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Bile acid pool size in hamsters during gallstone formation and after cholecystectomy

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With 2 figures

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The demonstration of a decreased bile acid pool size in gallstone patients as compared with normal controls has been interpreted in different ways. Originally, the hypothesis that the diminished pool size could be the chief causal factor in the production of lithogenic bile was universally accepted. Recent findings, however, have cast doubt on the validity of this hypothesis and led to totally different interpretations. In the present study an attempt is made to contribute to the resolution of this controversy by estimating the pool size in hamsters on lithogenic and antilithogenic diets before and after cholecystectomy.

Material and methods

Experiment I. Hamsters from our stock colony were randomly divided into four equal groups, two of which were used in the first experiment. The hamsters were individually caged and fed two different diets, i. e. commercial chow[1]) and the gallstone-inducing diet 284 respectively. For the composition of the latter diet the reader is referred to *Bergman & van der Linden* (1). The animals had free access to food and water throughout the experiment. After 7–8 days on these diets they were operated upon under Nembutal® narcosis. A midline incision was made. Through a small incision, a thin plastic tube with a small hole at the side was introduced into the duodenum. The tube was then manoeuvred so that the hole came to lie directly opposite the entrance of the common duct into the doudenum. The duodenum was ligated proximal to the tube and around the tube distal to the duct. These two ligatures were tied close to the common duct. In this way all bile leaving the duct was collected, there being only minimal contamination by duodenal secretions. Samples of bile were collected after 0.5, 1, 1.5 and 2 hrs.

Experiment II. In this experiment the two remaining groups of hamsters obtained by random division were used. The animals were cholecystectomized under Nembutal® narcosis. Ten days later they were put on the two above diets, i. e. chow and the lithogenic diet 284, the experimental conditions being the same as described in the previous experiment. After 7–8 days they were reoperated upon and bile was sampled as described above.

Bile acid analysis. Bile acids were quantitatively determined according to the method described by *Bruusgaard* (2). This method is a variation of the one originally described by *Iwata* and *Yamasaki* (3). Separation and quantitation of

[1]) Obtained from Harald Fors AB, Holmsund, Sweden

the major bile acids is achieved with a combined thin-layer chromatographic, colorimetric and enzymatic procedure.

Determination of bile acid pool size. Pool size was determined with the washout technique. *Dowling, Mark, Small & Picott* (4), using this technique in monkeys, found that a minimum plateau of bile acid secretion was reached after 4–5 hrs. In humans with an interrupted enterohepatic circulation the secretion levelled off after about 5–6 hrs (5). Our pilot studies showed that in hamsters the time required for reaching the minimum plateau was considerably shorter. In these pilot studies we interrupted the enterohepatic circulation in the same way as in experiment I. Already 1–1.5 hrs later secondary bile acids had virtually disappeared from the bile and bile salt secretion reached a lowest point after 1.5–2 hrs. As we found no systematic differences between samples collected after 1.5 or 2 hrs and later samples after 3, 4 and 5 hrs, we restricted our study to bile collected after 0.5, 1, 1.5 and 2 hrs. The average of the two last values was considered to provide an estimate of neosynthesis. The non-repleted pool size was calculated by substracting this baseline value from the values obtained for bile acid secretion after 0.5 and 1 hr. As in many cases the bile samples were rather small, pooling was necessary. On the average 2 animals contributed to each sample.

Results

The size of the bile acid pool in the four experimental groups in shown in fig. I. As seen in this figure there was a considerable decrease of the pool size after cholecystectomy, both in the group fed the lithogenic diet 284 and that fed chow. When subjected to the *Mann-Whitney* U test (6) this difference is statistically significant. Before cholecystectomy the pool size in the animals fed with gallstone-inducing diet was larger than in the controls, although this difference was not fully significant when subjected to the same test. After cholecystectomy there was no clear difference between the two groups.

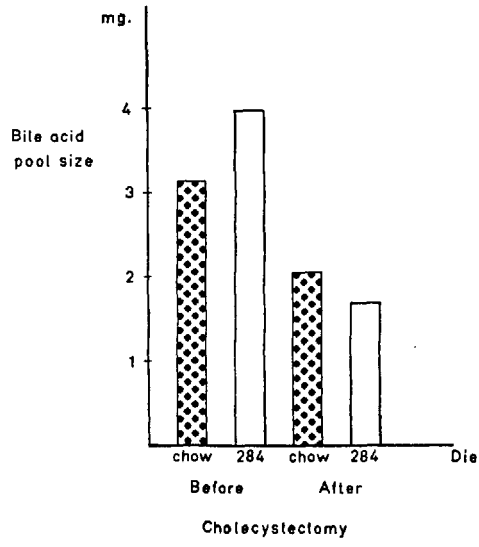

Fig. 1. Mean size of bile acid pool in hamsters fed the lithogenic diet 284 and hamsters fed chow before and after cholecystectomy.

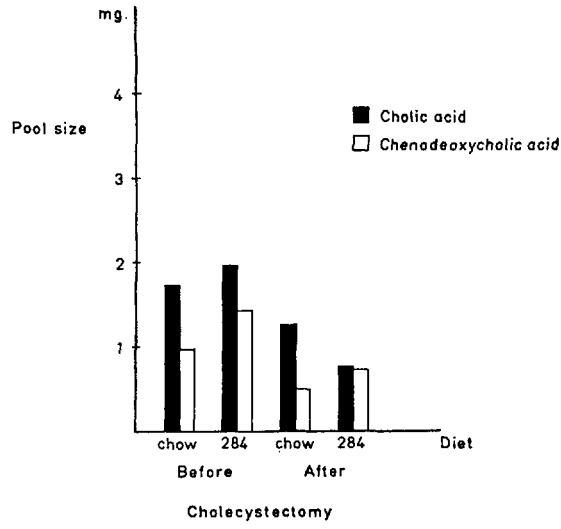

Fig. 2. Mean size of pool of cholic- and chenodeoxycholic acid in hamsters fed the lithogenic diet 284 and hamsters fed chow before and after cholecystectomy.

Fig. 2 shows the size of the pool of cholic and chenodeoxycholic acid. As seen in this figure, chenodeoxycholic acid constituted a larger part of the pool in hamsters fed a gallstone-inducing diet than in hamsters fed chow. This difference in the ratio between the pools of the two primary bile acids is not affected by cholecystectomy. In the present study it even seemed to be somewhat accentuated after the operation. The mean ratio of the pool sizes of all the animals fed the gallstone-inducing diet was 1.33 as compared with 1.97 for the animals fed chow. This difference was statistically significant.

Discussion

It was originally demonstrated by *Vlahcevic* and coworkers (7) that men with gallstone disease have a smaller total bile acid pool than control subjects without gallstones. This finding has subsequently been confirmed by others. For instance *Danzinger, Hofmann, Thistle* et al. (8) found a similar reduction in women with gallstones which was most consistent in the chenodeoxycholic acid part of the pool. Although the evidence sustaining this observation is highly convincing, opinions differ regarding its correct interpretation. Originally it was suggested that the small bile salt pool in patients with cholesterol gallstones might result in the secretion of lithogenic bile, i. e. bile with a relative excess of cholesterol over bile salt and phospholipid. The secretion of these two latter compounds is intimately interdependent, as bile salts play an obligatory role in the secretion of biliary phospholipids (9). However, the diminished pool size results in a decreased bile salt secretion only under the assumption that the number of enterohepatic circulations of bile salts is about the same in patients with gallstones and people without such stones. Some recent findings have made this assumption appear less likely. A number of studies (10, 11, 12)

have shown that after cholecystectomy the hepatic bile of gallstone patients is normalized as regards cholesterol solubility. This normalization takes place in spite of the fact that the bile acid pool does not expand (13). On this and other evidence *Low-Beer & Pomare* (14) based their intriguing hypothesis that the liver responds to changes in the enterohepatic cycling frequency of bile salts by adjusting the size of the cholate pool. In other words, the small bile salt pool found in gallstone patients and after cholecystectomy lacks significance, as there is also more frequent cycling of bile salts in these patients. *Vlahcevic, Bell* and coworkers, on the other hand (15), claim that following cholecystectomy bile remains saturated with cholesterol, and they (16) also maintain their hypothesis that a sufficiently large bile acid pool may be the essential factor in keeping cholesterol in solution.

The pathogenetic significance of the diminished bile salt pool has also been questioned on very different grounds. *Crook, Smith, McAllister* and *Mac Kay* (17) studied the effect of cholecystectomy on the size of the bile salt pool in dogs. They found that cholecystectomy resulted in a significant decrease of the pool, and they suggested that the reduced pool in gallstone patients could be due to a decrease of the capacity of the gallbladder owing to stones and subsequent inflammation. In other words, the decreased pool could be the result rather than the cause of the gallstones.

It is obvious that this problem can never be solved by the study of patients. For this purpose we would require access to two categories of people, i. e. normal controls and patients who are in the process of forming gallstones but who have not yet actually done so. These two groups would need to be studied before and after cholecystectomy. It goes without saying that normal controls are never subjected to cholecystectomy. As for patients in the process of forming gallstones, the number of studies in which patients without stones were claimed to have bile considered „lithogenic" by one criterion or other, is admittedly rather large. But in all these, often highly ingenious, studies not a single patient has been reported to have formed gallstones after his bile had been found to be „lithogenic". Now, for the acceptance of some criterion of „lithogenicity", the demonstration of at least one such patient seems to us a very modest demand indeed. As long as this unpretentious demand is not fulfilled, we do not feel justified in assuming that patients with „lithogenic" bile are actually in the process of forming gallstones. So two of the four categories of patients we would need are not accessible.

As the problem, therefore, cannot be solved by the study of patients, we have to rely on what information can be gained from an animal model. Hamsters are highly suitable, as they have been shown to rapidly form gallstones under certain dietary conditions. In the present study 4 groups were constructed. One group was given a gallstone-producing diet. This group would have formed gallstones if given enough time. One group was first cholecystectomized and then given the same diet. After cholecystectomy hamsters have been shown not to form gallstones (18). Finally one group with gallbladders and one cholecystectomized group were given a diet on which they are known never to form cholesterol gallstones. The results of our study confirm earlier observed differences in the bile acid

composition of hamsters on a lithogenic and an antilithogenic diet – more chenodeoxycholic acid in the former group (19). This difference should be taken into account when the present results are compared with those of *Robins* and *Fasulo (20)*. These authors studied only the cholate pool size and found it to be the same in hamsters fed a gallstone-inducing diet as in chow-fed controls. We found the total pool to be somewhat larger in the former group. This is due to the fact that in this group a greater part of the pool consists of chenodeoxycholic acid. Another difference between our results and those of *Robins* and *Fasulo* (20) is less easy to explain. Using an isotope dilution technique they found cholate pools of 8–9 mg as compared with total pools of 3–4 mg in the present study. Our results are, however, in better agreement with those of *Beher, Filus, Rao & Beher* (21) who in a comparative study of rodents found the bile acid pool of hamsters to be 2.3 mg.

After cholecystectomy there was a significant decrease of the pool size, both in hamsters on a lithogenic and those on an antilithogenic diet. This finding is in accordance with similar findings in dogs before and after cholecystectomy (17). Admittedly, observations on an animal model should be interpreted with great caution. Nevertheless our present results in hamsters – no diminished pool size during gallstone formation but a considerable decrease after cholecystectomy – raise the question whether any pathogenetic significance can be acribed to the diminished bile acid pool found in gallstone patients.

Summary

The size of the bile acid pool in hamsters under different dietary conditions was determined with a wash-out technique. Hamsters fed a gallstone-inducing diet had a somewhat larger pool than control animals fed chow. This difference was largely due to the smaller chenodeoxycholic acid pool in the controls. After cholecystectomy there was a significant decrease of the bile acid pool size both in hamsters fed a lithogenic diet and in chow-fed controls.

Zusammenfassung

Bei Hamstern unter verschiedenen Diätverhältnissen wurde die Größe des Gallensäurepools mittels einer Auswaschtechnik bestimmt. Hamster, die mit einer gallensteinfördernden Diät gefüttert wurden, hatten einen gegenüber den mit Normalnahrung behandelten Kontrolltieren leicht erhöhten Pool. Der Unterschied war hauptsächlich durch den kleineren Chenodeoxycholsäurepool der Kontrolltiere bedingt. Nach Cholezystektomie fand sich eine signifikante Verminderung des Gallensäurepools sowohl bei den mit gallensteinfördernden Diät gefütterten Hamstern wie auch bei den mit Normalkost gefütterten Kontrolltieren.

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