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Effect of dietary fibre on gallstone formation in hamsters*)

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With 1 figure and 2 tables

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A number of studies have shown that fibre may have an important effect on cholesterol and bile acid metabolism. In the present study we assessed the effect of different sources of dietary fibre on gallstone formation in hamsters. The compounds investigated were lignin which is present in plants as a reinforcing fibre, pectin which is found in most fruits and berries, and psyllium hydrocolloid which is derived from blond psyllium seed.

Material and Methods

Experiment I. Hamsters from our stock colony were randomly divided into four equal groups each consisting of 18 animals. The hamsters were individually caged and had free access to food and water. They were fed four different diets. The control group was given the gallstone-inducing diet 284 and the three others were fed the same diet but supplemented with 5 % W/W lignin, 5 % W/W pectin and 5 % W/W psyllium hydrocolloid respectively. For the composition of diet 284 the reader is referred to an earlier publication (1). After one month on these respective diets the surviving animals were operated upon under Nembutal® narcosis. A midline incision was made and the common duct was ligated at its distal end. About 15–20 minutes later the distended gallbladder was removed. The presence or absence of stones was noticed and the bile was collected. The concretions were classified by two independent assessors as 1) pure cholesterol stones, 2) pigmented stones, 3) mixed stones.

Slices of the liver were fixed in 10 % formalin solution, embedded in paraffin, sectioned and stained with haematoxylin-eosin and van Gieson's stain. Frozen sections of the liver were stained with Scharlach-Rot and Sudan Black B. At histological examination the assessor was unaware of the group to which the animals belonged.

Bile acids were quantitatively determined according to the method described by *Bruusgaard* (2). This method is a variation of the one described by *Iwata* and *Yamasaki* (3). Separation of the major bile acids is achieved with a combined thin-layer chromatographic, colorimetric and enzymatic procedure. Cholesterol was determined by the *Liebermann-Burchard* reaction. As most bile samples were small, pooling was necessary. As a rule two or three animals contributed to each bile sample.

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Pool size was determined in the unsupplemented group and in the group supplemented with psyllium hydrocolloid using the wash-out technique described by *Dowling, Mark, Small* and *Picott* (4).

Experiment II. Hamsters from our stock colony were randomly distributed into three equal groups each comprising 18 animals. Under similar experimental conditions as in experiment I, they were fed the gallstone provoking diet 284 without supplement and with supplements of lignin and psyllium hydrocolloid. Alcohol (95 % ethyl alcohol) was added to the drinking-water in rapidly increasing concentrations, a final concentration of 30 % being reached after 10 days. After 5 weeks the surviving animals were sacrificed and investigated as described above.

Results

Table 1 gives the numbers of surviving animals in experiment I in which gallstones were found. As seen in this table gallstones were present in 7 of 12 surviving animals in the control group. The stones in this group were either pure cholesterol or mixed stones in which cholesterol predominated. In the hamsters fed a pectin supplement similar gallstones were present in 5 of the 17 survivors. The difference between these two groups does not reach the level of significance.

In the groups fed a gallstone inducing diet supplemented with lignin and psyllium respectively none of the animals had stones. The difference between these groups and the controls is statistically significant ($p < 0.01$).

Fig. 1 gives the bile acid composition of the four dietary groups. The means of the three main bile acids are given as percentages of the total bile acids. As seen in this figure the bile acid composition of the hamsters fed a pectin supplement did not differ significantly from that of the controls. The bile of the lignin treated hamsters contained less chenodeoxycholic acid and more cholic acid than that of the controls. When the

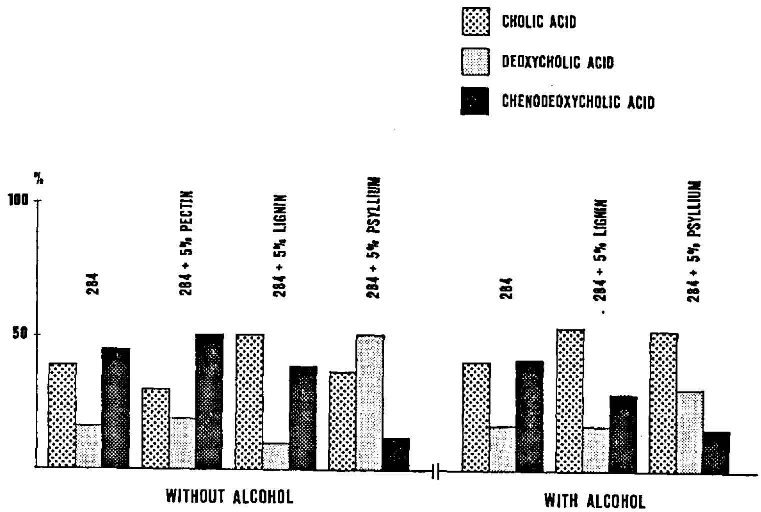

Fig. 1. Bile acid composition in hamsters fed different diets with and without alcohol in the drinking-water

Tab. 1. Gallstones in hamsters fed a gallstone-inducing diet with and without supplements. Figures denote number of animals.

Gallstones	C	P	M	Σ	No stones
Supplement					
Without	4	0	3	7	5
Pectin	3	0	2	5	12
Lignin	0	0	0	0	16
Psyllium	0	0	0	0	16

C = cholesterol stones; P = pigmented stones; M = mixed stones i. e. both C and P

cholic/chenodeoxycholic acid ratio was calculated for each bile sample this difference between these two groups was significant with the *Mann-Whitney* U test (5). Furthermore the bile of the lignin treated animals contained less deoxycholic acid than the controls and calculation of the cholic/deoxycholic acid ratio also revealed a significant difference.

When the bile acid pattern of the psyllium treated animals was compared with that of the controls there was a significant difference in the cholic/chendodeoxycholic acid ratio. This ratio was higher in the psyllium hydrocolloid treated group than in the controls. In contrast to the findings in the lignin trated hamsters the deoxycholic acid concentration was very high in the psyllium treated group. In this latter group deoxycholic acid constituted 51 percent of the total bile acids as compared with only 10 percent in the lignin treated group. The mean concentration of this bile acid was also clearly higher in the psyllium hydrocolloid group than in the controls and when the cholic/deoxycholic acid ratios were calculated for the individual bile samples and compared with those of the control group a significant difference was found with the *Mann-Whitney* U test ($p < 0.01$).

The size of the total bile acid pool was somewhat smaller in the psyllium hydrocolloid treated group than in the controls. The mean size was 2.4 mg and 2.9 mg respectively. This difference was not statistically significant.

The cholesterol concentrations showed large variations and due to these large variations and to the paucity of the available material the number of determinations was insufficient to allow for statistically valid conclusions. Still, the mean value in the pectin treated group was about the same as in the controls while lower mean values were found in the group treated with lignin and in the group treated with psyllium hydrocolloid.

The results of experiment II are given in table 2. When alcohol was added to the diet the supplements did not protect the hamsters against gallstone formation, more especially the formation of mixed stones rich in pigment. Such concrements were found in the great majority of the surviving animals. There were no significant differences between the dietary groups.

The bile acid composition of these hamsters is seen in fig. 1. With the unsupplemented gallstone inducing diet the addition of alcohol to the drinking-water did not result in a change of the bile acid pattern. In the

Tab. 2. Gallstones in hamsters fed a gallstone-inducing diet with and without supplements. 30 % alcohol in drinking-water. Figures denote number of animals.

Gallstones	C	P	M	Σ	No stones
Supplement					
Without	0	1	6	7	5
Lignin	0	3	8	11	6
Psyllium	2	0	15	17	1

group fed the lignin-supplemented diet, there was a rise of the deoxycholic acid and a comcomitant decrease of the chenodeoxycholic acid concentration. The rise of the deoxycholic acid concentration observed in the psyllium treated animals of experiment I was somewhat less marked when alcohol was added to the drinking-water. Instead of about 50 percent this bile acid constituted about 30 percent of the total bile acids. Still it was clearly higher than that of the hamsters fed the unsupplemented diet under similar conditions.

As described earlier (6) the histological examination revealed mild fatty degeneration of the liver in some of the alcohol treated hamsters both on supplemented and on unsupplemented diets. Liver histology was normal in all hamsters without alcohol in the drinking-water.

Discussion

The present study is part of the continuous search for substances which might be effective in the prevention of gallstones in hamsters. Admittedly, doubts have recently been raised regarding the suitability of the hamster as an animal model in this field of research. These doubts arose when it was found that the hamster reacted to chenodeoxycholic acid medication in a manner totally different from that of man (7). However, gallstone formation in the hamster has more similarities with that in man than any other experimental animal which hitherto has been studied. Therefore we felt justified in continuing our search for specific substances. We felt particularly justified in studying the bile acid composition for with the compounds which up to now could be tested both in the hamster and in man, analogous changes have been observed.

The study showed that, while pectin did not have a clear effect, lignin and psyllium hydrocolloid were effective in preventing the formation of cholesterol gallstones.

With both these substances there was a decrease of the mean cholesterol concentration of the bile but for reasons explained above this decrease was not statistically significant. In contrast to the similarity of the reaction of the biliary cholesterol concentration, the bile acid pattern reacted to these two substances in a dissimilar way.

With lignin we observed a decrease of the relative concentrations of chenodeoxycholic and deoxycholic acid. These changes are comparable to those observed with cholestyramine both in hamsters (8) and in man (9). But they are much less pronounced than with this latter substance. Therefore our results are not necessarily in disagreement with those of *Heaton*,

Heaton and *Barry* (10), who found no convincing effect of lignin in a study on two subjects. With cholestyramine the change of the bile acid composition is far less marked in man than it is in the hamster. As with lignin the change of the hamster's bile acid composition is much less clear than with cholestyramine, this change may in man be rather insignificant and not easily discernible in a study in only two subjects. As with cholestyramine the changes observed with lignin are explainable as due to interference with the absorption of newly formed deoxycholic acid and as due to preferential hepatic synthesis of cholic rather than chenodeoxycholic acid in a situation with excessive loss of bile salts.

The bile acid composition reacted to psyllium hydrocolloid in a totally different way. As with lignin and with cholestyramine there was an increase of the cholic/chenodeoxycholic acid ratio. However, the deoxycholic acid concentration rose which resulted in a significant decrease of the cholic/deoxycholic acid ratio. This is in agreement with recent findings in man. In T tube patients given psyllium hydrocolloid *Beher, Schuman* et al. (11) found a gradual increase of the deoxycholic acid concentration. This reaction of the bile acid pattern to psyllium hydrocolloid is much less easily explainable. It is hardly compatible with a bile acid sequestering role for the drug. Instead of increasing a sequestrant would lower the concentration of a secondary bile acid i.e. deoxycholic acid. Still, in rats the drug resulted in a decreased half life of cholate (12). Possible explanations may be sought in a change of the bacterial flora or in an affinity of the drug for cholic rather than deoxycholic acid.

The results in animals given alcohol in the drinking-water are in accordance with earlier observations (6). With alcohol, hamsters show a strong tendency to form pigmented and mixed stones. This tendency is not checked by the protection against cholesterol gallstone formation given by a dietary supplement. This is in agreement with our results with cholestyramine (7). Just as in earlier experiments in which we used a less strongly gallstone inducing diet, alcohol did not have any effect on the bile acid pattern of animals fed an unsupplemented strongly gallstone inducing diet. But, when a supplement was added there was a marked change. With lignin there was more deoxycholic and less chenodeoxycholic acid. With psyllium hydrocolloid we observed a decrease of the deoxycholic acid concentration. It is of interest to compare these findings with those we made when studying the effect of thyroxine on the bile acid composition (13). As with alcohol the effect of thyroxine was different with different diets.

When the hamsters were fed supplemented diets we found with alcohol a shift from chenodeoxycholic to cholic acid and the concomitant formation of gallstones rich in pigment. With cholestyramine a similar shift in the bile acid pattern is accompanied by prevention and dissolution of cholesterol gallstones (8). These observations confirm our impression that the formation of pigmented and of mixed stones rich in pigment follows rules which are totally different from those governing the formation of cholesterol gallstones.

As our primary interest is in cholesterol gallstone formation, let us reconsider the changes in the bile acid pattern accompanying its prevention. With the cholesterol gallstone preventing supplements lignin and psyllium hydrocolloid, there was a shift from chenodeoxycholic towards cholic acid; in addition we found with psyllium hydrocolloid a rise of the relative deoxycholic acid concentration. How these changes could affect the stability of micelles is a matter of conjecture. It has been claimed that a shift towards cholic acid would result in more stable micelles (14). On the other hand, bile rich in chenodeoxycholic acid has been shown to effect the slow dissolution of gallstones (15). Finally in *in vitro* experiments on the dissolution of human gallstones, the most effective solution was found to be unconjugated deoxycholic acid with lecithin (16).

This conflicting information reveals the paucity of our present knowledge and it underlines the necessity for further study. The interest on the effects of dietary fibre in man has hitherto centered mainly on its effect on human serum cholesterol. The results of the present study indicate that it may be worth while also to study its effect on human gallstone formation.

Summary

This study aimed at investigating the effect of different sources of dietary fibre on gallstone formation in hamsters. The substances studied were pectin, lignin and psyllium hydrocolloid. The two latter compounds protected hamsters against cholesterol gallstone formation. Lignin resulted in a decrease of the deoxycholic acid concentration and in a rise of the cholic/chenodeoxycholic acid ratio. These changes which are similar to those observed with cholestyramine suggest that lignin acts as a bile acid sequestrant. Psyllium hydrocolloid effected a similar shift of the cholic/chenodeoxycholic acid ratio but it also resulted in a rise of the deoxycholic concentration. This latter finding is not compatible with a bile acid sequestering role of this compound. The addition of alcohol to the drinking-water resulted in the formation of stones rich in pigment. Under these conditions the tendency to form such stones was not checked by either of the investigated substances.

Zusammenfassung

Das Ziel dieser Unterredung war, den Einfluß verschiedener Volumen von vergrößernden Stoffen der Diät auf die Gallensteinbildung beim Hamster zu studieren. Dabei wurden die Substanzen Pectin, Lignin und Psylliumhydrokolloid benützt. Die zwei letzteren schützten die Hamster gegen die Bildung von Cholesteringallensteinen. Lignin bewirkte eine Verminderung der Konzentration von Deoxycholsäure und eine Steigerung in der Chol-/Chenodeoxycholsäure-Konzentration. Ähnliche Veränderungen findet man bei Zufuhr von Cholestyramine, was für eine Bindung von Gallensäure bei Lignin sprechen könnte. Psylliumhydrokolloid bewirkte eine ähnliche Steigerung der Chol-/Chenodeoxycholsäure-Konzentration, aber auch eine Steigerung der Deoxycholsäurekonzentration. Der letztere Befund von Psylliumhydrokolloid ist nicht mit einer Bindung von Gallensäure zu vereinbaren. Bei Zugabe von Alkohol zum Trinkwasser wurden pigmentreiche Steine gebildet. Unter diesen Bedingungen wurde die Bildung von solchen Steinen bei keiner dieser angewandten Substanzen vermieden.

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