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The Effect of Lymphostasis on Bile Flow and Composition in the Rat

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Summary

The ligation of the thoracic duct and of the hepatic lymph vessels leads in the rat to a decrease of bile flow and bile salt output. Bilirubin excretion was temporarily reduced both in lymphostasis and in sham operated animals, but bilirubin concentration in the bile rose progressively and after 3 hours bilirubin output returned in both groups to the control level.

Acute occlusion of the lymph vessels of an organ leads to the accumulation of protein rich fluid in the tissue spaces, increase of interstitial fluid pressure and to alterations of tissue metabolism and function (16). The obstruction of the lymphatic drainage of the liver is followed by fluid accumulation in the Disse's and Mall's spaces, interstitial oedema, widening of open intercellular junctions of the lymphatic endothelium, vacuole formation in the liver cells, distension of the bile canaliculi and alterations of the microvili. Hepatic blood flow decreases and there are biochemical alterations revealed by the accumulation of enzymes of hepatic origin in the circulating blood (2, 9, 10, 12, 13, 14, 17). The above changes in chemical composition, anatomical organisation, perfusion, etc. may lead to alterations in the function of the organ. In the present experiments the effect of acute hepatic lymphostasis was studied on bile flow and bile salt and bilirubin excretion in the rat.

Material and Methods

The experiments were done on male CFY rats with a body weight of 300 to 420 g under ether anaesthesia. The common bile duct was approached by a midline abdominal incision. The duct was divided and cannulated. Atraumatic sutures (0/4) were introduced around the hepatic lymph vessels and the hilar lymph node. A longitudinal incision was made at the left side on the base of the neck. After the collection of control bile samples the sutures around the hepatic lymph vessels and lymph node were knoted. Consequently the thoracic duct was approached: the jugular vein was pushed laterally and the carotic artery pulled upward. The left clavicle was exarticulated and by this means the arch of the thoracic duct, which lies in the rat behind and below the clavicle could be visualised. In some animals the thoracic duct could be ligated directly in others the lymph vessel could be occluded only by a bulk ligature placed around the clavicle and the cervical part of the duct. The bile was collected after the occlusion of the lymphatics in 3 one hour periods. In the sham operated animals the same protocol was adopted, except that the ligatures around the lymphatics were not tightened.

Total bile acid concentration in bile was measured by a modification of the enzymatic method of *Murphy* et al. (15). Bilirubin concentration was estimated with the diazo reagent. The statistical analyses were made with the paired *t* test.

Results

After the ligature the lymph vessels in the liver hilum, which were previously barely visible became greatly distended and filled with a clear fluid. In these animals there was a mark-

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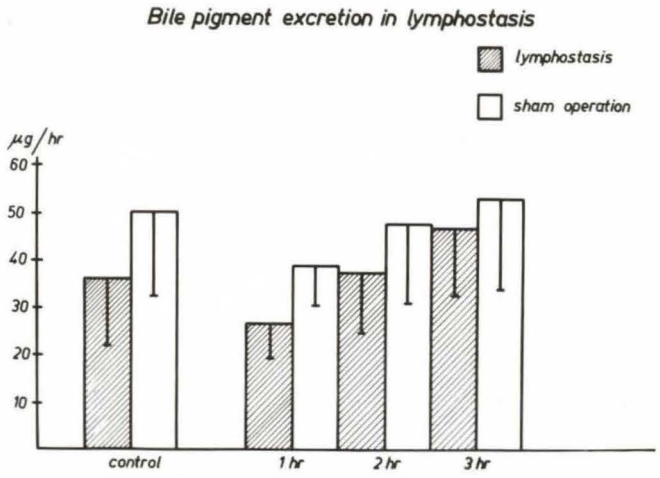

Fig. 1 Bilirubin fluxes (µg/hr) in the rat before and after ligation of the hepatic lymph vessels

ed and significant ($p < 0.01$) decrease of bile flow. In the 19 animals with lymphostasis bile output decreased in the first hour after the intervention by 40%, from 0.91 to 0.56 ml. In the 7 sham operated animals there was only a non significant 16% decrease. Bile salt concentration in the bile decreased in lymphostasis markedly. In the 3rd hour it was only 53% of the control value ($p < 0.01$). In the sham operated animals bile salt concentration was in the same period 89% of control ($p > 0.05$). On the other hand, bilirubin concentration increased both in lymphostasis and sham operated animals. In lymphostasis its biliary concentration was more than doubled, in the controls there was only a moderate 40% rise (Table 1). As a consequence bilirubin excretion, which decreased after the intervention significantly in both groups returned during the 3 hr observation period to the control value (Fig. 1). In lymphostasis bile pigment excretion was in the 3th hour even slightly higher ($p > 0.05$) than in the control period. On the other hand total bile salt excretion decreased markedly (in the 3rd hour by 72.5%) after the occlusion of the lymph vessel ($p < 0.001$). The moderate decrease of bile salt output observed in the sham operated group (34%) was on the borderline of statistical significance ($p \sim 0.05$) (Fig. 2).

Discussion

In the cat the occlusion of the lymph vessels in the liver hilum produces a marked lymph-

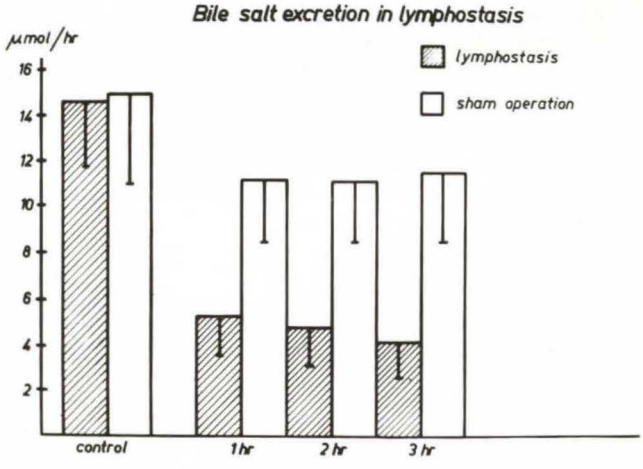

Fig. 2 Bile salt fluxes (µmol/hr) in the rat before and after ligation of the hepatic lymph vessels

Table 1 Effect of lymphostasis on bile composition and flow (N = 19)

Bile salts µMol/ml				Bilirubin µg/ml				Bile flow (ml)			
Control	1	2	3 hr	Control	1	2	3 hr	Control	1	2	3 hr
16.2±3.2	9.4±1.9	9.1±2.3	8.6±2.3	39.3±10.5	47.1±6.5	71.0±9.9	101.0±17.5	0.91±0.39	0.56±0.29	0.53±0.29	0.49±0.12
Scham operated animals (n = 7)											
14.7±3.2	12.4±2.8	12.7±2.4	13.1±2.2	49.7±11.0	45.2±13.3	59.1±21.3	69.5±10.7	0.99±0.17	0.86±0.29	0.80±0.23	0.80±0.26

oedema (2). In the rat, however, the ligation of the lymphatics accompanying the portal vein does not produce lymphostasis (5). It was shown in the dog that about 70% of the hepatic lymph is drained by the vessels around the portal vein the rest flows in the lymphatics leaving the organ with the hepatic veins and joining the thoracic duct (18). There are no quantitative data about the directions of hepatic lymph flow in the rat, but by ligating both the thoracic duct and the lymph vessels along the portal vein presumably an almost complete obstruction of the lymphatic drainage of the liver can be attained. This was confirmed by the marked distension of all visible lymphatics observed immediately after the completion of the surgical interventions. The rapid development of lymphostasis is explained by the high and constant rate of lymph formation in the liver. Consequently the changes in liver function, i.e. the decrease of bile production appear also very early, already in the first hour of lymphatic obstruction. The marked decrease in bile flow may be a consequence of decreased bile salt secretion. In the rat an apparently linear relation has been demonstrated between bile acid excretion rates and canalicular bile flow (3, 4). Considerable evidence has, however, accumulated, that as much as 60% of canalicular secretion in some species may be formed independently of bile salt transport (4, 6, 19), but in the present experiments the decrease of bile output was both in lymphostasis and in the sham operated animals nearly parallel to the decrease of bile salt secretion. Because of the rapidity of the reaction the decrease of bile acid output is probably due to some disturbance in the transport process of the bile salts across the hepatocytes and in their secretion into the canaliculi and not in the synthesis and conjugation or in the enterohepatic circulation (3, 7). Bile salt secretion seems to be somewhat depressed already by the surgical interventions for the occlusion of the lymph vessels. There is, however, a significant difference between the sham operated animals and the animals with acute lymphoedema of the liver. It can be concluded that hepatic lymphostasis reduces by some mechanism, which remains unclear bile salt secretion and consequently the rate of bile flow.

The transitory decrease in pigment excretion seems to be solely the consequence of the surgical interventions. A similar decrease was observed in the 1st hour both in lymphostasis and in the sham operated group. In both groups there is, however, a progressive increase in biliary bilirubin concentration. Consequently, bilirubin output returns in the 3rd postoperative hour to the control level.

Bilirubin glucuronide is rapidly excreted in the rat by an energy-dependent cell excretory process into the bile (8). Bile canaliculi seem to be the anatomical site of the excretion but the identity of bilirubin and bile salt excretion site does not involve a common excretory mechanism. A multiplicity of excretory mechanism was demonstrated in man (1) and in sheep (11) and in the present experiments, the differences in the reaction of lymphostasis speak again in favor of different excretory mechanisms.

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