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Effect of Distal Enteroctomy on Cholesterol and Bile Salt Levels in the Rat *

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The effect of 50 % or 80 % distal enteroctomy on cholesterol and bile salt levels in male Wistar rats have been investigated. Short time measurements showed that serum cholesterol levels were maximal after 20 days from 50 % intestinal resection and after 10 days from 80 % intestinal resection. This increase was maintained in 50 % resected rats 1 and 5 months after operation, whilts in 80 % resected group the values became normal. Portal blood and bile cholesterol levels remain almost normal except 5 months after 50 % intestinal resection. Bile salt concentration and bile salt output in the bile decrease after 1 and 5 months from 50 % intestinal resection and after 1 month from 80 % intestinal resection. These results together with data of fecal loss of bile salts indicate that in 50 % resected rats new steady states have been reached, with low levels of bile salts in the bile. One month after 80 % resection the fecal loss of bile salts was so high that the conversion of cholesterol into bile salts was increased. After 5 months from 80 % resection values in serum and bile were almost normal suggesting either an increase in extrahepatic cholesterol synthesis or a partial prevention of fecal loss that can be explained by the observed caecal enlargement.

Key words: Entero-hepatic circulation, Cholesterol, Bile salts, Intestinal resection.

It is well known that intestinal resection, by altering the entero-hepatic circulation (9, 11), decreases the input of bile salts into the liver (3, 10), which leads to an increase in hepatic synthesis of bile salts from cholesterol (7). This enhancement in bile salt synthesis, which is carried out by an increase in the activity of both 3-hydroxy-3-methyl glutaryl Coenzyme A reductase (5, 12) and 7, α-hydroxylase (4, 13, 17), affects both the hepatic (19, 23) and extrahepatic cholesterol synthesis (2). As a result the serum cholesterol levels are expected to be modified. Furthermore, serum cholesterol levels found after intestinal resection

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depend upon animal species (22), part and length of small intestine resected (20) and the time elapsed between the intestinal resection and cholesterol measurements.

To gain more insight into cholesterol turnover we have studied, as a function of time, the effects of either 50 % or 80 % intestinal resection on 1) the levels of cholesterol in serum, portal blood and bile, and 2) bile salt output in bile and faeces and bile salt content in portal blood.

Materials and Methods

Male Wistar albino rats weighing about 250 g maintained on a standard pellet diet and given tap water *ad libitum,* were used. The rats were randomly assigned to one of three groups: sham operation, and 50 % or 80 % distal small intestine resection.

Cholesterol levels in serum, portal blood and bile, and bile salts output in bile were measured 1 and 5 months after intestinal resection. Bile salts content in portal blood was only measured 5 months after intestinal resection. Serum cholesterol levels were also estimated 10, 20, 30 and 40 days after surgical operation.

Surgical procedure. — Rats were anaesthetized with i.p. pentobarbital sodium (4.5 mg/100 g b.w.) after 24 h fast. Laparotomy was performed and rats assigned for small bowel resection underwent either 50 % or 80 % small bowel resection by excision of small intestine beginning 1 cm distal of the ileocecal junction to the stomach. Prior to intestinal resection, the blood vessels of the resected intestinal segment were tied and sectioned, but the blood supply and innervation of the remaining intestine was carefully maintained. Intestinal continuity was re-established by an end-to-end anastomosis with Mersilene 3/0 thread. Finally, each layer, petitoneum, muscle and cutaneous was sutured separately with appropriate thread. Rats from the sham-operated group underwent simple mid-small intestinal transection without removal of any tissues and reanastomosis.

Assays. — Bile samples from each animal were obtained, under anesthesia with pentobarbital sodium after 24 h fast, by cannulating the common bile duct. Blood samples were extracted either from the heart or from the portal vein. Samples of blood 10, 20, 30 and 40 days were directly obtained from the tail.

Cholesterol was determined by the modified enzymatic technique after ALLAIN *et al.* (1). Faeces were collected during 3 days and heated for 24 h at 110°C and bile salts were extracted following the DE WAEL method (6). Bile salts in both bile and faeces were determined by an enzymatic method (8).

Statistics. — The significance of differences between mean values were analyzed by the two-tail Student's t-Test. Results are expressed as mean ±S.E.

Results and Discussion

Cholesterol changes after distal resection. — Serum cholesterol levels increase significantly after 1 and 5 months of 50 % intestinal resection (table I). These findings are in good agreement with those previously reported in dog (20). However, no significant changes in serum cholesterol levels in 80 % resected animals were found. Similar results have been reported in man (16).

To gain more insight into cholesterol turnover, the serum cholesterol levels were also determined 10, 20, 30 and 40 days after either 50 % or 80 % intestinal resection. After 50 % intestinal resection the maximun increase in serum cholesterol was found at 20 days (fig. 1), whilst the maximun increase after 80 % resec-

tion took place 10 days after operation (fig. 1), decreasing gradually with time and reaching normal values at 1 and 5 months. From these results it is apparent that even at minimal time elapsed after intestinal resection, the serum cholesterol levels show a great variability. On the other hand, portal blood cholesterol levels were increased 5 months after 50 % intestinal resection and only slightly 1 month after 80 % intestinal resection (table I). Since maximal serum cholesterol levels appear 10 days (80 % resection) or 20 days (50 % resection) after operation, this increase must be produced by a stimulation of liver cholesterol synthesis. In fact, a five-fold increase in the hepatic activity of the enzyme 3-hydroxy-3-methyl glutaryl Coenzyme A reductase, the rate limiting enzyme in cholesterol synthesis, after 8 days of interruption of entero-hepatic circulation, has been reported (14). Perhaps for this reason the greater the interruption of entero-hepatic circulation, the sooner the increase in serum cholesterol levels is.

Bile cholesterol levels increased after both 50 % and 80 % small bowel resection, the increase being greater at 5 months post-resection (table II). However, if the bile flow is taken into account, the bile cholesterol output decreased in 80 % resected animals 1 month after resection, whereas no significant differences were observed in the other experimental groups (table II).

Taken together, all these data, suggest that after 1 month of 50 % resection the increase in liver cholesterol synthesis is sufficiently high to compensate the cholesterol loss in faeces. After 5 months portal blood cholesterol levels increased (table I), suggesting that either its intestinal absorption was more efficient or that the intestine was synthesizing extrahepatic cholesterol (2).

Bile salt changes after distal resection. — In a steady state, the amount of fecal bile salts is assumed to represent the

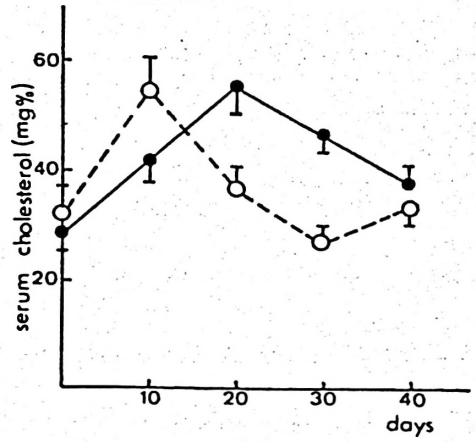

Fig. 1. *Time course of serum cholesterol levels in rats with 50 % (●——●) and 80 % (o- - -o) intestinal resection.*

amount of bile salt synthesized daily. From this definition, the control rats synthesized about 19 ± 2.9 μmol of bile salts in a day, which agrees with data reported by other authors (13). 1 month after 50 % resection, fecal bile salts output increased (38 ± 4.7 μmol/day v.s. 19 ± 2.9 μmol/day in control rats, $n = 10$ $p < 0.001$) and it did not significantly change 5 months (32.5 ± 5.1 μmol/day). In both cases bile flow, bile salt concentration and bile salt output decreased, being the decrease greater after 5 months than after 1 month (table II). Similar results were reported by other authors (14), after the interruption of entero-hepatic circulation. Furthermore, bile salts content in portal blood decreased from 0.5 ± 0.03 mM (sham) to 0.28 ± 0.03 mM ($p < 0.001$) 5 months after 50 % resection. This indicates that new steady states have been reached, with low levels of bile salts in the bile, because even though the bile salt synthesis was stimulated (4, 15), this was not sufficient to compensate the daily fecal loss. This imbalance appears to be more pronounced after 5 months post-resection.

Table I. *Cholesterol levels (mg %) in serum and portal blood.*
Analyses were made 1 month and 5 months after the intestinal resection. Data are mean values ± S.E. Number animals in brackets.

	Sham operation	50 % resected		80 % resected	
		1 month	5 months	1 month	5 months
Serum cholesterol	27.9 ± 1.6 (10)	46.7 ± 2.1* (6)	44.1 ± 1.4* (10)	26.9 ± 2.7 (6)	33.2 ± 2.3 (10)
Portal blood cholesterol	32.9 ± 2.5 (9)	33.6 ± 3.1 (9)	49.9 ± 3.4** (8)	37.7 ± 2.2 (8)	33.7 ± 2.4 (8)

* $p < 0.001$, ** $p < 0.005$, as compared to sham group.

Table II. *Bile flow, cholesterol and bile salt concentration in the bile.*
Analyses were made 1 month and 5 months after the intestinal resection. Data are mean values ± S.E. Number animals in brackets.

	Sham operation	50 % resected		80 % resected	
		1 month	5 months	1 month	5 months
Bile salt concentration (mmol/l)	39.9 ± 3.94 (9)	26.0 ± 1.50** (8)	21.3 ± 1.31* (10)	26.6 ± 2.60*** (6)	32.5 ± 6.00 (8)
Bile salt output (µmol/h)	38.4 ± 2.90 (9)	18.0 ± 2.30* (9)	8.5 ± 0.52* (10)	8.1 ± 0.50* (6)	28.6 ± 2.80 (8)
Bile flow (ml/h)	1.0 ± 0.08 (9)	0.8 ± 0.08 (8)	0.5 ± 0.04* (10)	0.3 ± 0.08* (6)	0.9 ± 0.03 (8)
Cholesterol concentration (mmol/l)	0.6 ± 0.04 (6)	0.7 ± 0.08 (8)	1.2 ± 0.13* (8)	0.7 ± 0.04 (6)	0.8 ± 0.07*** (8)
Cholesterol output (µmol/h)	0.6 ± 0.06 (6)	0.6 ± 0.01 (8)	0.6 ± 0.08 (8)	0.2 ± 0.05* (6)	0.8 ± 0.13 (8)

* $p < 0.001$, ** $p < 0.01$, *** $p < 0.05$, as compared to sham group.

One month after 80 % resection, the fecal loss of bile salts was higher (64 ± 13 µmol/day v.s. 19 ± 2.9 µmol/day in control rats, $n = 10$ $p < 0.001$), and bile salts output in the bile decreased by 79 %. Cholesterol output in the bile also decreased by 60 % (table II), though cholesterol level in portal blood was normal (table I), suggesting that the cholesterol in the liver had been converted into bile salts to compensate the fecal loss. However, 5 months after 80 % resection, a new situation emerged since fecal loss of bile salts decreased (46.5 ± 12 µmol/day v.s. 64 ± 13 µmol/day, $n = 10$ $p < 0.001$), bile salts concentration and

bile salts output in the bile were almost normal (table II), and the same occurred with the cholesterol levels in serum and portal blood (table I). These results could be explained either by an increase in extrahepatic cholesterol synthesis to be converted in bile salts or by a partial restoration of entero-hepatic circulation that prevents, in part, the fecal loss. Supporting the latter view are the findings that, after 1 month, the caecal tissue weights and caecal cholic acid absorption *in vivo*, were significantly increased in resected rats (21). The caecal tissue weihgts were far higher after 5 months (1.44 ± 0.11 g in sham operated v.s. 3.85 ± 0.3 g in resected rats, $n = 10$ $p < 0.001$). Furthermore, in 80 % resected rats, bile salts levels in portal blood were higher than those in 50 % resected rats (0.28 ± 0.03 mM in 50 % resected v.s. 0.37 ± 0.02 mM in 80 % resected rats, $n = 10$ $p < 0.05$). These observations suggest that the caecum could become an important place for bile salts reabsorption, as had been previously reported (18, 21).

Resumen

Se determinan en ratas macho las variaciones en los niveles de colesterol y sales biliares tras enteroctomía distal del 50 % y del 80 % del intestino delgado. Los niveles de colesterol en suero son máximos a los 20 y 10 días de la resección del 50 % y del 80 % respectivamente. Este incremento se mantiene al mes y a los 5 meses en las ratas sometidas a la resección del 50 %, mientras que en las del 80 % los valores se normalizan. Los niveles de colesterol en bilis y sangre portal, permanecen casi normales excepto a los 5 meses de la resección del 50 % del intestino delgado distal. La concentración y excreción biliar de sales biliares disminuye al mes y 5 meses de la resección del 50 % y al mes en las del 80 %. Estos resultados junto con los datos de excreción fecal de sales biliares, indican que en las ratas sometidas a una resección distal del 50 % se alcanza un nuevo estado de equilibrio con bajos niveles de sales biliares en bilis. Por otro lado, un mes después de la resección del 80 %, la excreción fecal es tan grande, que la transformación de colesterol en sales biliares se incrementa. Los valores obtenidos en suero y en bilis 5 meses después de la resección del 80 % son casi normales, sugiriendo un aumento en la síntesis extrahepática del colesterol o una pérdida menor de sales biliares en heces que se podría explicar por el gran aumento de tamaño que adquiere el ciego tras la exclusión masiva del intestino delgado.

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