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The relation between formation of gallstones rich in cholesterol and the solubility of cholesterol in aqueos solutions of bile salts and lecithin

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With 9 figures

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A series of studies from this laboratory deal with the composition of bladder bile from:

1. Hamsters with and without dietetically induced formation of gallstones (1, 2, 3).
2. Mice and chicks, i. e. species in which diets that are lithogenic for hamsters do not induce formation of gallstones (4, 5, 6).
3. Humans having gallstones of known cholesterol content but functioning gall-bladder (7), and
4. Humans having gastric or duodenal ulcer but normal liver and biliary tract (7).

The data determined in the bile were, besides pH, the millimolar concentrations of cholesterol, lipid-soluble phosphorus (= total phospholipids), and the individual major bile acids, the latter being determined by Sjövall's paper chromatographic methods (8, 9).

In the present paper we consider the question how far the above-mentioned data together with the limits for solubility of cholesterol in aqueous solutions of bile salts and lecithin established in our preceeding paper (10) are sufficient for explanation of the observed occurrence or non-occurrence of gallstones rich in cholesterol.

In our paper (10) the limits for solubility of cholesterol were presented in the form of curves having the molar ratio bile salts to cholesterol as abscissa and the molar ratio lecithin to cholesterol as ordinate.

Curves were presented for 100 millimolar solutions at pH 7.3 for the sodium salts of each of six different bile acids (glycodeoxycholic, taurodeoxycholic, glycochenode-oxycholic, taurochenodeoxycholic, glycholic and taurocholic) to which chromatographically purified egg lecithin was added in quantities varying from zero to approximately 80 millimoles per 100 millimoles of bile salt.

Corresponding curves valid for 50 millimolar and 200 millimolar solutions of a bile salt, sodium taurodeoxycholate, were not with certainty different from each other or from the curve established with a 100 millimolar solution of the same bile salt.

With either of two chromatographically purified egg lecithins of greatly different fatty acid pattern, the solubility curve for a 100 millimolar solution of a bile salt, sodium taurodeoxycholate, was the same.

With lecithin isolated from human bile and a 100 millimolar solution of a mixture of the six bile salts, in which the individual bile salts were distributed as in human bile, the solubility curve was practically identical with that obtained with a 100 millimolar solution of sodium glycochenodeoxycholate and chromatographically purified egg lecithin, and – what was just as important – the starting point of the curve on the horizontal axis, i. e. the number of millimoles of the bile salt mixture, about 34, required to dissolve 1 millimole of cholesterol in the absence of lecithin, could be calculated with fair approximation from the composition of the bile salt mixture and the solubility of cholesterol in solutions of the individual bile salts.

Analogous calculations of the number of millimoles of mixed bile salts required to dissolve 1 millimole of cholesterol when the molar precentages of the individual bile salts in the mixture are as in bladder bile of young hamsters on glucose diet, rice starch diet and „curative diet" (reference 1) yield results – near 35 – which are almost identical with the result calculated for a bile salt mixture corresponding to human bile – near 36.

Since the fatty acid pattern of the lecithin was found to be without influence, the curve established for a 100 millimolar solution of a bile salt mixture analogous to that characteristic of human bile is assumed to be valid also for 100 millimolar solutions of bile salt mixtures analogous to those characteristic of bladder bile from the above-mentioned groups of hamsters. Within rather wide limits, the curve is also assumed to be valid for higher and lower total concentrations of the bile salts.

Generally, it may be assumed that the solubility curve corresponding to any given mixture of the six bile salts of not too high or too low total concentration will be located between the two extremes – the curve for glycodeoxycholate and the curve for taurocholate – and that the starting point of the curve can be calculated with reasonable approximation when the composition of the bile salt mixture is known.

For a 100 millimolar solution of a bile salt mixture in which the six bile salts are distributed as in the bladder bile of hamsters on the lactose diet (reference 1) the calculated starting point of the solubility curve will be about 40. This means that the solubility curve for a bile salt mixture analogous to that characteristic of hamsters on the lactose diet will be placed between the curves for glycochenodeoxycholate and taurochenodeoxycholate (cf. fig. 1, reference 1). The reason for this slight displacement is related to the fact that the lactose diet causes a considerable decrease of the mean molar percentage of glycodeoxycholate, the bile salt having the greatest capacity for dissolving cholesterol.

Substituting – tentatively – lecithin for total phospholipids, the ratios total bile salts to cholesterol and total phospholipids to cholesterol determined in bladder bile from hamsters on the abovementioned diets can be compared with the curve indicating the limit for solubility of cholesterol in aqueous solutions of the appropriate bile salt mixture and lecithin.

The curve for mixed bile salts established in our previous paper (fig. 2 from reference 10) is reproduced in fig. 1 of the present paper, furnished, with radii indicating the ratios bile salts to lecithin equal to 1.25, 2.5, 5.0, 10.0, and 20.0 respectively.

If the single values of the ratios total bile salts to cholesterol and total phospholipids to cholesterol are plotted in the linear coordinate system used in fig. 1, the scattering of the points in the directions of the radii marked 5, 10 and 20 will be enormous. Therefore, it is more convenient to use logarithmic coordinates.

Fig. 2 shows, in logarithmic coordinates, a part of the abovementioned solubility curve and the single values of the ratios total bile salts to cholesterol and total phos-

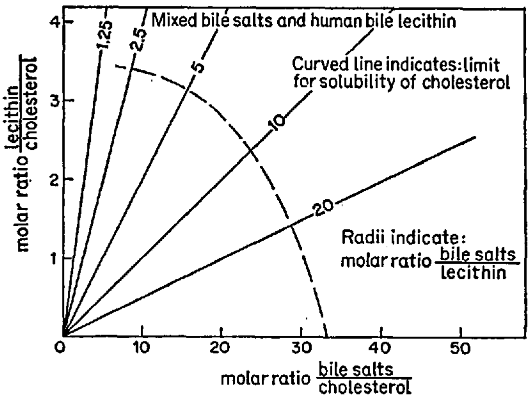

Fig. 1. Limit for solubility of cholesterol in a 100 millimolar aqueous solution of a mixture of sodium salts of the six bile acids Glycodeoxycholic (GD), Taurodeoxycholic (TD), Glycochenodeoxycholic (GCD), Taurochenodeoxycholic (TCD), Glycocholic (GC) and Taurocholic (TC), to which are added lecithin from human bile in amounts varying from zero to approximately 80 millimoles per 100 millimoles of bile salt mixture. The molar percentages of the single bile salts in the mixture correspond to those found in human bile. *Abscissa:* molar ratio of total bile salts to cholesterol. *Ordinate:* molar ratio of lecithin to cholesterol. Rectilinear (cartesian) coordinates. The figures on the radii indicate molar ratios of total bile salts to lecithin.

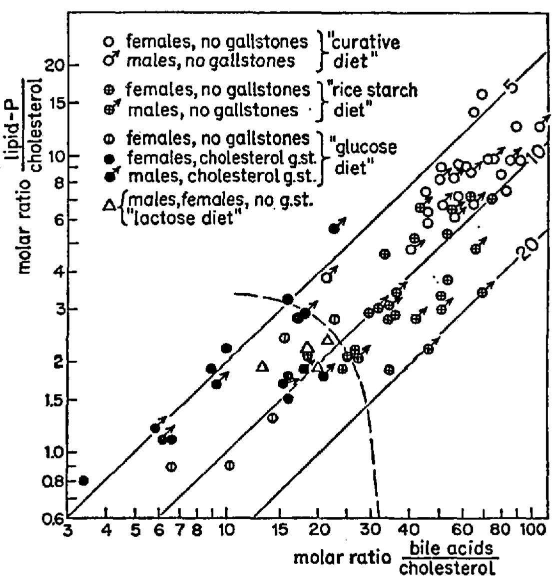

Fig. 2. Molar ratios of total bile salts to cholesterol (abscissa) and molar ratios of total phospholipids to cholesterol (ordinate) in bladder bile of *young hamsters* on glucose diet, lactose diet, „curative diet“ and rice starch diet (reference 1). For explanation of symbols see the inscribed text. The curved line represents limit for solubility of cholesterol (from fig. 1) when total phospholipids is equal to lecithin. Logarithmic coordinates.

pholipids to cholesterol for the young hamsters on glucose diet, „curative diet", rice starch diet and lactose diet (reference 1). (In the logarithmic coordinate system, parallel lines forming an angle of 45° with the axes replace the radii which in the rectilinear system indicate the ratios bile salts to lecithin.)

In fig. 2, points above or to the right of the curve represent unsaturated solutions of cholesterol whereas points below or to the left of the curve represent supersaturated solutions of cholesterol.

It is seen that, with one exception which may be due to an incorrect determination, all the points representing hamsters on the glucose diet (which produces abundant formation of cholesterol gallstones), are located below or on the curve, whereas all the points representing hamsters on the „curative diet" (which prevents formation of gallstones) are located above the curve in continuation of the „band" on which the points for the hamsters on the glucose diet are found.

The points representing hamsters on the rice starch diet (with which formation of gallstones is rare) are located above and to the right of the curve, with the exception of two points that are found to the left of the curve but very close to it. The points for the hamsters on rice starch diet are not found on a „band" in strict continuation of that on which the points for the hamsters on the glucose diet are found but tend to be displaced toward the line representing the ratio bile salts to total phospholipids = 20.

The points representing hamsters on the lactose diet are located below and to the left of the curve but not so far from it as are many of the points for hamsters on glucose diet. As already mentioned, the curve with which the points for the hamsters on lactose diet are to be compared is not exactly the same as that indicated in the figure, but one which is displaced slightly to the right of it. This further stengthens the impression that the points for hamsters on the lactose diet are located in the supersaturated region in spite of the fact that occurrence of gallstones in hamsters on lactose diet is rare.

Figs. 3 and 4 show the results of a study on bladder bile from groups of young mice given the glucose diet, the rice starch diet and a diet containing 62.3% glucose and 12% rice starch (5). Fig. 3 applies to male mice, fig. 4 to female mice.

The mice had no gallstones. The bile of mice contains almost only taurine-conjugated bile acids. The major bile acid in this species is taurocholic acid (11). Therefore, the solubility curve found for taurocholate is the one with which the single values of the ratios bile acids to cholesterol and lipid-soluble phosphorus to cholesterol shall primarily be compared. (The solubility curve from fig. 2 is also plotted in figs. 3 and 4).

It is seen that all the points are located above the solubility curve marked TC, and therefore correspond to unsaturated solutions of cholesterol in aqueous solutions of taurocholate and lecithin.

Further, it is seen that almost all the points referring to mouse bile occur in the space between the two lines indicating the ratios bile salts to phospholipids equal to 2.5 and 1.25 resp. In this region there is very little difference between the solubility curve for taurocholate and the solubility curve for the mixture of bile salts taken from fig. 2.

Fig. 5 shows the results of a study on bladder bile from groups of young chicks (cockerels) given the glucose diet, the rice starch diet, the glucose diet modified by incorporation of gelatin, and a normal commercial chicken ration (6).

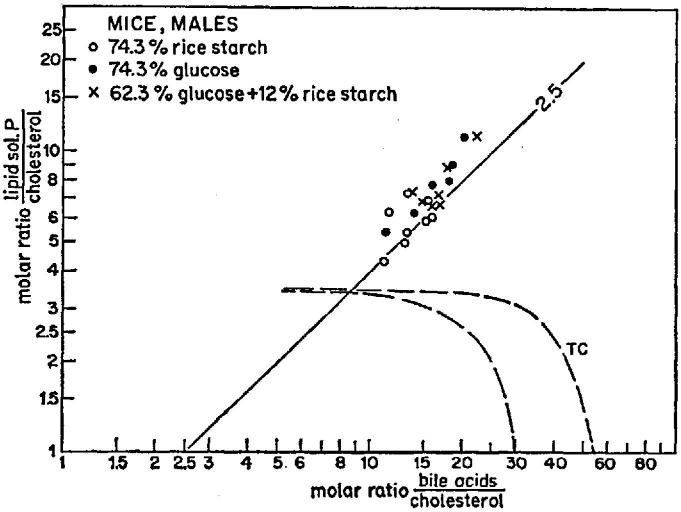

Fig. 3. Molar ratios of total bile salts to cholesterol (abscissa) and molar ratios of total phospholipids to cholesterol (ordinate), in bladder bile of *young male mice* on glucose diet, rice starch diet and a diet containing 62.3% glucose and 12% rice starch (reference 5). For explanation of symbols see the inscribed text. The curved line marked TC represents the limit for solubility of cholesterol in a solution of sodium taurocholate (100 millimolar) and varying amounts of lecithin. The curved line without marking represents the limit for solubility of cholesterol in a solution of a mixture of bile salts (100 millimolar) and lecithin (the same curve as in fig. 2). Logarithmic coordinates.

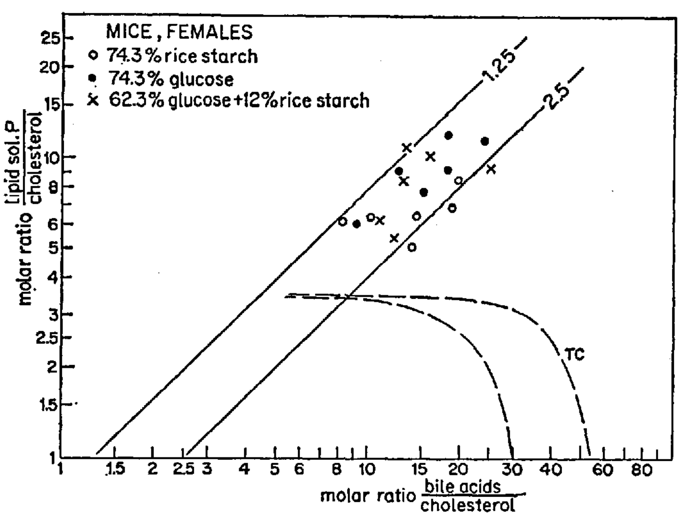

Fig. 4. *Young female mice*, same ratios and curves as in fig. 3.

The chicks had no gallstones. The bile acids of chicks are conjugated with taurine (12). The major bile acid is taurochenodeoxycholic acid; taurocholic acid and smaller amounts of other taurine-conjugated bile acids e. g. allocholic acid also occur (12). The solubility curves for cholesterol in aqueous solutions of taurochenodeoxycholate and of taurocholate (both with lecithin) are plotted in fig. 5. together with the single values of the ratios bile acids to cholesterol and phospholipids to cholesterol.

It is seen that all the points are located at a considerable distance above both solubility curves, and therefore, correspond to unsaturated solutions of cholesterol. In chicks, the bile phospholipids have been found to contain a considerable amount of phosphatidylethanolamine (14–24 mole per cent of the sum of lecithin and phosphatidyl ethanolamine) (reference 13). But, as already stated, this has not led to formation of gallstones. The scattering of the points in fig. 5 is considerable. The lowest and highest single values are 35–500 for the ratio bile acids to cholesterol, 5.2–45 for the ratio phospholipids to cholesterol, and 2.5 – about 25 for the ratio bile acids to phospholipids. Such a wide range of the last mentioned ratio has not been found for any of the other species whose bladder bile has been examined in this laboratory.

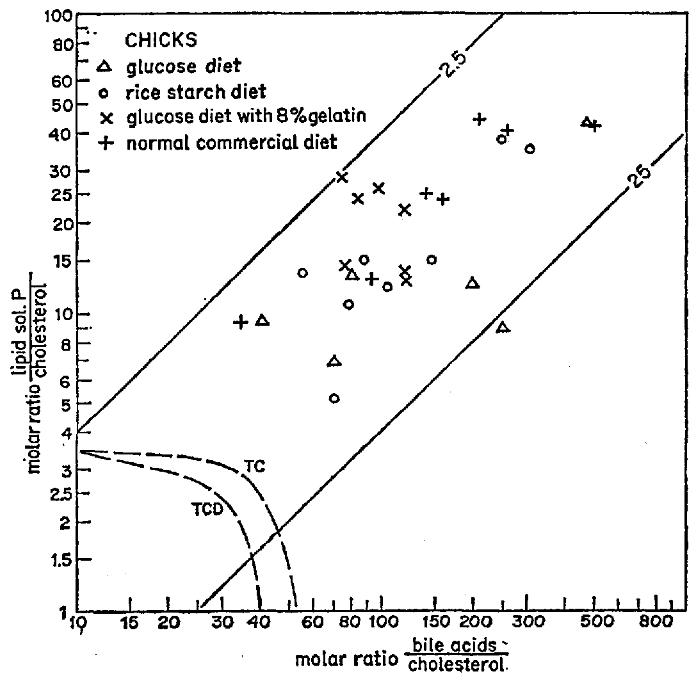

Fig. 5. Molar ratios of total bile salts to cholesterol (abscissa) and molar ratios of total phospholipids to cholesterol (ordinate) in bladder bile of *young chicks* (cockerels) on glucose diet, rice starch diet, glucose diet with 8% gelatin, and normal commercial diet (reference 6). For explanation of symbols see the inscribed text. The curved lines marked TC and TCD represent limits for solubility of cholesterol in aqueous solutions of taurocholate (100 mM) and taurochenodeoxycholate (100 mM) respectively with lecithin in varying amounts. Logarithmic coordinates.

In the absence of further evidence one might have concluded from the results described so far, that – apart from the peculiar observations made with the lactose diet – the ratios between bile salts and cholesterol and between phospholipids and cholesterol in the bladder bile, generally, are sufficient for explanation of occurrence or non-occurrence of cholesterol gallstones. The data for hamsters on diets causing formation of cholesterol gallstones were comparable to saturated or supersaturated solutions of cholesterol in aqueous solutions of bile salts and lecithin, whereas the data for hamsters on diets not causing formation cholesterol gallstones (with exception of the lactose diet and a few single values for hamsters on rice starch diet) corresponded to unsaturated solutions of cholesterol, and the same applied to the data found for mice and chicks not having gallstones. The fact that a few of the hamsters on glucose diet had no gallstones in spite of the fact that their data corresponded to supersaturated solutions of cholesterol is no matter for concern, since e. g. accidental non-retention of precipitated particles might afford an explanation.

However, analyses of bladder bile from hamsters reared on modified diets for production of cholesterol gallstones show that formation of such gallstones can occur under circumstances where the ratios bile salts to cholesterol and phospholipids to cholesterol indicate unsaturation with respect to cholesterol when judged merely by comparison with the solubility curve for cholesterol in simple aqueous solutions of bile salts and lecithin.

In the study (2) the diet for production of gallstones in young hamsters was changed by replacing the carbohydrate component (74.3% glucose) by 12% rice starch and 62.3% glucose. Thereby the prevalence of cholesterol gallstones was not materially reduced, amorphous gallstones occurred in a few of the animals, but the ratio bile salts to cholesterol and phospholipids to cholesterol were increased as compared with the results obtained with the glucose diet in the study (1). Addition of small amounts of cupric sulfate, manganous sulfate and potassium iodide seemed to enhance the ratios bile salts to cholesterol and phospholipids to cholesterol further, but did not materially interfere with the occurrence of cholesterol gallstones. This is particularly evident from the experimental series G 94, (reference 2) the results of which are plotted in fig. 6 together with the solubility curve from fig. 2.

In a later study (3) a basal diet containing 12% rice starch, 52.3% glucose and 10% butter-fat was used for production of cholesterol gallstones in young hamsters. The ratios bile salts to cholesterol and phospholipids to cholesterol from this experiment are plotted in fig. 7 together with the solubility curve from fig. 2. It is seen that cholesterol gallstones appeared even though in most cases the ratios plotted were in the region corresponding to unsaturated solutions of cholesterol when compared with the solubility curve.*)

*) In a group of hamsters in the study (2) in which 3% filtrol treated cod liver oil was incorporated into the fat-free cholesterol gallstone producing diet, a measure resulting in non-occurrence of cholesterol gallstones and appearance of amorphous gallstones in some of the animals, all the points representing the ratios bile salts to cholesterol and phospholipids to cholesterol will be on the side of the solubility curve corresponding to unsaturated solutions if plotted in the figures. The same applies to another group of hamsters in the same study, in which the animals received the stock diet during the entire feeding period and thereby avoided formation of cholesterol gallstones, and to a group of hamsters in the study (3) in which formation of cholesterol gallstones was drastically reduced by substituting the fat of a high linoleic acid margarine for butter fat.

It may be argued that the values for total bile acids in the foregoing studies are a little too low because of the existence of small amounts of bile acids which cannot be determined by the methods used. But correction of an error of this kind would only displace the points a little to the right side and that would not bring any of the points appearing in fig. 6 and 7 into the saturated or supersaturated region.

It may be argued, further, that the total amounts of phospholipids is not the same as lecithin because also other phosphatides are present in hamster bile. This argument is more relevant, since lysolecithin, sphingomyelin and small amounts of phosphatidylethanolamine have been found in bile from hamsters as well as in bile from mice ((14) and unpublished results from our laboratory.) This question will be dealt with in separate studies. (The already mentioned relatively high amount of phosphatidylethanolamine in the bile of chicks is a feature characteristic of this species, or possibly of birds in general; it does not apply to hamsters and mice).

A third objection could be that some of the bladder biles have total concentrations of bile salts below 50 millimolar for which the solubility curve drawn might not be valid. In fig. 2 ten of the points referring to hamsters on glucose diet are from samples having total concentrations of total bile salts (TBS or TBA) lower than 50 mM (the lowest being 19.5 mM). Since, however, the influence of these lower concentrations would consist in a moderate elevation of the solubility curve in the region in question (the exact magnitude of the elevation has not been determined) this would only strengthen the impression that the points are located in the saturated or supersaturated area. Only one of the points referring to the „curative diet" in fig. 1 is from a sample having a concentration of TBA lower than 50 mM (46.2), and the location of this point is such that a moderate change of the solubility curve cannot bring in into the supersaturated area. Two of the points referring to the rice starch diet are from samples having a concentration of TBA lower than 50 mM (33.2 and 41.0) but only one of these points could be brought into the supersaturated area by moderate elevation of the solubility curve.

In fig. 6, one of the points referring to bile from hamsters having cholesterol gallstones and receiving the diet without added Cu, Mn, I is from a sample having a total concentration of TBA below 50 mM (41.4 mM) but this point is already within the supersaturated area. Three of the points referring to hamsters having cholesterol gallstones and receiving the diet with added Cu, Mn, I are from samples having concentrations of TBA below 50 mM (45.5, 44.1, 39.4) but the locations of these points are such that only one of them might fall within the supersaturated area by a moderate elevation of the solubility curve in the region in question.

In fig. 7, none of the points are from samples of bile having a concentration of TBA lower than 50 mM.

Finally, it could be assumed that each bile sample should be compared with a solubility curve of its own different from the curve derived from the average composition of the bile salt mixture in the group to which the sample belongs. But in no case could the solubility curve for any of the bile salt mixtures in question be so extremely situated as the curve for taurocholate, and even if the curve for taurocholate was used as reference, seven or eight of the points referring to biles with cholesterol gallstones in fig. 6, and all the points indicating biles with such gallstones in fig. 7 would still appear as unsaturated.

Since formation of cholesterol gallstones is not compatible with unsaturation (or with largely uninterrupted unsaturation during the period of time in which animal receives the gallstone producing diet) other factors than total phospholipids and bile

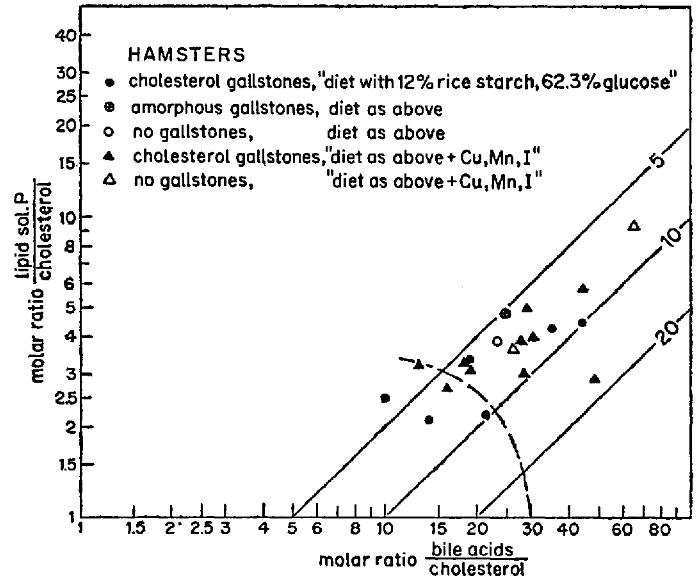

Fig. 6. Molar ratios of total bile salts to cholesterol (abscissa) and molar ratios of total phospholipids to cholesterol (ordinate) in bladder bile of *young hamsters* on diet with 62.3% glucose, 12% rice starch and no added fat, and on the same diet with addition of small amounts of cupric sulfate, manganous sulfate and potassium iodide (reference 2). For explanation of symbols see the inscribed text. The curved line represents solubility limit for cholesterol in a solution of a mixture of bile salts (100 millimolar) and lecithin (the same curve as in fig. 2). Logarithmic coordinates.

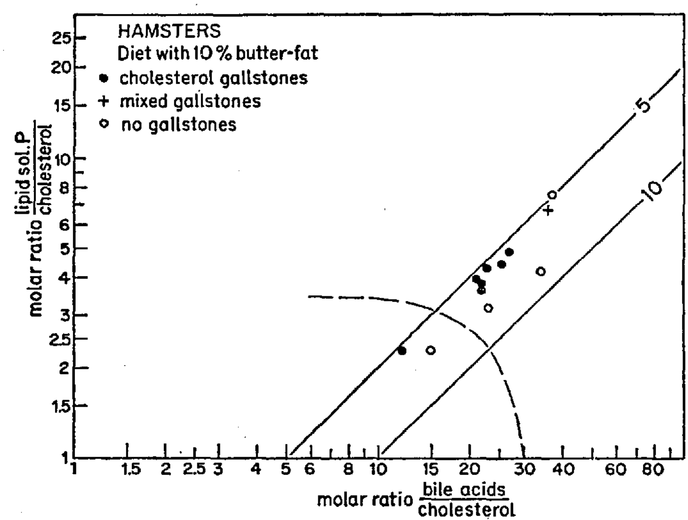

Fig. 7. Molar ratios of total bile salts to cholesterol (abscissa) and molar ratios of total phospholipids to cholesterol (ordinate) in bladder bile of *young hamsters* on diet with 52.3% glucose, 12% rice starch and 10% butter fat (reference 3). For explanation of symbols see the inscribed text. The curved line represents limit for solubility of cholesterol in a solution of a mixture of bile salts (100 millimolar) and lecithin (the same curve as in fig. 2). Logarithmic coordinates.

salts must be assumed to influence the limit for solubility of cholesterol in the bile of hamsters. (An ultimate alternative might be that all the hamsters having cholesterol gallstones in spite of apparent unsaturation were in a state of recovery from the condition during which the gallstones were formed, but this seems unlikely).

In the paper dealing with the composition of human biles (7) it was mentioned that roughly half of the bladder biles from peptic ulcer patients not having diseases of liver and biliary tract (group A) and roughly half of the bladder biles from cholelithiasis cases with functioning gall-bladder (group B) would be considered supersaturated when compared with the solubility limit for cholesterol in aqueous solutions of bile salts and lecithin. The solubility curve which is now available is plotted in the diagrams fig. 8 and 9 showing the ratios bile acids to cholesterol (TBA/C) and phospholipids to cholesterol (P/C) for the two groups of human bladder bile. It is seen that in both groups *more* than half of the points are located below the solubility curve, i. e. in the area corresponding to supersaturated solutions of cholesterol.

Thus, the absence of cholesterol-rich gallstones in humans cannot be explained merely on the basis of unsaturation with respect to cholesterol in the simplified solvent system (water, bile salts, lecithin) judged by the ratios determined. In the already discussed groups of young hamsters reared on modified diets for production of cholesterol gallstones (figs. 6 and 7), the problem was, largely, the opposite, viz. that the occurrence of cholesterol gallstones could not be explained on the basis of supersaturation with respect to cholesterol in the simplified solvent system. The possible occurence of other biliary phosphatides besides lecithin was mentioned as one of the factors worthwhile of consideration in the search for an interpretation of these results. In human bile other biliary phosphatides than lecithin occur in such low amounts that their presence can be excluded *a priori* as a factor of importance (cf. tables 4, 5 and 6 in reference 7).

Underestimation of the amount of total bile acids in the human biles due to presence of small amounts of bile acids not determinable by the methods used cannot be a factor of importance either, since correction for an error of this kind would only mean a slight displacement of the points in figs. 8 and 9 to the right side, and in the part of the diagram where the points representing human biles are found, the curve indicating the limit for solubility of cholesterol is even closer to be a horizontal line than in the region where the points representing biles from hamsters on glucose diet and „curative diet" are located.

In the group of patients having gallstones (Group B, reference 7) four had concentrations of total bile acids lower than 50 mM (38.5, 33.6, 33.6, 39.1). These were all found in the subgroup of 20 cases in which the gallstones contained between 50 and 100% cholesterol. None of the 26 patients in the whole group had concentrations of total bile acids above 200 mM.

In the group of patients not having gallstones (Group A, reference 7) none of the 27 cases had concentrations of total bile acids below 50 mM. Five had concentrations of total bile acids above 200 mM (215.3, 252.6, 218.5, 240.7, 217.6) (these were all found among the 19 cases having duodenal ulcer).

The tendency towards low concentrations of total bile acids in the group of patients having cholesterol-rich gallstones and the tendency towards high concentrations of total bile acids in the group of patients not having gallstones are reflected in the group of hamsters receiving the extremely efficient diet for production of cholesterol gallstones (74.3% glucose, no added fat) and the group of hamsters receiving the „curative diet" respectively. Thus, it seems that low concentrations of

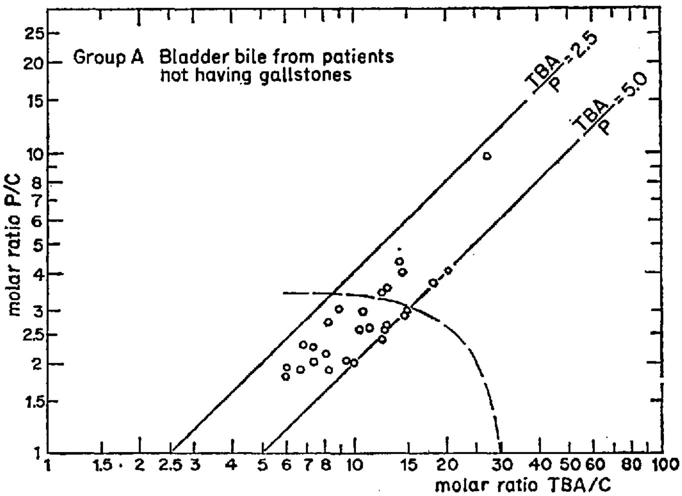

Fig. 8. Molar ratios of total bile salts to cholesterol (abscissa) and molar ratios of total phospholipids to cholesterol (ordinate) in bladder bile of 27 *patients operated for peptic ulcers and having normal liver and biliary tract* (Group A, reference 7). The curved line represents limit for solubility of cholesterol in a solution of a mixture of bile salts (100 millimolar) and lecithin (the same curve as in fig. 2). Logarithmic coordinates.

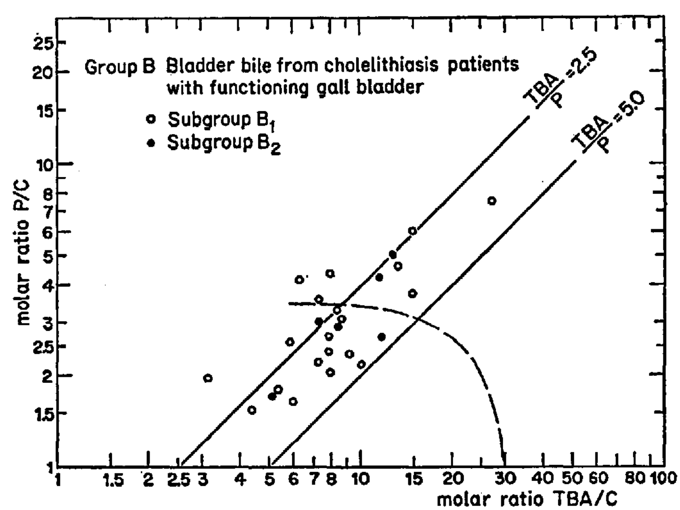

Fig. 9. Molar ratios of total bile salts to cholesterol (abscissa) and molar ratios of total phospholipids to cholesterol (ordinate) in bladder bile of 26 *cholelithiasis patients having functioning gall bladder*. (Group B, reference 7).

The gallstones from the 20 patients in subgroup B_1 contain between 54.7% and 97.5% cholesterol. The gallstones from the 6 patients in subgroup B_2 contain between 0.1% and 43.0% cholesterol. The curved line represents limit for solubility of cholesterol in a solution of a mixture of bile salts (100 millimolar) and lecithin (the same curve as in fig. 2). Logarithmic coordinates.

total bile acids is a factor giving further impetus to formation of cholesterol gallstones under circumstances where conditions for formation of such gallstones are already present.

In consequence of the results of the present study, attempts should be made to establish the identity of components which may alter the solubility of cholesterol in bile, otherwise determined by the concentrations of bile salts and lecithin, and of components which may prevent or delay precipitation of cholesterol from supersaturated biles.

Finally, it may be mentioned that if instead of the solubility limits for cholesterol determined in our study (10) we had used the solubility limits presented by NEIDERHISER and ROTH (15), half of the points referring to hamsters on glucose diet, as well as all the points referring to hamsters on lactose diet, „curative diet" and rice starch diet (fig. 2) would have been assumed to represent unsaturation. Further, all the points shown in figs. 3–7 would have been judged to represent unsaturatution, and the same would have applied to the majority of the points in each of figs. 8 and 9 (the human bladder biles). If we had used the solubility limits presented by ADMIRAND and SMALL (16), the majority of the points referring to hamsters on glucose diet, and all the points referring to hamsters on lactose diet, „curative diet", and rice starch diet (fig. 2) would have been considered to represent unsaturation. All the points in figs. 3–7 would have been judged to represent unsaturation, and the same would have applied to the majority of the points in each of the figs. 8 and 9 referring to human bladder biles.

Zusammenfassung

I.

Die in früheren Arbeiten bestimmten molaren Verhältnisse zwischen Gesamt-Gallensalzen (Gesamt-Gallensäuren, TBA) und Cholesterin (C), und zwischen lipid-löslichem Phosphor (Gesamt-Phospholipiden, P) und Cholesterin in Blasengalle von jungen Hamstern, Mäusen und Küken, die mit verschiedenen Experimental-Nahrungen gefüttert waren, wurden mit den kürzlich bestimmten Löslichkeitsgrenzen von Cholesterin in wässerigen Lösungen von geeigneten Gallensalzen, oder Gemischen von Gallensalzen, und Lecithin verglichen.

Wenn Gesamt-Phospholipide als Lecithin betrachtet wurden, entsprachen die molaren Verhältnisse TBA/C und P/C Sättigung oder Übersättigung unter Umständen, wo die Häufigkeit von Cholesterin-Gallensteinen hoch war, und Nicht-Sättigung unter Umständen, wo die Häufigkeit von Cholesterin-Gallensteinen gleich Null oder sehr niedrig war, *in den folgenden Fällen:*

1. Reichliches Vorkommen von Cholesterin-Gallensteinen bei Hamstern, deren Nahrung 74.3% Glucose, aber keinen Zusatz von Fett enthielt.
2. Nicht-Vorkommen von Cholesterin-Gallensteinen bei Hamstern, deren Nahrung 74.3% Reisstärke, aber keinen Zusatz von Fett enthielt.
3. Nicht-Vorkommen von Cholesterin-Gallensteinen bei Hamstern, deren Nahrung 28,3% gemahlenen, polierten Reis, 36% Trockenhefe, 10% Schweineschmalz und eine kleine Menge von Kupfersulfat enthielt.
4. Nicht-Vorkommen von Cholesterin-Gallensteinen bei Hamstern, deren Nahrung aus „Stock diet" bestand.
5. Seltenes Vorkommen von Cholesterin-Gallensteinen bei Hamstern, deren Nahrung 3% mit Filtrol behandelten Dorschlebertran enthielt.
6. Niedrige Häufigkeit von Cholesterin-Gallensteinen bei Hamstern, deren Nahrung 10% linolsäurereiches Margarine-Fett enthielt.
7. Nicht-Vorkommen von Cholesterin-Gallensteinen bei Mäusen und Küken, welche mit

Nahrungen, die bei Hamstern Cholesterin-Gallensteine hervorrufen, oder mit anderen Nahrungen gefüttert wurden.

In den folgenden Fällen stimmten die Verhältnisse TBA/C und P/C mit der beobachteten Häufigkeit von Cholesterin-Gallensteinen *nicht* überein:

1. Niedrige Häufigkeit von Cholesterin-Gallensteinen bei Hamstern, deren Nahrung 74.3% Lactose, aber keinen Zusatz von Fett enthielt. Hier entsprachen die Verhältnisse einer moderaten Übersättigung.
2. Vorkommen von Cholesterin-Gallensteinen bei Hamstern, deren Nahrung 62.3% Glucose, 12% Reisstärke, kleine Mengen von Cuprisulfat, Manganosulfat und Kaliumiodid, aber keinen Zusatz von Fett enthielt. Hier entsprachen die Verhältnisse Nicht-Sättigung in der Galle von der Mehrzahl der Tiere, welche Cholesterin-Gallensteine hatten.
3. Vorkommen von Cholesterin-Gallensteinen bei Hamstern, deren Nahrung 52.3% Glucose, 12% Reisstärke und 10% Butterfett enthielt. Hier entsprachen die Verhältnisse Nicht-Sättigung in der Galle von beinahe allen Tieren, welche Cholesterin-Gallensteine hatten.

II.

Die früher veröffentlichten Werte der Verhältnisse TBA/C und P/C in menschlichen Blasengallen wurden auch mit der kürzlich bestimmten Löslichkeitsgrenze von Cholesterin in wässerigen Lösungen von Gallensalzen (einem Gemisch von Gallensalzen, ähnlich dem in menschlicher Galle vorkommenden) und Lecithin (aus menschlicher Galle) verglichen. In der Mehrzahl der Fälle entsprachen die Verhältnisse Übersättigung, gleichgültig ob die Gallen von Patienten, welche cholesterinreiche Gallensteine und fungierende Gallenblase hatten, oder von *ulcus pepticum*-Patienten, deren Leber und Gallenwege normal waren, herrührten.

Summary

I.

The previously determined molar ratios between total bile salts (total bile acids, TBA) and cholesterol (C) and between lipid-soluble phosphorus (total phospholipids, P) and cholesterol, in bladder bile of young hamsters, mice and chicks reared on different experimental diets were compared with the recently established limits for solubility of cholesterol in aqueous solutions of suitable bile salts (or mixtures of bile salts) and lecithin.

When total phospholipids were considered equal to lecithin, the molar ratios TBA/C and P/C corresponded to saturation or supersaturation when the prevalence of cholesterol gallstones was high, and to unsaturation when the prevalence of cholesterol gallstones was nil or very low, *in the following cases:*

1. Abundant occurrence of cholesterol gallstones in hamsters receiving a diet containing 74.3% glucose and no added fat.
2. Absence of cholesterol gallstones in hamsters receiving a diet containing 74.3% rice starch and no added fat.
3. Absence of cholesterol gallstones in hamsters receiving a diet containing 28.3% ground polished rice, 36% dry yeast, 10% lard and a trace of cupric sulfate.
4. Absence of cholesterol gallstones in hamsters receiving stock diet.
5. Low prevalence of cholesterol gallstones in hamsters receiving a diet containing 3% filtrol treated cod liver oil.
6. Low prevalence of cholesterol gallstones in hamsters receiving a diet containing 10% of the fat from a high linoleic acid margarine.
7. Absence of cholesterol gallstones in mice and chicks receiving diets causing formation of cholesterol gallstones in hamsters, or other diets.

The molar ratios TBA/C and P/C were *not* in accordance with the prevalence of cholesterol gallstones in the following cases:

1. Low prevalence of cholesterol gallstones in hamsters receiving a diet containing 74.3% lactose and no added fat. Here the ratios corresponded to moderate supersaturation.
2. Occurrence of cholesterol gallstones in hamsters receiving a diet containing 62.3% glucose, 12% rice starch, small amounts of cupric sulfate, manganous sulfate and potassium iodide, but no added fat. Here the ratios corresponded to unsaturation in the bile from most of the animals having cholesterol gallstones.
3. Occurrence of cholesterol gallstones in hamsters receiving a diet containing 52.3% glucose, 12% rice starch and 10% butter fat. Here the ratios corresponded to unsaturation in the bile from almost all the animals having cholesterol gallstones.

II.

The previously reported values for the ratios TBA/C and P/C in human bladder biles were also compared with the recently established limits for the solubility of cholesterol in aqueous solutions of bile salts (a bile salt mixture of a composition corresponding to that of human bile) and lecithin (from human bile). Here the ratios corresponded to supersaturation in the majority of the cases whether the bile was obtained from patients having gallstones rich in cholesterol, and functioning gall bladder, or from patients having peptic ulcers and normal liver and biliary tract.

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Errata in reference 1: in table 6 under the heading GCD, line 15: instead of 19.8 read 19.0; under the heading Total bile acids, line 15: instead of 152.3 read 157.3.

Errata in reference 2: in table 6 under the heading Total bile acids, line 2: instead of 26.1 read 26.2, line 3: instead of 47.0 read 42.3.

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