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Kinetics of bile acid metabolism in experimental blind loop syndrome

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SUMMARY In an experimental model of the contaminated small bowel syndrome, Eriksson's washout technique was used to study bile salt metabolism. Bile salt secretion rate was increased from 204·7 ± SD 36·5 μmol/100 g body weight/24 h to 329·9 ± 146·6, pool size from 33·4 ± 5·4 μmol/100 g BW to 42·5 ± 7·2, and biliary flow from 6·6 ± 1·72 ml/100 g BW/24 h to 8·4 ± 2·1. Detailed analysis of the washout curves gave indirect evidence of a short-circuit jejunohepatic shunt in the model contaminated small bowel syndrome. Parameters of this shunt were calculated with 9·1 μmol/100 g BW for 'short-circuit' pool size and 13·8 for its circulation frequency.

Bacterial overgrowth in the small intestine that leads to bile salt deconjugation has been shown to be the principal cause of malabsorption in the blind loop or, as it may be more suitably termed, the contaminated small bowel syndrome (Donaldson, 1965; Drasar *et al.*, 1966; Tabaqchali and Booth, 1966; Rosenberg *et al.*, 1967; Tabaqchali *et al.*, 1968; Gorbach and Tabaqchali, 1969). Many pathogenic mechanisms are secondary to intraluminal bacterial utilization of foodstuffs and vitamins, diminished fat absorption, as well as to mucosal damage with reduced enzymatic activity (Tabaqchali and Booth, 1970; Ament *et al.*, 1972; Gracey *et al.*, 1973).

While bacterial deconjugation of bile salts is well established in this syndrome, both in experimental studies and human pathology, little information is available regarding the consequences of deconjugation upon bile acid metabolism and kinetics in the enterohepatic circulation (EHC). The ease of non-ionic passive absorption of deconjugated bile acids in the jejunum suggests the generation of a short-circuit circulation (jejunohepatic shunt) in the blind loop syndrome, in addition to the regular EHC with its active transport in the ileum (Dietschy *et al.*, 1966; Heaton, 1972).

Experimental data of bile acid kinetics in an animal model of the contaminated small bowel syndrome are presented which give direct evidence of the short-circuit shunt and which allow the deranged bile salt metabolism to be quantified. Emphasis is put on an increase in the size of the bile acid pool in conditions of bacterial overgrowth.

Methods

EXPERIMENTAL ANIMALS

Adult female Wistar rats (Mura: WIST SPF 67 Han) from a long established colony (Mus Rattus, D-8011 Brunnthal, FRG) weighing 190-300 g were used throughout the course of this study. The mean weight of the animals was 229·1 ± SD 38·34 g in the control and 245·8 ± 24·6 g in the blind loop group. Under light ether anaesthesia, self-filling, antiperistaltic intestinal loops 8 cm long with the base approximately 12 cm distal from the biliary papilla were constructed (Cameron *et al.*, 1950; Lambert, 1965). The intestinal *cul-de-sac* was markedly distended with contaminated overflow into the upper jejunum after four to five weeks, at which time kinetic studies of bile acid metabolism were performed. Vitamin B_{12} (0·1 μg) was injected intraperitoneally every week to obviate pernicious anaemia in the blind loop group. Experimental (n = 14) and control (n = 15) animals were housed under identical conditions and fed a standard rat diet (Rat-Muis-Hamster 1110; Hope Farms BV; Woerden, Netherlands).

STUDIES OF BILE ACID METABOLISM

Parameters of bile acid metabolism were studied by a slight modification of Eriksson's washout technique in animals with acute bile fistula (Eriksson,

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1957). An experimental approach was used similar to that recently reported by Mok *et al.* (Mok *et al.*, 1974). After bile duct cannulation (Clay-Adams PE 10 tubing), bile was sampled with an automatic fraction collector in hourly intervals for 12-24 hours while animal activity was restrained in modified Bollman cages. The rats were studied in the non-fasting state. Bile acid concentrations were determined enzymatically in duplicate by the 3α-hydroxysteroid dehydrogenase method (Talalay, 1960; Iwata and Yamasaki, 1964).

Bile acid secretion rate over 24 hours was calculated from the first hour sample. Bile acid pool size was estimated as the sum of hourly collected bile salts until the 'low point' during the washout procedure was reached. The 'basal' synthesis rate at this point was subtracted from the sum. Circulation frequency of the bile acid pool was calculated by dividing secretion rate by pool size. Bile salt parameters are expressed in μmol/100 g body weight and given as mean values ± SD and ± SEM. Results were statistically analysed using Student's t test. P values < 0·05 are considered to be significant.

Results

BILIARY FLOW

Biliary flow in the experimental animals with blind loop syndrome was markedly increased from 8·41 ± SD 2·08 ml/100 g BW/24 h compared with 6·56 ± 1·72 in the control group (t = 2·619; P < 0·01). It should be mentioned that this increased flow was recorded over the entire period of collection (Fig. 1, Table 1).

BILE ACID SECRETION RATE

Bile acid output over 24 hours was increased in the blind loop animals with 329·85 ± SD 146·62 μmol/100 g BW/24 h compared with 204·72 ± 36·51 in the control group. The increase of 61 % is highly significant (t = 3·206; P < 0·0025) (Figs. 2 and 3; Table 1).

BILE ACID POOL SIZE

The increase of 27% from 33·43 ± SD 5·38 μmol/100 g BW in the control group to 42·49 ± 7·22 in

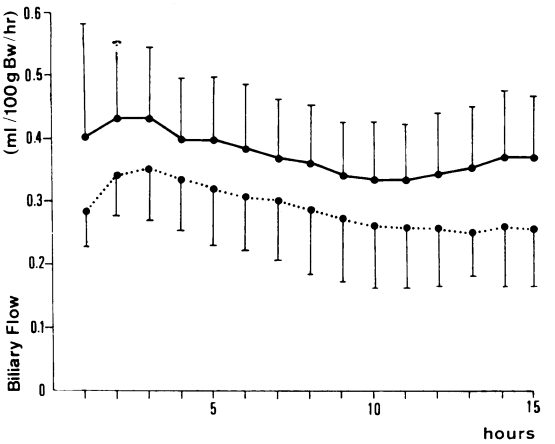

Fig. 1 *Biliary flow (means ± SD) during 15 hours after interruption of the enterohepatic circulation in control* (dotted line) *and blind loop rats* (straight line).

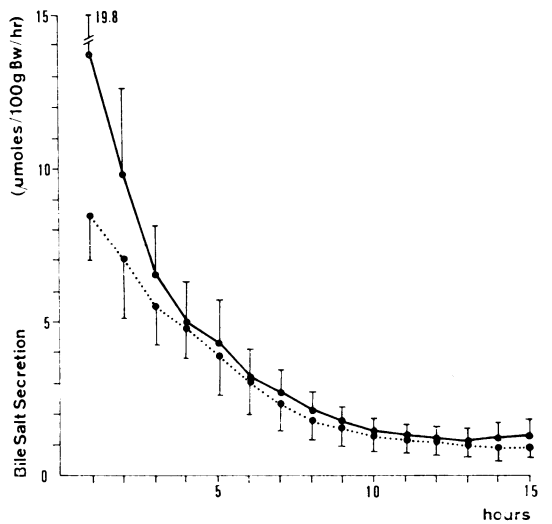

Fig. 2 *Bile salt secretion rates (means ± SD) during 15 hours after interruoption of the enterohepatic circulation in control* (dotted line) *and blind loop rats* (straight line).

	Control			*Blind loop*			*Statistical significance*
	X	± *SD*	± *SEM*	*X*	± *SD*	± *SEM*	
Secretion rate (μmol/100 g BW/24 h)	204·72	36·51	9·43	329·85	146·62	39·20	P < 0·0025
Pool size (μmol/100 g BW)	33·43	5·38	1·39	42·49	7·22	1·93	P < 0·0005
'Basal' synthesis (μmol/100 g BW/24 h)	18·69	4·30	1·11	22·31	7·25	1·94	NS
Circulation frequency (cycles/24 h)	6·2	1·38	0·36	7·6	2·34	0·63	P < 0·05
Bile concentration (first h) (μmol/ml)	31·08	5·78	1·49	39·60	12·07	3·23	P < 0·0125
Biliary flow (ml/100 g BW/24 h)	6·56	1·72	0·44	8·41	2·08	0·56	P < 0·01

Table 1 *Effect of experimental blind loop syndrome on parameters of bile acid metabolism*

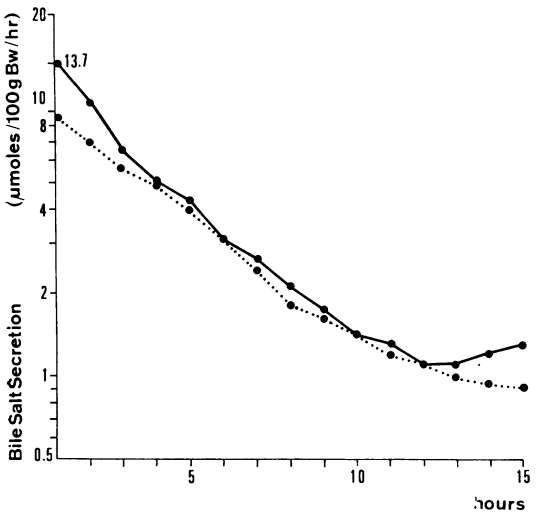

Fig. 3 *Semilogarithmic plot of bile salt secretion rates (same mean values as in Fig. 2).*

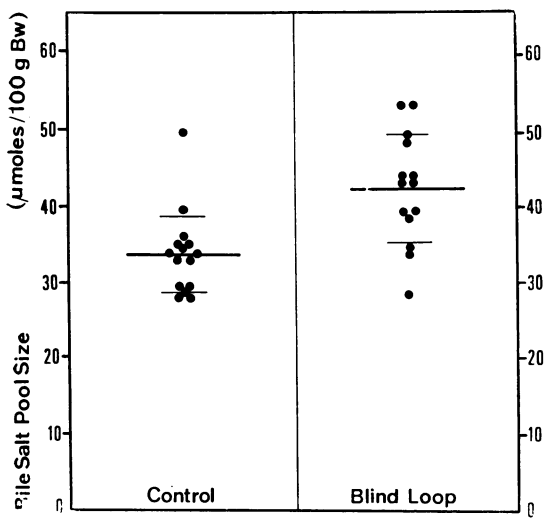

Fig. 4 *Bile salt pool size (means ± SD) in control and blind loop rats.*

the experimental blind loop animals is highly significant ($t = 3{\cdot}851$; $P < 0{\cdot}0005$). Values for bile acid pool size are shown in Fig. 4 and Table 1.

BILE ACID SYNTHESIS RATE

The increase in the amount of bile salts excreted at the 'low point' is slight and insignificant. In the blind loop animals, a 'basal' synthesis rate of 22·31 ± SD 7·25 μmol/100 g BW/24 h and 18·69 ± 4·30 in the control group was measured ($t = 1{\cdot}649$; $P < 0{\cdot}1$) (Table 2).

Amount of bile salts deconjugated per day	125·13 μmol/100 g BW/24 h
'Short-circuit' pool size	9·06 μmol/100 g BW
Circulation frequency of 'short-circuit' pool	13·8 cycles/day

Table 2 *Calculated data of short-circuit jejunohepatic shunt*

FREQUENCY OF CIRCULATION OF BILE ACID POOL

The number of enterohepatic circulations was 6·2 ± SD 1·38 in the control group versus 7·6 ± 2·34 in the blind loop group. The increase of 1·4 cycles per day is significant ($t = 1{\cdot}980$; $P < 0{\cdot}05$) (Table 1).

Discussion

These studies show highly significant increases of both bile acid secretion rate and pool size in an experimental model of the stagnant loop syndrome. Bile salts are cycled more frequently in the enterohepatic circulation and bile flow is markedly enhanced.

The operative technique of creating an experimental model for small intestinal bacterial overgrowth by Cameron's '*cul-de-sac*' method is well established (Cameron *et al.*, 1950; Gracey *et al.*, 1974). The direct method of evaluating bile salt metabolism by Eriksson's washout technique, on the other hand, gives rise to criticism. The assumption of an unchanged bile acid secretion rate after acute interruption of the EHC by bile duct cannulation has been shown to be valid for monkeys (Dowling *et al.*, 1968; Dowling *et al.*, 1970). These results were the basis for determining bile acid output in rats from the first two hour collection periods in the studies of Mok *et al.* (1974a). While their fractionation time is short in comparison with that of other authors (Eriksson, 1957; Myant and Eder, 1961), our data based on one hour sampling periods give more detailed information. The decay of bile salt secretion rate during the washout procedure follows a logarithmic function beginning from the first hour. This indicates that calculation of bile acid secretion rate in rats should be done from the first hour sample only. Consequently, our secretion rates and the number of enterohepatic cycles are higher than those of Mok *et al.* (1974a). The linear decay of bile salt secretion rate in the semilogarithmic plot indicates

that the assumption of an unchanged secretion rate immediately after bile duct cannulation is valid in the application of the washout technique in rats as well as in monkeys.

The estimation of bile acid pool size from samples of the washout procedure implies an unchanged bile acid synthesis during the time period before the 'low point' is reached. In case of an increased synthesis by the liver due to feedback mechanism in the interrupted EHC, the pool size would have been slightly underestimated in these studies. In comparing Eriksson's technique with Lindstedt's isotope dilution method for pool size determination in monkeys, Mok *et al.* could indeed demonstrate that the 'exchangeable' pool is underestimated by the washout technique (Lindstedt, 1957; Mok *et al.*, 1974b). But isotope dilution by these authors has shown an unchanged basal rate of synthesis.

These limitations to absolute physiological data do not interfere with our findings of relative pathophysiological differences of pool size and circulation frequency between the two experimental groups studied. The small difference in 'basal' synthesis rate between blind loop rats and controls allows comparison of difference of pool size and circulation frequency.

The analysis of the washout curves shows that the greater proportion of the bile acid pool in blind loop rats is washed out in the first four hours. The exponential decay of bile salt secretion rate is much steeper in the initial collection periods. After four hours, both groups decline at an identical rate. This suggests more ready absorption of bile salts from the gut for a limited period of time during the uptake from the remaining bile salt bulk in the intestine after interruption of the EHC. The location of this facilitated absorption is speculative but it is most probably the jejunum. If absorption of bile salts were increased either by active transport or passive diffusion or both in the ileum, the decay of bile salt secretion rate would be steeper compared with the controls. No change in the speed of the secretion rate during the washout procedure itself would be observed. No analysis of bile salt composition at various sites of the small intestine has been carried out in these experiments. In clinical studies on patients with bacterial contamination of the upper jejunum, Tabaqchali and Booth (1968) showed that absorption of deconjugated bile salts takes place in the jejunum, while in lower parts of the intestine only conjugated bile salts were found. One could assume, on the other hand, that bile salts have been completely deconjugated at the site of the intestine with bacterial overgrowth and that ileohepatic circulation has been interrupted. If this condition existed in our experimental model, no change in the steepness of the logarithmic decay could occur in these animals. Only a parallel shift of the last part of the curve towards the abscissa would be expected after partial consumption of bile salts by deconjugation. The fact that the last part of the decay curve in the blind loop animals equals that of the controls suggests a quantitatively maintained ileal absorption. This would mean that, in our experiments, ileohepatic circulation is preserved and jejunohepatic shunting would be superimposed upon this physiological condition.

If this interpretation is accepted, one may quantify the jejunohepatic shunt in the animals with contaminated small bowel syndrome. The amount of bile salts deconjugated per day may be calculated as the difference of the secretion rates of blind loop (BL) and control (Contr.) animals: Bile salts deconjugated per day = Secretion rates (BL − Contr.) The difference between the pool sizes of the two groups would give the size of the 'short-circuit' pool. By dividing bile salts deconjugated per day through 'short-circuit' pool size the circulation frequency of the jejunohepatic shunt may be calculated (Table 2).

The sixty-one per cent increase of bile salts secreted seems to be secondary to rapidly circulating bile salts. Bile flow in the blind loop group is significantly increased and remains elevated throughout the entire washout procedure. The fact that flow does not decrease after the short-circuit shunt has been washed out indicates that the increased flow is not driven by osmotic forces during higher bile salts secretion rates. This may rather be due to ultrastructural changes in hepatocytes. Experiments on this subject are proceeding and will be the subject of future communications.

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