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Effect of Fasting and Ileal Resection on the Concentration of Deoxycholic Acid in Rat Portal Blood[1] (39382)

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Despite the development of interest in the enterohepatic circulation of bile acids, little is known about their composition in portal blood. Most information has come from studies on rats (1-3) in whom cholic acid is the predominant bile acid. The amounts of other bile acids appear to vary considerably, there being a 30-fold difference in concentration of deoxycholic acid in studies (2) and (3). Although this could be partly explained by the different strains of animals used, it is also possible that feeding may have had an effect.

This study has been designed to investigate the effect of starvation on the composition and concentration of portal blood bile acids. Since the distal small intestine is the main site of active transport of bile acids (4), the effect of small bowel surgery on the "secondary" bile acids formed by bacteria in the gut has been examined.

Methods. Male and female Sprague-Dawley rats (250-300 g) were used. When required, portal blood (2-3 ml) was withdrawn from the superior mesenteric vein after ligation of the portal vein under ether anesthesia.

In the study of the effect of fasting, polyethylene cannulas (PP20, Portex Ltd., Kent, U.K.) were introduced into the splenic vein [similar to method A of Gallo-Torres and Ludorf (5)] and into the bile duct under pentobarbitone anesthesia immediately prior to the study. Purified [24-^{14}C]deoxycholic acid (52 μCi/μmole. Radiochemical Centre, Amersham, U.K.) (containing less than 0.4% as cholic acid) was then administered by two routes to rats which were either fasted (20 hr) or allowed to feed *ad libitum*. In the first experiment, 12 nmole were infused in isotonic saline (53 μl/min) over a 30-min period into the superior mesenteric vein. Bile was collected for 5-min periods during the infusion and then for a further 10 min. In the second experiment, a needle was positioned in the duodenum via the pylorus and [24-^{14}C]deoxycholic acid (24 nmole, 0.5 μCi, in saline) was injected as a bolus. Bile was collected for 5 hr, the animals being maintained under light pentobarbitone anesthesia.

In the study of intestinal resection animals were operated on under ether anesthesia. A laparotomy was performed in control animals and their intestines were divided and resutured. Distal ileal resections (20 and 50 cm) were performed 1 cm from the ileal caecal valve, end-to-end anastomosis being used to restore intestinal continuity. Animals were allowed to feed *ad libitum* on a standard laboratory diet postoperatively and portal blood was sampled 7 days later, between 9:00 and 10:00 AM. Control animals gained weight (mean 11%) over the 7-day period whereas animals in the experimental groups all lost weight: 20-cm ileal resection (5%), 50-cm ileal resection (9%), and right hemicolectomy combined with 20-cm ileal resection (22%).

Isolation of bile acids. Bile acids in serum (0.2-1.0 ml) and bile (50-100 μl) were isolated by liquid solid extraction using the resin XAD-7 (Rohm & Haas Ltd., Croydon, Surrey, U.K.) in a similar manner to that described for XAD-2 (6) except that 0.004 *M* sodium hydroxide was used to wash the resin since water alone caused elution of bile acids.

Analysis of biles. The bile acids were separated by tlc before and after hydrolysis with cholyl glycine hydrolase (7). The solvent systems used were chloroform:methanol:acetic acid:water (65:20:10:5, v/v) (8) for the conjugated bile acids, and 2,2,4-trimethylpentane:di-isopropyl ether:acetic

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acid:propan-2-ol (2:1:1:0.4, v/v) for the deconjugated bile acids. The bile acids were located by spraying with iodine which was then sublimed. The marked zones were scraped into scintillation vials and 0.5 ml of methanol was added to elute the bile acids followed by 10 ml of a scintillation cocktail [2 volumes of toluene, containing 2.5 diphenyloxazole 4 g/liter and 1,4 di-2-(5 phenyloxazolyl) benzene 0.05 g/liter, and 1 volume Triton X-100]. The positions of the individual bile acids were confirmed by spraying a separate track with 1 *M* sulphuric acid and heating to 120° for 15 min.

Analysis of sera. A portion of the initial extract was acidified (pH 1) with 6 *M* hydrochloric acid and extracted three times with diethyl ether (3 volumes) in order to examine the unconjugated bile acids. The total bile acids (conjugated and unconjugated) were examined by deconjugation of a second portion of the initial extract with cholylglycine hydrolase (7). The resulting unconjugated bile acids were converted to their methyl esters by treatment overnight with methanol containing acetyl chloride (7.5%, v/v). After addition of internal standard, 5α-cholestane, the methyl esters were treated with pyridine, hexamethyldisilazane, and trimethylchlorosilane (9:3:2, v/v) (9) to form their *O*-trimethylsilyl ether derivatives. These were purified after dilution with *n*-hexane (1 ml) by washing with water (1 ml) to remove pyridine and excess silylating reagent. Gas chromatography was carried out in a 5-ft × 1/4-in. O.D. glass column containing 1% Hieff-8BP on Gas Chrom Q (100–120 mesh) (Applied Science Laboratories, State College, Pennsylvania) at 235° with a nitrogen flow rate of 60 ml/min. Detection was by flame ionization and quantitative data obtained by comparison of peak height ratio to 5α-cholestane with standards. Recoveries through this procedure were checked by the addition of known amounts of glycine and taurine conjugates of each bile acid (10 and 50 nmole) to bile acid-free serum for every analysis and an appropriate correction made. They were shown to vary between 75–85% for di- and trihydroxy bile acid conjugates and 55–65% for lithocholyl conjugates. Recovery of the unconjugated bile acids present in serum was greater than 95%. The sum of the individual bile acid concentrations has been reported as the total bile acid concentration.

Bile acids isolated from the serum from portal blood were dissolved in chloroform:methanol (1:1, v/v) and analyzed by tlc using the system 2-methylbutyl acetate: propanoic acid:propan-1-ol:water (4:3:2:1, v/v) (10) in order to examine the state of conjugation. Bile acids were located by spraying with 1 *M* sulphuric acid heating at 120° for 15 min and viewing the fluorescence generated by exposure to 354-nm light.

Liver histology was examined by light microscopy. The pathologist had no previous knowledge of the type of operation that had been performed.

Results. Fasting (20 hr) led to no significant change in the total concentration of serum bile acids in portal blood (150.8 ± 20.4 → 193.6 ± 24.9 μM) in male animals. There was, however, a sharp rise in the deoxycholic acid concentration (7.7 ± 2.1 → 26.9 ± 4.0 μM, $p < 0.005$): this increase was reflected mainly in changes in the concentration of conjugated deoxycholic acid (Table 1).

To ascertain whether the increase in deoxycholic acid was due to a failure in 7α-hydroxylation by the liver [24-^{14}C]deoxycholic acid was infused into the superior mesenteric vein and bile collected. A total of 85% of the administered isotope was recovered in the bile of both fed and fasted animals within the period (40 min) of the experiment. Additional experiments were performed in which the isotope was injected into the upper small intestine. Recoveries of greater than 80% of the administered radioactivity in the bile occurred within 5 hr.

Analysis of the biles revealed no difference in the percentage of isotope hydroxylated to cholic acid between fed and fasted animals in the infusion study (Table 2). The values observed (2.5–8.9%) were much less than those reported previously (30–40%) (11, 12) when the isotope was given orally, although Hoffman, Iser and Smallwood observed no conversion of deoxycholic acid to cholic acid when it was infused into the portal vein (13). In order to see whether this difference was due to the method of admin-

TABLE I. EFFECT OF FASTING (20 HR) ON THE CONCENTRATIONS (μM) OF SERUM BILE ACIDS IN MALE RAT PORTAL BLOOD.[a]

Total	Cholic acid		Deoxycholic acid	
	Conj.	Unconj.	Conj.	Unconj.
Fed (6)				
150.8 ± 20.4	102.7 ± 20.7	40.4 ± 6.7	4.0 ± 1.3	3.7 ± 0.9
Fasting (6)				
193.6 ± 24.9	132.3 ± 18.7	34.4 ± 8.6	17.8 ± 3.0	9.1 ± 1.4
			$P < 0.005$[b]	$P < 0.01$[b]

[a] Results given as mean ± standard error.

[b] Test of significance, unpaired Student's t test. Where no p value is given, the difference was not significant at the 5% level.

TABLE II. CONVERSION OF [24-^{14}C] DEOXYCHOLIC ACID TO CHOLIC ACID.[a]

Route of administration	Total biliary radioactivity in cholic acid (%)	
	Fed animals	Fasted animals
Portal vein (4)	4.9 (2.5-8.1)	4.8 (2.5-8.9)
Intestine (2)		
1 hr	7.4 (6.4-8.3)	4.1 (4.1-4.2)
1-5 hr	7.8 (6.6-9.0)	8.6 (7.1-10.5)

[a] Mean and range given.

istration [24-^{14}C]deoxycholic acid was injected into the duodenum, but again there was only a little 7α-hydroxylation to cholic acid (4.1% fasted and 7.4% fed): the proportion of labeled cholic acid did not change during the experiment (Table 2).

Effect of intestinal resection. Since fasting had a marked effect on the serum concentration of deoxycholic acid in portal blood it was decided not to starve other experimental animals before sampling blood in order to examine the effects of bowel surgery alone.

There was a wide scatter of results in control animals although the mean total serum bile acid concentration in portal blood (139 μM in male animals, 137 μM in female animals) was similar to that observed in the fasting study. Chenic acid was a prominent bile acid in female animals (mean 17 μM) but not in male animals. Traces of lithocholic acid (0.5 μM) were observed in female animals, but hyodeoxycholic acid and the α- and β-muricholic acids were not detected in any of the animals (limit 0.5 μM).

Resection of the ileum in male rats, both 20 and 50 cm, caused a significant fall in the mean concentration of total cholic acid (134 μM to 24 and 17 μM, respectively, $p < 0.001$) whereas that of deoxycholic acid rose (from 5.6 μM to 12.7 and 9.1 μM, respectively), mainly because of unconjugated deoxycholic acid (Table 3) although this rise was not significant. Similarly in female animals with a 20-cm resection there was a significant decrease in the mean total cholic acid concentration (115 to 45 μM, $p < 0.02$) and a rise in the total deoxycholic acid concentration (3.7 to 16 μM), although again not significantly. In addition the mean concentrations of total chenic acid[3] and lithocholic acid were increased from 17 to 42 μM and 0.5 to 6.5 μM, respectively. Consequently there was only a small decrease in the total serum bile acid concentration in portal blood (from 138 to 111 μM). Analysis by tlc showed that conjugated cholate in the portal blood from all the sera was present as cholyltaurine.

Male animals who had a right hemicolectomy in addition to an ileal resection also had reduction in the total bile acid concentration. However, deoxycholic acid was absent from the sera of portal blood in all animals except one. All these animals had diarrhea.

When the bile acid composition in portal blood was examined 6 months after surgery in a small series (three animals in each group), similar qualitative changes were obtained to those seen after 1 week. However, the concentration of all bile acids were higher than those observed in the younger animals. In controls the mean total bile acid concentration was 275 μM and in animals with ileal resection was 121 μM (20 cm) and 70 μM (50 cm). For deoxycholic acid the mean concentrations in animals with ileal

[3] Chenic acid: abbreviated name for 3α,7α-dihydroxy-5β-cholan-24-oic acid.

TABLE III. SERUM BILE ACID CONCENTRATIONS (μM) IN PORTAL BLOOD OF MALE AND FEMALE ANIMALS 7 DAYS FOLLOWING SURGERY.[a]

	Sex	No. of rats	Total bile acid concentration	Cholic acid		Deoxycholic acid		Chenic acid		Lithocholic acid	
				Total	Unconj. (%)	Total	Unconj. (%)	Total	Unconj. (%)	Total	Unconj. (%)
Control	M	9	139 ± 21.3	133 ± 20.6	28	5.7 ± 1.4	39	<0.5	–	<0.5	–
	F	4	137 ± 24.3	115 ± 20.4	10	3.7 ± 1.4	46	17.3 ± 4.1	8	0.5 ± 0.3	tr
Ileal resection											
20 cm	M	8	37 ± 6.5	24.4 ± 5.4	29	12.7 ± 3.6	71	<0.5	–	<0.5	–
			$p < 0.001$[b]	$p < 0.001$[b]		NS[e]					
50 cm	M	8	27 ± 6.2	17.4 ± 3.8	47	9.1 ± 3.5	75	<0.5	–	<0.5	–
			$p < 0.001$[b]	$p < 0.001$[b]		NS[e]					
20 cm	F	4	111 ± 19.0	46 ± 7.3	44	16.0 ± 3.9	50	42.0 ± 9.3	38	6.5 ± 2.8	35
			NS[c]	$p < 0.02$[c]		NS[c]		$p < 0.05$[c]		NS[c]	
Ileal resection (20 cm) and right hemicolectomy	M	7	52 ± 12.4	49.8 ± 12.4	16	[f]	–	<0.5	–	<0.5	–
			$p < 0.01$[b]	$p < 0.01$[b]							
			NS[d]	NS[d]							

[a] Mean ± standard error given.
[b] Unpaired Student's t test against male control animals.
[c] Unpaired Student's t test against female control animals.
[d] Unpaired Student's t test against male animals with 20-cm ileal resection.
[e] Deoxycholic acid was found in one animal only. NS, not significant.

resection were 27 μM (20 cm) and 10 μM (50 cm): whether this was due to increases in age and mean body weight is not known.

Histological examination of the rat liver showed only minor changes. One week after ileal resection slight fatty changes were observed in female rats. Similar observations were made in male rats 6 months after surgery.

Discussion. The concentrations of total bile acids in the serum of portal blood of control rats covered a wide range (66–273 μM) of the same order observed by Okishio and Nair (3): the very low values previously observed by Cronholm and Sjövall (2) were not encountered. The extended sampling period (up to 30 min) used by these investigators (2) compared with 30–40 sec in our study could have been responsible for this difference. All studies were unanimous in showing that cholic acid was the major bile acid present in portal blood. Qualitative tlc analysis showed that cholic acid was conjugated almost entirely with taurine, this being consistent with the known composition of rat biliary bile acids. Other bile acids were present in trace amounts in male animals, but only deoxycholic acid was in a concentration exceeding 0.5 μM. However, in female animals 10% of the total bile acids consisted of chenic acid which is consistent with a previous report of a sex difference in the chenic acid pool size in the rat. (14).

An overnight fast (20 hr) caused a marked increase in the concentration of deoxycholic acid in male animals from 8 to 27 μM which is in good agreement with the results of Cronholm and Sjövall (2) for fed animals and Okishio and Nair (3) for fasted animals. Since the increase was mainly due to deoxycholyltaurine it seemed unlikely that increased bacterial action caused by slowed intestinal transit could explain this effect, as it is generally accepted that bacterial deconjugation of bile acids precedes their dehydroxylation although evidence for direct dehydroxylation has been reported in man (15). It has been previously shown (11, 12) that deoxycholic acid is converted to cholic acid by hepatic 7α-hydroxylation so that the possibility has to be considered that fasting may have decreased the capacity of the liver for 7α-hydroxylation and thus in-

creased the amount of circulating conjugated deoxycholic acid. However, in experiments in which isotopic deoxycholic acid was administered either via the superior mesenteric vein or into the small intestine, analysis of the biles revealed no difference in the amount of labeled cholic acid between fed and fasted animals. A decreased rate of enterohepatic cycling of bile acids is a further possible explanation, but it is difficult to reconcile with the fact that deoxycholic acid was in the conjugated form.

It has been concluded from these observations on fasting that, as that rat is mainly a nocturnal eater and does not normally fast for long periods, studies on bile acid metabolism should normally be made in fed animals. This procedure was accordingly followed in our investigations on ileal resection which demonstrated that short (20 cm) and long (50 cm) terminal ileal resection caused similar reductions in the total serum bile acid concentration in portal blood. This was largely due to a decrease in the concentration of cholyltaurine which is consistent with a previous report of a threefold fall in the cholyltaurine pool size following resection from 21 μmole/100 g body wt to 7.2 μmole/100 g body wt (16) and supports the view that the distal small intestine is necessary for its conservation.

Since taurine conjugates of each of the bile acids were present in the serum of portal blood this would suggest that significant absorption of these polar bile acids can occur in the absence of the terminal ileum *in vivo*, as conjugation is unlikely to occur in the intestine. The mechanism of their reabsorption is uncertain, although active transport has been reported in the third quarter of rat small intestine (17). In addition the rate of absorption of cholyltaurine from the jejunum following ileal resection has been shown *in vitro* to be twice that in controls (18), and that this is not just because of jejunal hyperplasia but reflects a greater ability to concentrate cholyltaurine against a concentration gradient (19).

The deoxycholic acid concentration was raised in both male and female animals following ileal resection, mainly in the unconjugated form, although the scatter of the data prevented a significant difference being observed. This would follow from an increased rate of production of deoxycholic acid by anaerobic bacteria caused by greater amounts of unabsorbed cholyltaurine entering the colon, and a greater intestinal conservation of the unconjugated over the conjugated forms by passive non-ionic diffusion (20). No explanation can be offered for the apparent increase in the concentration of chenic acid in female rats.

Although the combination of a right hemicolectomy with resection of the terminal ileum did not influence the concentration of cholyltaurine it resulted in the disappearance of deoxycholic acid from the portal blood. Despite the intestinal hurry observed in these animals not all bacterial activity towards bile acids was suppressed since some unconjugated cholic acid was present. In man a similar operation also results in a diminution in deoxycholic acid synthesis (21).

Bile acids have been shown to be hepatoxic when given in large doses in experimental studies (22–26). Since ileal resection resulted in a reduction in the total serum bile and concentration in portal blood and only moderate rises in the concentration of secondary bile acids, it was therefore not surprising that no histological damage was detected in the livers of these animals.

Summary. Bile acids in the serum of rat portal blood have been examined. Fasting (20 hr) caused a marked increase in the deoxycholic acid concentration, mainly of deoxycholyltaurine. Studies with [24-^{14}C]deoxycholic acid failed to show that this was due to decreased rehydroxylation by the liver. Resection of the terminal ileum caused a three- to fourfold reduction in the total bile acid concentration in male animals, cholyltaurine being most affected although it remained the predominant bile acid. In contrast the concentration of deoxycholic acid increased in half the animals. In female animals the concentrations of chenic acid and lithocholic acid also increased so that the total bile acid concentration only decreased slightly. Ileal resection combined with a right hemicolectomy caused the disappearance of deoxycholic acid.

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