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Dept. of Surgery, Centrallasarettet, Östersund, and
Dept. of Pathology I, University of Umeå (Sweden)

Change in bile composition during gallstone formation in gerbils

W. van der Linden and *F. Bergman*

With 1 figure and 2 tables

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In an earlier study (1) we described the formation of cholesterol gallstones in gerbils fed certain diets. In that paper we concentrated on histological changes and due to the relative paucity of material, we were unable to present conclusive data concerning changes in bile composition. In the present study we aimed at investigating these latter changes and at comparing them with those found in other animal models.

Material and methods

Mongolian gerbils of both sexes with a mean weight of 60 g were randomly divided into two groups. One group comprising 38 animals were fed with chow*) to which 1 per cent W/W cholesterol and 0.5 per cent W/W cholic acid were added. The other 28 animals served as controls and were fed chow. All animals were individually caged and had free access to food and water. After 1 month 17 animals of the experimental group were sacrificed. After 3 months the surviving animals of the experimental group were sacrified and so were the animals of the control group. At autopsy the gallbladder was removed and bile was collected for analysis. As the bile samples were small, pooling was necessary in some cases. The number of investigated samples in the three groups (controls, 1 month exposure and 3 months exposure) were 12, 8 and 10, respectively.

Bile samples were assayed for cholesterol, bile acid, and phospholipid content. Cholesterol was determined according to *Abell* (2). Lipid phosphorus was determined according to the method of *Dryer* as described by *Henry* (3). Finally, bile acids were determined according to the method described by *Bruusgaard* (4).

Results

Table 1 gives the composition of the gallbladder bile for gerbils fed chow during three months and for those fed with chow supplemented with cholesterol and cholic acid during 1 month and 3 months respectively. The different values are given als percentage molar fractions of the total sum of bile acids and lipids. As seen in the table, after 1 month on the experimental diet there is already a considerable increase of the cholesterol molar fraction as compared with the controls. With the Mann-Whitney U test this difference is significant at the 0.01 level. The increase in cholesterol takes place at the expense of the bile acid fraction, which shows a corresponding decrease ($0.05 > P > 0.01$).

*) *Harald Fors*, Holmsund, Sweden

Table 1. Molar concentrations of three main bile components, expressed as percentages of their sum, in gallbladder bile of gerbils.

Diet and duration	Cholesterol	Phospholipids	Bile acids	N[1])
chow 3 months	4.97 ± 0.36	5.73 ± 0.63	89.30 ± 0.70	12
cholest.-cholic ac. 1 month	9.83 ± 0.74	6.77 ± 1.71	83.41 ± 2.24	8
cholest.-cholic ac. 3 months	21.86 ± 2.52	6.44 ± 0.95	71.70 ± 2.52	10

[1]) Number of bile samples analyzed.

After 3 months on the experimental diet when cholesterol gallstones start to appear, the changes in bile composition have become even more pronounced. The cholesterol fraction has now risen to very high values while the decrease in bile acids has also become significant at the 0.01 level. The phospholipid molar fraction shows a slight increase, but the differences between the one and three months values and the controls are not statistically significant.

As a result, apart from the expected fall of the phospholipid/cholesterol and bile acid/cholesterol ratios, there is also a considerable decrease in the bile acid/phospholipid ratio. This latter ratio is 17.10 for controls as compared with 15.77 after one month and 12.89 after three months on the experimental diet.

Discussion

There are at present five animal models with reliable information on the changes in bile composition which precede or accompany the forma-

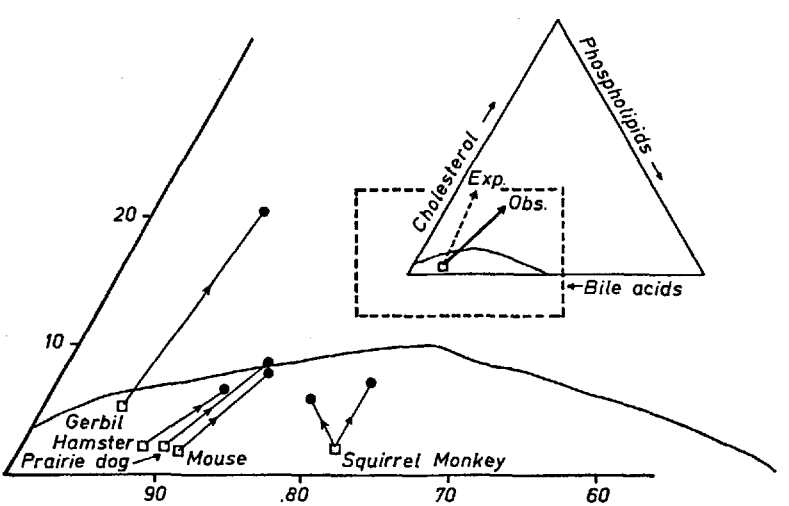

Fig. 1. Change of bile composition at induction of cholesterol gallstones in different animals. The arrows in the small triangle give the general direction of the observed changes and the direction expected on the assumption of an unaltered bile acid/phospholipid ratio.

Table 2. Phospholipid molar fraction (Lip P) and Bile acid/phospholipid ratio (BA/Lip P) under conditions of gallstone prevention and -formation in five animal models.

	Prevention			Formation		
Diet	Lip P	BA/Lip P	Animal model	Lip P	BA/Lip P	Diet
starch fat-free	7.9%	11.4	hamster (5)	12.3%	6.6	glucose fat-free
chow 72 d	10.5%	8.8	mouse (6)	10.6%	5.7	cholest.-chol. ac. 72 d
chow	21.4%	3.6	squirrel monkey (7)	18.0%	4.3	840 C
				23.6%	3.0	840 C > 9 mo
				21.6%	3.3	high butter+
				31.3%	1.9	high butter+ > 9 mo
cholest. free 14 d	9.4%	9.4	prairie dog (8)	14.3%	5.4	high cholest. 14 d
chow 3 mo	5.7%	17.1	gerbil	6.4%	12.9	cholest.-chol. ac. 3 mo

tion of stones. These changes are essentially the same. There is in all five models an increase of cholesterol, expressed as a percentage molar fraction of the total sum of bile acids and lipids. This increase takes place at the expense of the bile acid fraction. The phospholipid fraction shows a tendency towards higher values. A seen in table 2 there is only one exception to the rule i.e. the squirrel monkey on the less lithogenic diet 840 C. But, after prolonged exposure to the diet, this animal also conforms to the general rule: No decrease of the phospholipid fraction. The non-significant increase of the phospholipid fraction found in our gerbils is therefore in accordance with the upward trend of this fraction, when cholesterol gallstones are induced in animals. Also the fall of the bile acid/phospholipid ratio accompanying gallstone formation in gerbils is in agreement which the findings in other models. As we have stressed elsewhere, the information which can be derived from animal studies is for the most part highly ambiguous, but here, for once, there is no ambiguity at all (9).

In figure 1 the pathways of the five animals when they move from gallstone prevention to lithogenicity, is given in a triangular diagram. The figure shows that the general direction of the pathways deviates clearly from what would be expected under the assumption of an unaltered bile acid/phospholipid ratio.

The present study showed that in the gerbil as well as in all other animal models, hitherto described, the change in bile composition, which precedes the formation of cholesterol stones, is essentially a change in the relationship between cholesterol and bile acids. Whatever their role in the solubilization of cholesterol, phospholipids show a tendency to higher values when gallstones are induced. The study of gallstone formation in animals has given numerous examples of the pitfalls of extrapolation. Still, the unanimity of the results obtained in five different models is impressive.

Summary

Mongolian gerbils form cholesterol gallstones when fed with chow supplemented with cholesterol and cholic acid. The change in bile composition accompanying gallstone formation was found to consist of an increase of the cholesterol fraction at the expense of the bile acid fraction of the total sum of bile acids and lipids. The five animals models for gallstone formation on which reliable information regarding bile composition is now available, were compared. A similar change was found in all five models while the phospholipid fraction showed a tendency to higher values. In all five models the bile acid/phospholipid ratio decreases when gallstones are induced.

Zusammenfassung

Mongolische Gerbils bilden Cholesterolgallensteine, wenn sie mit einer Diät gefüttert werden, der Cholesterol und Cholinsäure zugefügt sind. Die Veränderung der Gallenzusammensetzung, die die Gallensteinbildung begleitet, besteht aus einer Steigerung der Cholesterolfraktion auf Kosten der Gallensäurefraktion der Summe von Gallensäuren und -lipiden. Die fünf Tiermodelle für Gallensteinbildung, über welche man jetzt zuverlässige Auskünfte hat, wurden verglichen. Alle fünf Modelle zeigten dieselbe Veränderung. Die Phospholipidfraktion zeigte eine Tendenz zu höheren Werten. In allen fünf Tiermodellen sank die Gallensäuren-/Phospholipid-Quote, wenn Gallensteine sich entwickelten.

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Authors' address:

Dept. of Pathology I, Lasarettet, Umeå, Sweden