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Relative Changes in Bile Secretion During Rat Liver Regeneration

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The influences of partial hepatectomy (66 %) on some aspects of rat biliary secretion were studied at different time intervals after surgery (0, 40, 96, 192 and 384 h). Bile salt independent and bile salt dependent fractions were determined. During the first intervals (40 and 96 h) bile salt independent fraction clearly decreased after which it slowly recovered (192 h) until control levels were reached (384 h). These results are interpreted as proof of a faster compensatory hyperplasic regeneration in zone I of the hepatic acinus than in zone III.

The extraordinary power of regeneration of the rat liver is well known. It is also known (1), that the intensity and rate of regeneration are variable in the different zones of the hepatic parenchyme, and that is much greater in zone I of the acinus (periportal) than in zone III (centrilobular). Furthermore, the metabolic capacity of the hepatocytes also varies according to the zone (9) and the same occurs with the functional capacity with respect to biliary secretion (5). Thus the study of biliary secretion during liver compensatory hyperplasic period following partial hepatectomy may shed light on the mechanism and processes involved in this secretion.

Materials and Methods

A total of 75 adult Nestle rats, of both sexes, with a weight range of 200-250 g were used in the survey. The animals were divided at random into 5 groups of 15 animals; a control group (I) and four others (II, III, IV and V). All animals underwent surgery twice. In the experimental groups the first time to carry out partial hepatectomy (66 %) and the second in order to cannulate the common bile duct. The latter operation was performed at different time intervals after hepatectomy

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(group II at 40 h; III at 96 h; IV at 192 h and V at 384 h). The animals of the control group underwent the same manipulation except that the first was a sham operation.

The animals were fasted for 24 h prior to surgery, though water was provided *ad libitum*. Under light ether anesthesia the middle and left lateral lobes of the liver were removed through a medial laparotomy (8). After closing the incision as usual the animals were placed in individual cages. Twenty four hours after surgery they were given free access to food (Dieta Sanders - Mouse. Madrid).

The second operation was carried out on the animals after the aforementioned time intervals. In all cases access to food was stopped 40 h prior to surgery. The animals were anaesthetized I. p. with 20 % ethyl urethane, after which medial laparatomy was carried out, the pylorus was tied and the common bile duct was cannulated.

The cannulated animals, still under anesthesia, were placed in individual cabins with adjustable temperature control, thus affording the possibility of maintaining a rectal temperature of 38 ± 1°C throughout experiments. Bile was collected for 2 h in previously weighed plastic vials and bile flow was determined by the difference in weight. The samples were stored at —20°C until they were analized. Two hours after the collection of bile had begun the animals were killed and their livers removed for later histological controls.

Total bile salts were determined by a colorimetric method (11) and were expressed as mg/ml of cholic acid. Chloride was determined potentiometrically.

Microscopic studies were carried out on histological sections of material fixed in formaldehyde and embedded paraffin. The sections were stained with hematoxilineosine and with Masson's staining method. The material for electron microscopy was fixed in buffered glutaraldehyde, postfixed in 1 % osmiun tetroxide and embedded in epoxyresin (13). Ultra thin sections were made with a LKB ultramicrotome, were contrasted with uranyl acetate and lead citrate and examined in a Phillips EM 300 electron microscope.

The data were compared by an analysis of variance. When the analysis indicated that a significant difference existed, the means were compared by the Student «t» test. The least-Squares method was applied to determine the regression lines. The correlation coefficients were calculated and the statistical level of significance of each case was determined.

Results

Table I shows the values of bile flow and the concentration and output of bile salts and chloride on the bile of all groups. Bile flow is not significantly altered at 40

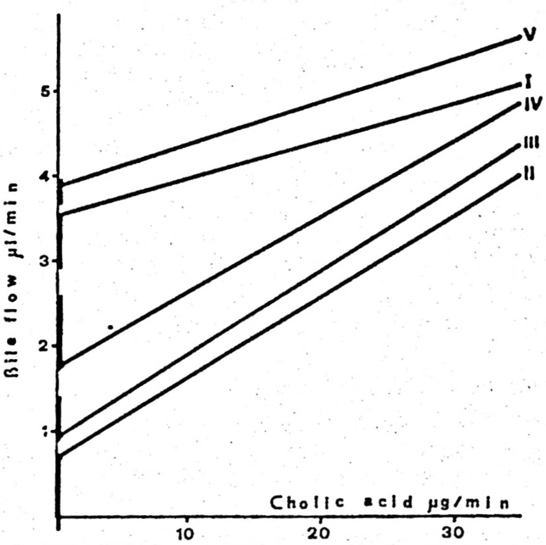

Fig. 1. *Relationship between bile flow and bile salt output in rat at different times after hepatectomy.*
I control, II 40 h, III 96 h, IV 192 h, and V 384 h. The equations of the lines are given in table II. Extrapolations to a zero bile salt output indicate BSIF. Y-intercept confidence limits (95 %) are shown.

Table I. *Effects of hepatectomy, at different times from surgery, on rat biliary secretion.*

Experimental group	Hours after hepatectomy	Number of animals	Flow (µl/min)	Cholic acid (mg/ml)	Cholic acid (µg/min)	Chloride (mEq/l)	Chloride (µEq/min)
I	Control	15	5.5±0.2	8.0±0.5	44±3	105±2	0.58±0.02
II	40	10	5.1±0.4	9.3±0.5	46±2	101±2	0.51±0.03
III	96	9	7.8±0.4*	9.1±0.2	70±3*	97±2***	0.76±0.03*
IV	192	7	6.6±0.5**	8.5±0.5	55±4	97±2***	0.64±0.05
V	384	8	6.0±0.2	7.0±0.5	43±4	96±1*	0.58±0.03

* $p < 0.001$; ** $p < 0.005$; *** $p < 0.01$.

h, though on the contrary is increased at 96 and 192 h after hepatectomy, returning to normal values after 384 h. The concentration of cholic acid did not show any significant change with the exception of a tendency to increase in the first period after hepatectomy. Cholic acid output is only increased to a significant extent at the third interval (192 h) due to the concommitant increase in flow and in concentration. Chloride concentration decreased only slightly though this was significant.

Figure 1 shows the statistical correlation between bile salt outputs and bile flows in the different groups (3). This representation makes it possible to calculate the fraction of bile flow which is independent of bile salt (BSIF).

At this point it should be pointed out that in the light of recent works revised by ERLINGER (2) it is possible that the BSIF «may be influenced by bile acids (and so not truly independent)». In this sense the use of the terms (BSIF) and (BSDF) of the bile salts described in this work are for orientative purpose but not in the quantitative sense. The corresponding numerical values are shown in table II, where for each average bile flow the regression line slope appears together with the absolute value of BSIF which is given by the y-intercept, the absolute value of the BSDF calculated by difference, and the percentage values for both fractions (4). The slope of the correlation clearly increased after hepatectomy, except for the last period. The BSIF decrease both in average and in percentage values, showing a progressive tendency towards recovery; this is in fact reached at 384 h. The behaviour of the bile salt dependent fraction is of course completely the opposite.

The most outstanding data registered in our histological study under the light microscope are the following: The sinusoid

Table II. *Relationship between flows (yi) and bile outputs (xi) in rats at different times from hepatectomy.*

m = slopes; b = $\bar{y}$ intercept (equivalent to BSIF in absolute values expressed as µl/min). n = number of pair of samples; BSIF % percentual fraction of flow independent of bile salt (BSIF % $= \frac{b \times 100}{\bar{y}}$); $\bar{y}$ = mean flow values in animals of each experimental group.

Experimental group	Hours after hepatectomy	m	b	n	$\bar{y}$	BSIF %	Significance p<
I	Control	0.043	3.56	14	5.49	65	0.05
II	40	0.095	0.68	10	5.08	13	0.05
III	96	0.098	0.92	9	7.81	12	0.01
IV	192	0.089	1.73	7	6.57	26	0.05
V	384	0.050	3.86	8	6.00	64	0.001

after hepatectomy are greater in size, reaching a maximum at 96 h and the greatest blood content at 192 and 384 h. The size of the central veins are also increased and there are no changes in the morphological aspects of canaluculi and bile ductules. As may be expected, liver regeneration is evident at all intervals, specially in the earlier ones. Examination under electron microscope revealed a clear accumulation of lipid-containing vesicles together with zymogen granules. At 96 h, tight-junction type structures are again clearly appreciable.

Discussion

In agreement with what has been reported by other authors (9), the bile flow does not decrease significantly at 40 h after hepatectomy. Bearing in mind that at that moment liver regeneration is still very incomplete, specially in fasting animals (14), it is evident that bile flow per weight unit of liver is indeed increased and that the increase in absolute values is clear and significant at 96 h. These facts could be explained according to the hypothesis put forward by KLAASSEN (9), whose results are similar: the possible commencement of activity of previously quiescent hepatocytes and the maintenance of the blood flow which might be limiting for secretion; if liver weight is reduced and blood flow is maintained, hepatic blood perfusion per gram of tissue will be increased. Indeed, our histological data suggest even greater blood contribution in these conditions.

The higher bile flow could be due to increases in the BSDF, in the BSIF, or to both. In 1974 KLAASSEN (9), suggested that according to his results in which the concentration of bile salts increased, it was possible that the dependent fraction were responsible for this. A preliminary explanation would be that since blood flow is increased, the contribution of bile salts and other metabolites to the hepatocytes is greater facilitating their extraction from plasma. Furthermore, liver regeneration is particularly intense in zone I (9) and the hepatocytes of this zone are those which are principally involved in the BSDF (5). Finally, the capacity, of osmotic exchange of the bile salts may be increased as suggested by the greater slope of the regresion lines (table II); this is in agreement with the lesser concentration of cholesterol and phospholipids in bile that have been described (9), and with the accumulation of lipids in hepatocytes observed by others (1, 12). All this suggests that a greater amount of bile salts is being secreted in a non-mycelar fashion (7).

The bile salt independent fraction decreases sharply and significantly in contrast to recently reported results (10). The discrepancy may be apparent rather than real: Firstly, these authors obtained a marked increase in BSIF after 24 h of hepatectomy, an interval which did not enter into our calculations and, in fact, the increase was much smaller at 48 and 72 h. Furthermore, if expressed as percentage these increases dissappear. Finally too, the data were calculated per gram of liver, which makes comparison difficult and is possibly the main cause for this discrepancy.

The decrease in the bile salt independent fraction might be due to an inhibition of the Na - K - ATPase dependent systems, though this seems improbable in the light of published results (10) that show such activity increasing. Another possibility could be an inhibition in the transport of HCO_3 which would be in agreement with decreases observed in the concentration of chloride (table I), since the excretion of both anions seems to be parallel (6). Nervetheless, the most immediate explanation may be derived from Bucher's hypothesis (1) in the sense that regeneration is delayed in zone III of the acinus. The hepatocytes of this zone would be te main elements for the formation of the bile salt independent fraction (5). In the last period studied by us, 384 h, when regeneration has presumably reached all zones of the acinus, not only the flow and composition of the bile return to normal, but also the balance between the two fractions is recovered.

Resumen

Se estudian los efectos de la hepatectomía parcial (66 %) sobre la fracción independiente (BSIF) y la fracción dependiente (BSDF) de las sales biliares en rata a diferentes intervalos de tiempo (0, 40, 96, 192 y 384 h).

A las 40 y 96 h la BSIF disminuye claramente y posteriormente se recupera lentamente (192 h) hasta alcanzar los valores de control (384 h). Estos resultados se interpretan como una prueba del desarrollo de una regeneración hiperplásica compensadora más rápida en la zona I del acino hepático que en la zona III.

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