the pancreatic juice—a situation that also existed in the control samples (Figs. 5 and 6).

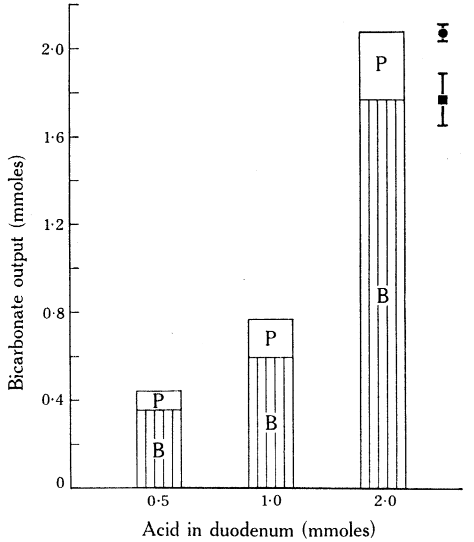

Fig. 6.—Relationship between the amount of hydrochloric acid infused to the duodenum, and the resultant increment in output of bicarbonate in bile (B) and pancreatic juice (P). Each block represents the average of two values from each of three sheep. Symbols at right represent ± S.E. for a single infusion rate for bile and pancreatic juice.

IV. Discussion

Secretin stimulates secretion of water and electrolytes, particularly bicarbonate, in bile and pancreatic juice of sheep as of most other animals (Magee 1961; Harper 1967; Wheeler 1968; Heath 1970). However, in dogs the effect of secretin on bile flow is only 10–20% of the effect on the flow of pancreatic juice, and the concentration and output of bicarbonate is much higher in pancreatic juice (Pak *et al.* 1966; Wheeler 1968). Our observations indicate that the position is reversed in the sheep; infusions of secretin into the portal vein stimulated the volume and bicarbonate output of bile to a greater extent than that of pancreatic juice.

The route by which secretin enters the blood stream is not known, but experiments on dogs have shown that infusions of secretin to the jugular vein stimulate pancreatic secretion to a greater extent than portal infusions (Harper 1967), although this effect is more marked at low infusion rates (Way, Johnson, and Grossman 1969). Chey, Lee, and Lorber (1970), who infused synthetic secretin into the portal vein and a peripheral vein, concluded that the liver is an important site of inactivation of secretin in the dog. In our experiments on sheep, little difference was apparent between responses to portal and to jugular infusions. It is possible that, although the role of the liver in the metabolism of secretin is not known, it may vary between species; this may be responsible for some of the differences observed during the infusion of secretin to animals of different species.

The effects of secretin were found to be quantitatively different from those recorded in the dog. If our results with sheep are compared with those in which pure, natural secretin was infused into dogs (Jones and Grossman 1969; Fawcett 1970), it can be shown that comparable doses of secretin stimulate a much greater output of volume and bicarbonate in bile in the sheep than in the dog. However, the response of the pancreas to secretin is less in the sheep than in the dog. When allowances are made for the differences in body weight, it can be estimated that comparable doses of secretin increase the flow of pancreatic juice in the dog five to ten times more than in the sheep, and that the concentration and output of bicarbonate from the dog pancreas is about six times greater than that from the sheep pancreas (see Fawcett 1970). In rats, on the other hand, the response of the pancreas to secretin is much less than in sheep, and the liver is quite unresponsive to infusions of secretin (Shaw and Heath 1972).

In sheep, which are ruminant herbivores infusion of acid or gastric juice into the duodenum increases the flow of bile. This increase in bile flow is associated with a decrease in the concentrations of chloride and total solids in bile (see Harrison 1962), and is similar to that seen after infusions of secretin. The volume flow and the concentration and output of bicarbonate in pancreatic juice also increase after infusion of acid into the duodenum, but this response is small compared with that in dogs and in pigs (Magee 1965). The infusion of acid into the duodenum of animals such as dogs, pigs, and human beings is a potent stimulus to the secretion of water and bicarbonate in pancreatic juice and bile, and this effect is mediated in part by release of secretin from the duodenal mucosa (Preshaw, Cook, and Grossman 1966; Wormsley 1970). Acid in the duodenum may stimulate release of cholecystokinin in addition to secretin in some animals (Hong, Nakamura, and Magee 1970; Wang and Grossman 1951; Magee 1961), and it has been reported that cholecystokinin potentiates the action of small amounts of secretin on the volume and bicarbonate output of pancreatic juice in dogs (Spingola, Meyer, and Grossman 1970). However, no information is available on the effects of cholecystokinin on the secretion of water and electrolytes into bile or pancreatic juice in sheep.

When acid entered the duodenum of sheep in which the enterohepatic circulation of bile salts was interrupted, the increase in the output of volume and bicarbonate in bile was much greater than that in pancreatic juice. Under normal conditions, however, the enterohepatic circulation is intact, and the secretion of biliary bicarbonate and volume is stimulated to a greater extent. These results indicate that the liver must play a much more important role than the pancreas in the entry of bicarbonate into the duodenum in sheep.

V. Acknowledgments

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