Bile Production in Fasted and Fed Primates

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Previous experiments have suggested differences in the bile salt independent flow rate between monkeys and dogs, and in the ability of bile salt secretion to influence bile flow. The present study was performed to determine whether the availability of food could have influenced these results. Bile flow and bile salt secretion rates were studied in fasted and fed primates. In the fasting experiments, sodium cholate, sodium taurocholate, and monkey bile were infused into the duodenum; only sodium cholate was infused in the feeding experiments. The results showed that bile flow was linearly related to the bile salt secretion rate in both fasting and feeding animals. Bile salt independent flow is extremely variable during fasting and is increased and stabilized by feeding. These studies indicate that the differences found in previous experiments are probably attributable to variations in experimental design.

RECENT ADVANCES in the understanding of bile production may soon permit measurement of the different components of bile flow, in various types of biliary tract obstruction, and in other diseases of the liver or bile ducts. However, certain important differences in experimental findings have occurred, which may make such analyses difficult. These differences, which are attributable to variations in species or methods of study, are outlined below, and have been mentioned in a recent review of the subject.[15]

Intravenous infusion of bile salts, producing a steady rate of bile salt secretion by the liver, is associated with fluctuating bile flow in the fasted dog.[12] The fluctuation in bile flow is eliminated, if anticholinergic agents are infused concurrently with the bile salts.[6] Linear correlation between bile flow and bile salt secretion has been demonstrated under these conditions, by varying the rate of bile salt infusion.[6] Extrapolation of the relationship between bile salt secretion and flow suggested that bile flow was negligible at very low bile salt secretion rates.[6] Dowling[2] studied the effect of bile salt secretion on bile flow in the fed rhesus monkey. Linear correlation between bile salt secretion rate and bile flow was observed, although anticholinergic agents were not administered. At very low bile salt secretion rates, there was still considerable bile flow. It is not clear why anticholinergic agents are necessary to demonstrate the linear relationship between bile flow and the bile salt secretion rate in the dog, but not in the monkey, or why large bile flows occur at low bile salt secretion rates, in the monkey, but not in the dog. Although these differences could have been due to species variation, it seemed possible that they were related to the selection of fasted or fed models.

Nahrwold studied the effect of increasing bile salt secretion rate on bile flow, by infusing bile salts into the duodenum, in both fasting and fed dogs.[5] Bile flow was not affected in the former, but a significant increase occurred in the latter. It is difficult to explain why the fasted dogs in this experiment had no increase in bile flow, when the bile salt secretion rate was increased, since both Wheeler[6] and Dowling[2] demonstrated a good correlation between bile flow and the bile salt secretion rate. Furthermore, there was an appreciable bile flow at low bile salt secretion rates in both fasted and fed dogs in Nahrwold's experiments,[5] a finding similar to that in fed monkeys,[2] but different from that in other fasted dogs.[6]

The purpose of the present experiment was to deter-

Submitted for publication June 20, 1973.

Supported by Grants from the Medical Research Council of Canada, The J. P. Bickell Foundation and the Toronto Western Hospital Research Foundation.

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mine whether the described differences were related to species variation, or to other factors, such as the availability of food. The experiments of Wheeler[6] and those of Nahrwold,[5] both conducted in the dog, suggested that factors, other than species variation, accounted for the differences. It was hoped that clarification of these differences would help in the planning of studies of abnormal states affecting the liver and bile ducts.

Methods

Animal Model

Female rhesus monkeys (4–6 kg) were adapted to restraining chairs. They were fed monkey biscuits (Teklad Inc.) 30 gm/kg/day and housed in a room with diurnal variation in lighting. Room lights were on from 7 A.M. to 7 P.M. daily.

At operation, the supraduodenal bile duct was divided and the lower end was closed. The upper end of the bile duct was intubated in order to allow collection of all bile produced by the animal. A tube duodenostomy was also performed. The cystic duct was divided and tied, and the gallbladder aspirated. The procedure was similar to that described in detail by Dowling.[2]

Two weeks were allowed for recovery from the surgical procedure. During this period, the combined bile duct and collecting tube dead space was measured by the BSP method of Barber-Riley.[1] In the second week of the recovery period, the enterohepatic circulation of bile salts was maintained by constant intraduodenal bile salt infusion (Sodium cholate, 6–8 mmoles/24 hrs).

Infusion Solutions

The sodium cholate infusions were made from a commercial preparation (Sigma). This material was more than 95% pure by GLC. Solutions were prepared in distilled water to contain 1.0 mmole, 2.0 mmoles, 4.0 mmoles, 6.0 mmoles, or 8.0 mmoles/45 ml. Sodium chloride was added to the bile salt to produce solutions which were approximately isotonic (range 275–295 mOs/l Advanced Instruments Osmometer).

"Sodium taurocholate" (Sigma) was shown to be a crude preparation, containing approximately 60% sodium taurocholate and a variety of other conjugated bile acids, by GLC and TLC. The crude bile salt was used to make solutions containing 2.0 mmoles, 4.0 mmoles or 8.0 mmoles/45 ml.

The monkey bile used had been obtained during intraduodenal sodium cholate (Sigma) infusions in several monkeys. The bile contained 90% cholic acid and 10% chenodeoxycholic acid, by GLC. The bile acids were more than 90% conjugated, mostly with glycine (TLC). The bile was frozen at −20 C until the day of the experiment.

Experimental Procedure

Fasting Experiments. Each animal was given monkey biscuits (30 gm/kg) and water in the late afternoon (4–5 P.M.), on the day before a study. Two hours later, any remaining biscuits and water were removed. The intraduodenal bile salt, or monkey bile infusion was started at 5 P.M. and continued throughout the night and the next day, at one rate. A syringe pump (Harvard Apparatus Co.) and a roller pump (Holter Co.) were used to administer the bile salt solutions and monkey bile respectively. Bile sampling was started at 8 A.M. and continued to 4–5 P.M. The animals were then fed again and a new infusion rate established.

The sodium cholate was infused at a rate of 6.0 mmoles, 4.0 mmoles and 2.0 mmoles/24 hr, on three successive days in five animals; the volume infused on each day was 45 ml. Isotonic saline (45 ml/24 hr) was infused on the fourth day. In one animal, (C), sodium cholate was infused at 8 mmoles/24 hr, instead of 6.0 mmoles/24 hr. In two animals receiving sodium chloride infusions, an extremely slow bile flow was recorded. Since spontaneous biliary tract obstruction had occurred during very slow bile flows in previous experiments, the saline infusions were discontinued at very slow bile flows and sodium cholate infused at 1.0 mmole/24 hr. on a subsequent day. The entire experiment (four days of sampling) was repeated in all five animals, from several days to several weeks later. In some animals a third experiment was performed. The number of experiments done in each animal is shown in Table 1. Bile flow and bile salt concentrations were measured in each sample. Sodium taurocholate was infused at the rate of 2.0 mmoles, 4.0 mmoles and 8.0 mmoles/24 hrs. on three successive days in three animals. These experiments were repeated several weeks later in two of the animals. Monkey bile was infused on three successive days at 50 ml, 100 ml, and 200 ml/24 hr (approximately 2.0 mmoles, 4.0 mmoles, and 8.0 mmoles/24 hr) in each of four animals. The experiment was repeated several weeks later and in one animal it was repeated on a third occasion.

Feeding Experiments. The animals were fed 10 gm monkey biscuits and 30 ml water every two hours

TABLE 1. *Number of Experiments in Each Animal (Each Experiment Done over Three or Four Infusion Days)*

	Animal				
Fasting Experiments	A	B	C	D	E
1. Sodium Cholate	3	3	3	3	2
2. Sodium Taurocholate			1	2	2
3. Monkey Bile	3	2	2	2	
Feeding Experiments					
1. Sodium Cholate	1		2	1	1

throughout the day, beginning at 7:30 A.M. At 5 P.M. they were given only 20 gm/kg monkey biscuits, so that the total daily intake was approximately equal to that of the fasting group. The study was otherwise conducted as for the fasting experiment, except that only saline or sodium cholate infusions were used. In three animals, three infusion rates were used over three successive days. In a fourth animal (C), the study was continued over six days (three rates, two days at each rate). The feeding experiment was performed before the fasting experiments in one animal, between the fasting experiments in one animal and following the fasting experiments in two animals.

Bile Sampling Technique

In order to minimize errors due to bile duct and tubing dead space, the technique described below was adopted. Bile flow was calculated from the volume of bile produced during a sampling period. Chemical composition was determined in bile samples collected in the following manner. The volume of bile equal to the dead space volume, produced at the beginning of the first daily sample, was discarded. An identical volume, obtained from the initial outflow in the second sampling period, was added to the first sample. During the third and subsequent sampling periods, the initial outflow equal to the dead space volume was also added to the previous sample. Collection was continued at the end of the day to collect the dead space volume for the final sample.

TABLE 2. *Fasting Experiments: Bile Flow and Bile Salt Secretion Rates on Four Successive Days During Sodium Cholate Infusions (Animal A)*

Day	Infusion	Bile Flow ml/24 hr	Bile Salt Secretion mmoles/24 hr
1.	6 mmoles/24 hr sodium cholate	117.6	5.9
		117.6	5.7
		106.0	5.8
		110.4	5.5
		121.6	5.8
		142.4	6.8
2.	4 mmoles/24 hr sodium cholate	156.0	5.4
		153.6	6.0
		144.0	6.0
		115.2	4.8
		103.2	4.7
		70.4	4.0
		96.0	5.3
3.	2 mmoles/24 hr sodium cholate	108.0	4.5
		129.6	4.4
		101.6	3.1
		117.6	3.2
		99.8	2.6
4.	Saline	100.8	1.3
		67.2	1.1
		91.2	1.4
		72.0	1.1

Chemical Analyses

Bile salt concentration was measured by the enzymatic method.[2] Bile BSP concentration was determined as described by Wheeler.[13] To measure conjugated and unconjugated bile salts, undiluted bile (10 μl) was applied to silica Gel G thin layer chromatograms and developed with toluene: glacial acetic acid: water. 50:50:10 (v.v.v.).[11] Each sample was run on two lanes. One lane was stained with phosphomolybdic acid spray. The unstained lane was divided into conjugated and unconjugated areas, each area scraped, and the silica gel washed with methanol (10 ml). An aliquot of the methanolic solution was evaporated to dryness at 70 C. Methanol (0.1 ml) was added and bile salt concentration determined. The internal standards for conjugated and unconjugated bile salts (Supelco) were pure by GLC.

Gas liquid chromatography* of standards and infusion solutions was performed by methods recently described.[16]

Statistical Methods

Results were analyzed for difference by the Wilcoxon rank test. Bile flow was plotted against the bile salt secretion rate, and linear regression lines and correlation coefficients calculated. The standard error of the estimate (S.E.E.), a measure of vertical dispersion or scatter about the linear regression line, was also determined.

Results

Fasting Experiments

Bile Flow. Considerable variation in bile flow was observed during each experimental day, in all three types of infusion. Peak flow rates occurred at random times during the eight hour sampling period. The variation is shown in one representative four-day experiment (Table 2).

Bile Salt Secretion Rate. Variation in the bile salt secretion rate also occurred, but usually was less than that seen in the bile flow (Table 2). The bile salt secretion rate at the highest rates of infusion (6–8 mmoles/24 hrs) was usually the same as the infusion rate ($\pm$1.5 mmole/24 hr). At the lower infusion rates, the bile salt secretion rate was 0–2 mmoles/24 hr above the infusion rate, probably due to the contribution from hepatic synthesis which would be high at the low infusion rates. In some samples, the bile salt secretion rate differed from the infusion rate by 3–4 mmoles/24 hr. The bile salt secretion rate is equal to the hepatic bile salt synthesis rate during saline infusions when no bile salts are being absorbed from the intestine. The bile salt secretion rate showed little variation during saline infusions (Table 2), indicating that the bile salt synthesis rate was fairly

* Courtesy of Drs. M. M. Fisher and I. M. Yousef.

steady. No differences in the results for bile flow or bile salt secretion rate were found between initial experiments and subsequent experiments done weeks later. This demonstrates that the results of studies commenced in the third week after surgery were not related to a failure to recover from the operative procedure.

Bile Flow to Bile Salt Secretion Rate Relationship. Bile flow was plotted against the bile salt secretion rate, as previously described[12] (Figs. 1–3). In each case, there was an increase in bile flow as bile salt secretion rate increased. At any particular bile salt secretion rate, there was a large fluctuation in bile flow. The extent of this fluctuation was similar across the whole range of recorded bile salt secretion rates, in those studies in which the most data was gathered (three or more experiments at one infusion rate, figs. 1A-D and 3A). The data fell roughly between parallel lines (not shown) with no tendency for convergence of the lines at low bile flows. When only one or two experiments were performed at one infusion rate, the fluctuation of bile flow was not always similar at all bile salt secretion rates; neither was it consistently greater at high, medium or low bile salt secretion rates.

Linear regression analysis (Table 3) showed a highly significant correlation (usually $p < 0.001$) between the bile flow and the bile salt secretion rate. The large dispersion of the data from the linear regression line is demonstrated by the high values for the S.E.E. (Table 3). The mean bile salt independent flow* is defined by the Y-axis intercept of each linear regression line. The large fluctuation in bile flow at any bile salt secretion rate (vertical dispersion), suggested that in individual samples there was considerable variation from this mean bile salt independent flow.

Conjugation of Bile Salts. During the sodium cholate infusions, the bile salts in the bile collected from the five animals were 87%–95% conjugated, except in one animal (A), in which the bile salts were 72–77% conjugated. During the bile and sodium taurocholate infusions, the bile salts were almost 100% conjugated, again with the exception of animal A (70–90%). Since the bile salts in the latter two infusions were more than 90% conjugated, it is suggested that this animal was deconjugating the infused bile salts, either in the intestine or in the bile, due to bacterial contamination. There was no correlation between the degree of conjugation and the fluctuations in bile flow at any bile salt secretion rate observed in the fasting experiments.

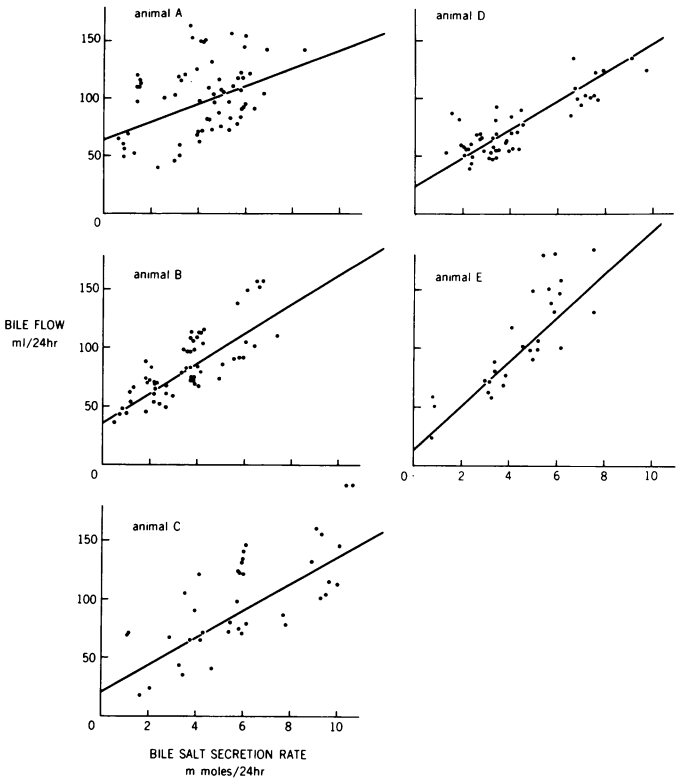

FIG. 1. Fasting experiment. Bile flow vs. bile salt secretion rate in samples obtained from animals receiving intraduodenal sodium cholate infusions. Line shown is linear regression line.

Feeding Experiments

Bile Flow. Bile flow was less variable during each experimental day in the feeding experiment than in the fasting experiment (Table 4). When variations in flow occurred, they were usually well related to changes in the bile salt secretion rate. Bile flow was increased during the feeding experiments.

Bile Salt Secretion Rate. The relationship between the bile salt infusion rate and the bile salt secretion rate was similar to that seen in the fasting experiments, except that large differences between the two rates, occasionally observed in the fasting experiments were rare in the feeding experiments.

Bile Flow to Bile Salt Secretion Rate Relationship.

* The term bile salt independent flow used in this study indicates that part of the total collected bile flow, not dependent upon bile salt secretion. It does not indicate that the flow originates exclusively from the bile ductule or the canaliculus. Nor does it account for any portion of flow, which is independent of bile salt secretion, and which is absorbed in the ductule, since that would not be found in the total collected flow. The use of the term bile salt independent flow and the method of detection of this flow, in the studies cited in the introduction, are identical to the term and method in the present study. The portion of bile salt independent flow reabsorbed in the bile ductule may be estimated using canalicular clearance techniques,[4,14] but since the purpose of the present experiment was to explain the differences in the studies described, canalicular clearance techniques were not used here. The values for the slopes and Y-axis intercepts are presented for completeness. The significance of these values is limited by the scatter of the data and the presence of unconjugated cholic acid in the bile during cholic acid infusions.

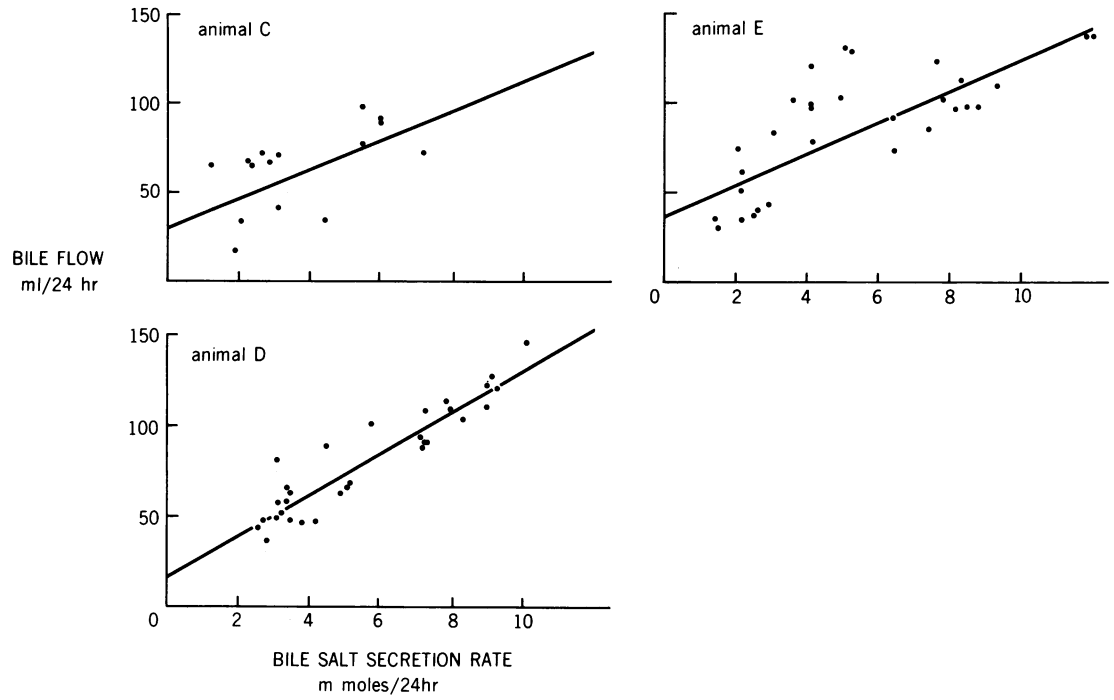

FIG. 2. Fasting experiment. Bile flow vs. bile salt secretion rate in samples obtained from animals receiving intraduodenal sodium taurocholate infusions. Line shown is linear regression line.

Bile flow was plotted against the bile salt secretion rate (Fig. 4). There was an obvious increase in bile flow as the bile salt secretion rate increased. In comparison with the fasting studies, little fluctuation was observed in the bile flow at any particular bile salt secretion rate. The linear regression data is shown in Table 5. The linear correlation coefficients were highly significant ($p < 0.001$) and significantly higher than the linear correlation

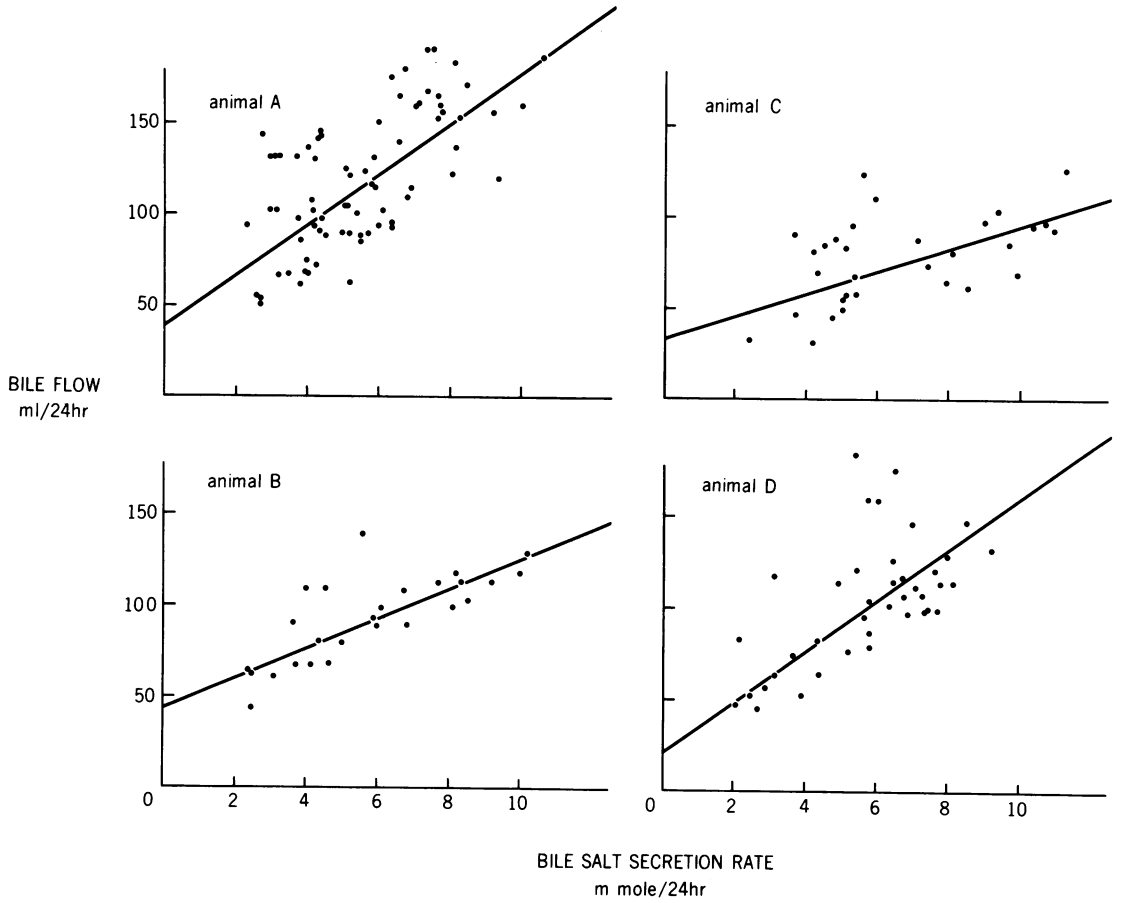

FIG. 3. Fasting experiment. Bile flow vs bile salt secretion rate in samples obtained from animals receiving intraduodenal monkey bile infusions. Line shown is linear regression line.

TABLE 3. *Fasting Experiments: Linear Regression Data and Standard Error of the Estimate (Measure of Scatter) During Sodium Cholate, Sodium Taurocholate and Bile Infusions*

		A	B	C	D	E
Infusion	Slope	7.57	12.72	11.54	12.19	20.54
	Y intercept	64.15	34.44	20.57	23.41	14.41
	Linear correlation coefficient ("r")	.479	.815	.760	.865	.896
Sodium	p for "r"	<0.001	<0.001	<0.001	<0.001	<0.001
Cholate	Standard error of the estimate (S.E.E.)	26.53	15.72	29.22	14.43	21.92
	Slope	—	—	7.68	11.58	8.84
	Y intercept	—	—	29.60	15.98	35.85
Sodium	Linear correlation coefficient ("r")	—	—	.567	.932	.784
Taurocholate	p for "r"	—	—	<0.05	<0.001	<0.001
	Standard error of the estimate (S.E.E.)	—	—	19.32	10.00	21.42
	Slope	13.76	8.12	6.25	14.25	—
	Y intercept	39.11	44.34	33.12	20.42	—
Bile	Linear correlation coefficient ("r")	.674	.778	.611	.760	—
	p for "r"	<0.001	<0.001	<0.001	<0.001	—
	Standard error of the estimate (S.E.E.)	27.44	14.37	20.98	24.75	—

coefficients of the comparable fasting experiments ($p < 0.01$).

The mean bile salt independent flow (Y-axis intercept) was larger in the feeding experiment than in the comparable fasting experiment, in the same animal. Because there was very little fluctuation in bile flow at any bile salt secretion rate, the bile salt independent flow in each sample must have been very close to this mean. The reduction in the vertical dispersion from the linear regression line is indicated by the S.E.E., which was four to five times less than the comparable fasting values obtained during sodium cholate infusions. The difference was significant ($p < 0.01$). These results demonstrate that frequent small feedings result in an increased and stabilized bile salt independent flow.

TABLE 4. *Feeding Experiment: Bile Flow and Bile Salt Secretion Rate on Three Successive Days During Sodium Cholate Infusion*

Day	Infusion	Bile Flow	Bile Salt Secretion Rate
1.	6 mmoles/24 hr sodium cholate	218.4	7.7
		216.0	7.7
		201.6	6.3
		201.6	6.8
		198.4	6.6
		190.4	6.8
		211.1	7.6
2.	4 mmoles/24 hr sodium cholate	193.6	5.7
		194.4	5.9
		172.8	5.2
		182.4	5.3
		187.2	5.9
		182.4	5.4
		176.0	5.8
3.	Saline	116.4	1.5
		109.2	1.3
		122.4	1.5
		111.6	1.4

Note constancy of bile flows and close relationship between changes in bile salt secretion rate and change in bile flow (Animal A).

Conjugation of Bile Salts. Findings were similar to the fasting experiments.

Discussion

The pertinent observations in this experiment may be summarized as: 1) Bile flow is linearly related to the bile salt secretion rate in both fasted and fed primates; 2) Bile salt independent flow is extremely variable in fasted primates, during infusions of conjugated and unconjugated bile salts, or bile; and 3) Bile salt indepen-

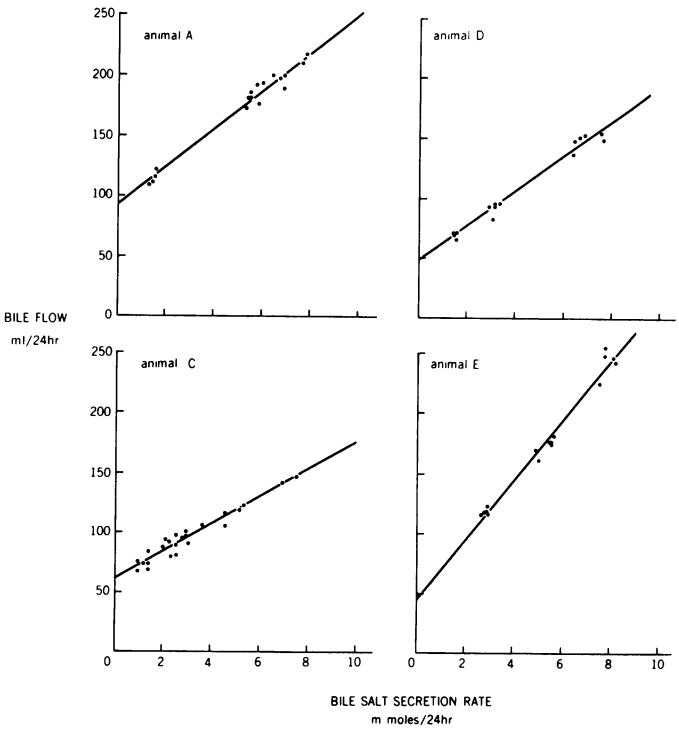

FIG. 4. Feeding experiment. Bile flow vs bile salt secretion rate in samples obtained from animals receiving intraduodenal sodium cholate infusions. Line shown is linear regression line.

TABLE 5. *Feeding Experiments: Linear Regression Data and Standard Error of the Estimate During Sodium Cholate Infusions*

		A	B	C	D	E
Infusion	Slope	16.10	—	11.45	14.51	24.61
	Y Intercept	92.64		60.90	48.66	45.33
Sodium	Linear Correlation					
Cholate	Coefficient ("r")	.988	—	.966	.989	.988
	p for "r"	<0.001	—	<0.001	<0.001	<0.001
	Standard Error of the Estimate S.E.E.	4.95	—	5.30	5.55	7.44

Note high correlation coefficients and small standard errors compared to data in Table 3.

dent flow is increased and stabilized by steady feeding.

The following explanations of the differences in experimental findings described in the introduction may be made, as a result of the observations in the present experiment. Wheeler[12] found that bile flow fluctuated at one bile salt secretion rate in the fasted dog, not receiving anticholinergic agents. This result is similar to our own in fasted primates. No attempt to correlate bile flow to bile salt secretion was made under these conditions in the dog, although, as shown above, significant correlation does exist in the fasted primate, despite considerable dispersion of the data. Instead, anticholinergic agents were administered to the dogs and bile flow was stabilized.[6] The anticholinergic agent used (Piptal, Lakeside Laboratories) completely suppresses gastric acid secretion.[3] The latter is a potent stimulant of secretin release from the duodenum,[12] which in turn stimulates bicarbonate rich ductular secretion.[7] Bicarbonate secretion in the bile was considerably reduced when Piptal was used,[6,12] suggesting that the stabilization of bile flow at one bile salt secretion rate was achieved by suppression of bile salt independent flow, originating from the bile ductules. This is further supported by examination of the linear correlation data between bile flow and bile salt secretion in the dogs receiving Piptal.[6] There is little dispersion of the data points from the linear regression lines and the Y-axis intercepts are close to zero, indicating that there is little or no bile salt independent flow. Since Wheeler[6] obtained stabilization of bile flow by suppressing bile salt independent flow, and Dowling[2] used a fed animal in which bile salt independent flow is high and stable (confirmed by our experiments), one must conclude that the difference in bile flow at low bile salt secretion rates in these two experiments is not due to species variation, but to the use of a fasted model receiving an anticholinergic agent, by Wheeler, and to the use of a fed model by Dowling.

Nahrwold[5] found significant bile flow in fasted dogs at low bile salt secretion rates. This would be expected since anticholinergic agents were not used. This finding is similar to our own in fasted primates. There was a much larger bile flow at low bile salt secretion rates in fed dogs, a finding similar to Dowling's experiments[2] and the present experiments in fed primates.

Finally, there is Nahrwold's interesting observation that fasted dogs given an intraduodenal bile infusion do not respond with an increase in bile flow, although the bile salt secretion rate by the liver rises.[5] This finding appears to contradict Wheeler's,[6] Dowling's[2] and our own conclusion that there is always linear correlation between bile flow and the bile salt secretion rate. However, the intraduodenal bile infusion was associated with a large decrease in the hepatic bicarbonate output,[5] indicating that the ductular secretion of bile was inhibited by the intraduodenal bile infusion. It is not surprising that the ductular bile salt independent flow would decrease under these conditions since the release of secretin is dependent on a low pH and the bile that was infused into the duodenum would have had a slightly alkaline pH. Wheeler's demonstration that alkalinization of the duodenum may be associated with a drop in bicarbonate output and bile flow,[12] supports this. It is likely that, in Nahrwold's experiment, the increase in bile salt secretion was indeed associated with an increase in the bile salt dependent flow, but that this was offset by a decrease in the bile salt independent flow, so that total flow remained unchanged. This explanation was first suggested by our own fasting experiments in which there are numerous examples of samples having the same bile flow at different bile salt secretion rates. This is due to the fact that total flow in a sample with a small bile salt dependent flow and a large bile salt independent flow, may equal total flow in a sample with a large bile salt dependent and a small bile salt independent flow.

The combined infusion of food and bile in Nahrwold's experiment was associated with an increase in flow and bicarbonate output. Obviously, the addition of food more than compensated for the inhibiting effect of alkaline bile infusion on ductular flow.

The results of the present experiment may be useful in planning studies of bile formation, especially in abnormal states such as biliary tract obstruction. Food intake must be similar in control and experimental periods of a study, if the changes found are to be attributed to factors other than food intake. Sometimes, this may be difficult when drugs affecting appetite are given or when the animal is made uncomfortable (eg. biliary tract obstruction).[9] Fasting for prolonged periods may itself affect composition and is therefore not acceptable.[8] The use of intestinal intubation to provide stable food intake throughout the experiment may be a suitable method of overcoming this problem. Recently, we have studied the effect of repeated intermittent biliary tract obstruction.[10] The animals were fed at 8 A.M., during both the control

and experimental periods. In the experimental period, the animal was obstructed from 6 P.M. to 8 A.M. and allowed to recover from obstruction from 8 A.M. to 6 P.M. The animals ate equally well during control and experimental periods, since obstruction was relieved just prior to feeding. We were able to compare control and experimental bile flows during similar periods of the day using this technique.

Anticholinergic agents have been very useful in stabilizing bile flow so that relationships between bile flow and bile salt secretion could be analyzed.[6] These drugs should probably be avoided, except for this specific reason, since they eliminate bile salt independent flow, which may be a desirable parameter to investigate. Also, it has not been clarified whether anticholinergic agents have other undesirable effects, eg. on bile composition.

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