Bile Acid Enteroliths; with an Account of a Recent Case

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Enteroliths consisting mainly of choleic acid have been reported on at least ten previous occasions, the first by Mörner & Sjöqvist (1908; quoted by Mörner, 1923). Mörner (1939) gives a list of cases known to him up to that time. The only case reported from this country is the one described by Raper (1921), the remainder having been found in Sweden. Chitre & Puradare (1944) described an enterolith weighing 185.5 g. removed from a Hindu woman, which was said to contain over 70 % of glycocholic acid, but no details of the analysis nor physical constants of the bile acid were given.

On 21 March 1948, Mr G. Armitage, Honorary Surgeon to the Leeds General Infirmary, removed two stones from the intestine of a woman aged 73, and submitted them to me for analysis. One of the stones was found in a duodenal diverticulum, and the other lower down the small intestine; it was thought that this too had been formed in a diverticulum, but later escaped from it and gave rise to the symptoms of intestinal obstruction which were the outstanding clinical features of the case. A more complete account of the clinical and radiological aspects of the case will be published elsewhere (Armitage, Fowweather & Johnstone, 1949).

The stones were yellowish brown, of irregular shape, with a roughness similar to that of the skin of an orange. The smaller stone weighed 26 g. with an overall length of 41 mm. and overall width of 21 mm. On sawing into halves, it was seen that there was a central zone, rectangular in section, measuring 19 × 7 mm. of a much darker colour than the rest; the demarcation line between central and outer zones was not very sharp. The larger stone weighed 29.5 g., had overall dimensions of 47 and 45 mm. and a darker central zone, oval in section, measuring 30 × 20 mm.

EXPERIMENTAL

Analysis was begun on the outer zone of the smaller stone; the inner zone appeared to differ from it only quantitatively. Later the larger stone was examined; the whole of one half was ground up and analyzed, and the central zone of the other half analyzed separately. At the centre of the larger stone was a thin irregular piece of vegetable material measuring 20 × 5 mm., thought to be a portion of a fruit skin; nothing of this kind was found within the smaller stone.

Cholesterol was tested for by the Liebermann-Burchard reaction, which was found to be negative. The Pettenkofer reaction, however, was strongly positive. The material con-

tained only a very small amount of mineral matter, was soluble in hot ethanol, and readily soluble in alkali, giving a solution which frothed readily, the froth persisting for some time. It did not melt at 100°. It was evident that the stone consisted largely of bile acid.

Lipids were removed from a weighed quantity of dry, powdered material by extraction in a Soxhlet with light petroleum. The residue was extracted with ethanol and the extract evaporated to dryness.

RESULTS

The following figures were obtained:

	A. Smaller stone, outer zone	B. Larger stone, whole of one half	C. Larger stone, inner zone
Water	1.80	0.56	0.20
Ash	0.40	0.34	0.30
Free fatty acids	1.85	2.04	13.29
Neutral fat	0.30	0.44	4.91
Ethanol extract (mainly bile acid)	92.20	93.71	74.05
Food residues, etc. (by difference)	3.45	2.91	7.25
	100.00	100.00	100.00

The ethanol extract contained a small amount of bile pigment.

The dried ethanol extracts were redissolved in ethanol and titrated with 0.1 N-NaOH, using phenolphthalein as indicator, showing the equivalent weights to be: A, 379.5; B, 382.8; C, 386.2. A further portion of the petroleum-extracted material was dissolved in ethanol, treated with animal charcoal and filtered. The filtrate was evaporated to small bulk, from which most of the dissolved material crystallized out. The crystals were filtered off, washed with ethanol and dried in a vacuum desiccator. The resulting product was entirely colourless and had the following characteristics:

	A	B	C
Melting point	185–186°	185–186°	186–187°
Equiv. wt.	378.8	377.4	382.1
$[\alpha]_D^{20}$	+48.7°	+47.5°	Not determined

Wieland & Sorge (1916) gave the melting point of choleic acid as 186–188°, and its optical rotation as +48.47°. Raper (1921) found for the recrystallized ethanol extract from his stone a melting point of 186.5–187.5° and optical rotation of +50.52°; the latter figure is higher than most other recorded figures; he stated that the molecular weight of his

product, determined by titration with 0.1 N-NaOH, was 384.9. The cholic acids normally encountered are complexes containing eight molecules of deoxycholic acid and one molecule of a higher fatty acid. Wieland & Sorge (1916) consider that natural cholic acid contains both palmitic and stearic acids. The equivalent weights of the palmitic and stearic complexes are 376.9 and 380 respectively.

The similarity in appearance and behaviour, as well as in physical constants, of the ethanol extracts from the materials *A*, *B* and *C* indicated a close similarity in composition. Moreover, the evidence obtained indicated that the extracts consisted largely of natural cholic acid, and the crystalline products obtained from them showed a high degree of purity. This was confirmed by a determination of the deoxycholic acid content of one of them by the method of Kawaguchi (1938). It showed a value of 92.3 %, while the average figure for the deoxycholic acid content of natural cholic acid obtained by Wieland & Sorge (1916) was 91.8 %.

In the cases already reported, it has been shown that cholic acid was the principal constituent of the ethanol extract of the stones examined, but little or no information has been given as to the nature and quantity of other substances present. In the present case attempts were made to obtain more precise information on this point. Bilirubin was certainly present in small amount, sufficient to give a definite yellow colour to the ethanol extracts. That some other material was also present was indicated by the fact that the mother liquor and washings after separation of the crystalline products from decolorized extracts of *A*, *B* and *C*, when added together and concentrated, yielded a crystalline product of melting point 180–182°, i.e. about 5° below the melting point of pure cholic acid. Experiments were therefore made on the whole of the ethanol extract. (Material *B* was used for the purpose.) When submitted to the Gregory & Pascoe (1929) reaction the extract gave a definitely positive result. This reaction, especially in the more recent modifications of Reinhold & Wilson (1932) and Irvin, Johnston & Kopala (1944) has been shown to be highly specific for cholic acid. Using the last-named method the amount of cholic acid in the whole ethanol extract was found to be approximately 10 %. An almost identical result was given by the method of Kawaguchi.

The molecular weight of cholic acid ($C_{24}H_{40}O_5$) is 408. Hence admixture of cholic acid with choleic acid will cause the equivalent weight to be higher than for pure choleic acid, and the equivalent weights of the total extracts have, in fact, been shown to be higher than for the crystallized products; a mixture containing 90% of choleic acid and 10 % of cholic acid would have an equivalent weight of 382. It is unlikely that the conjugated cholic acids, glycocholic

and taurocholic acids, are present to any appreciable extent, for their molecular weights are 465 and 515 respectively. Even small amounts would therefore have a considerable effect on the equivalent weights.

It may reasonably be accepted, therefore, that the total ethanol extract of the stones consisted of bile acid together with a small amount of bile pigment, and that the bile acid contained approximately 90 % of choleic acid and 10 % of cholic acid; the conjugated bile acids were not present to any appreciable extent.

In bile much the greater proportion of the bile acids is present in the conjugated form, and as salts (mostly sodium), while in the enteroliths they are present as free acids, and unconjugated. An experiment to determine the pH at which certain bile acids are precipitated from solutions of their sodium salts gave the following results:

Bile acid	pH at which precipitation occurred
Choleic acid	7.0
Cholic acid	5.8–6.0
Glycocholic acid	4.6–4.8

Josephson (1933) performed somewhat similar experiments with a number of bile acids with similar results. He found the dissociation constants of deoxycholic acid, cholic acid and glycocholic acid to be 3.8×10^{-7} , 64.6×10^{-7} and 355×10^{-7} respectively; for the tauro acids the constants are higher than for the glyco acids.

DISCUSSION

True enteroliths of any kind are rare. The ease with which many foreign bodies pass through the normal alimentary canal makes it obvious that for enteroliths to form and grow in size some mechanical condition must be present which allows the retention of concretions within the intestine in their early stages. In almost all the reported cases such a mechanical condition was noted; in some the stones were obviously related to the presence of diverticula, in a few to stricture. The presence of foreign bodies is also likely to be favourable to stone formation since their presence will cause stasis (an important factor in stone formation) either in a diverticulum or in the lumen of the intestine, and they will also act as a nucleus around which solid material may be deposited. Helström (1936) found a plum stone at the centre of a choleic acid enterolith. Raper (1921) found tissue resembling fruit skin; similar material was found in the larger of the two stones in the present case, and Chitre & Puradare (1944) found a tamarind seed.

There must be in addition special conditions which determine the formation of bile-acid stones. With one exception the reported cases already referred to have been choleic acid stones, and the exception, that of Chitre & Puradare (1944), is not sufficiently well

documented to place the identification of the bile acid concerned entirely beyond doubt. This finding at first seems surprising since the conjugated bile acids are normally present in much greater concentration than choleic acid. McGee & Hastings (1942), in experiments on human subjects, found that the pH of the upper part of the small intestine was between 6.2 and 6.8 except in a few isolated instances. The experiment already described shows that choleic acid is the only one of those examined which will be precipitated within this range, and thus offers a convincing explanation why choleic acid is precipitated in preference to other bile acids. It is probable that in some cases the acidity in this region exceeds the normal (e.g. where the amount of hydrochloric acid discharged from the stomach into the duodenum is greater than usual as in conditions associated with hyperchlorhydria), and may reach a degree of acidity sufficient to cause precipitation of cholic acid also, but the attainment of an acidity sufficient to precipitate glyco or tauro acids is very much less likely.

The formation of stones within the intestine would appear to require the precipitation of the material concerned in appreciable quantity, yet it is believed that unconjugated bile acids are present in bile in quite small amounts. Doubilet (1936) gives some results of 'typical examples' of differential bile acid analysis; these show, in human gall-bladder bile, 0.890 % of unconjugated acids, out of a total of 9.751 %, and in human fistula bile 0.413 % out of a total of 1.717 %. Not all of the unconjugated acids is deoxycholic acid, capable of combination with the free fatty acid in bile or in the intestine to form choleic acid. I have not seen any separate figures for unconjugated cholic and deoxycholic acid in bile, but it is generally believed that the total cholic acid exceeds the total deoxycholic acid. Wieland & Revery (1924), who noted the presence of chenodeoxycholic acid in human bile, gave the proportions of cholic:deoxycholic:chenodeoxycholic acid as 3:1:1. Doubilet's (1936) figures for human gall-bladder bile show 4.255 % of cholic acid and 5.496 % of deoxycholic acid; he makes no mention of chenodeoxycholic acid, but, if any were present, it would be included in the figure for deoxycholic acid. (Here it may be noted that Nagaki & Ohya (quoted by Saba, 1940) claim that chenodeoxycholic acid is often found to predominate in human bile.) Figures for deoxycholic acid therefore, in cases in which examination for, or determination of, chenodeoxycholic acid is not made, may well be too high. Chenodeoxycholic acid, like cholic acid, has little tendency to form co-ordination compounds (Sobotka, 1937*a*); hence its presence will not result in the formation of choleic acid. If, then, the proportion of the unconjugated deoxycholic acid in bile bears any relation to that of the total deoxycholic acid, the

amount of the former (and therefore of choleic acid) is normally very small; it is in fact so small as to indicate that deposition and retention within the intestine, in quantities sufficient to form the enteroliths which have been described, is unlikely to occur. Raper (1921) suggested that the material first deposited was glycocholeic acid and that glycine was subsequently split off from this by bacterial action. There are, however, three difficulties in accepting this explanation. First, there is not, according to Sobotka, any real evidence that the conjugated bile acids form choleic acids. Secondly, the evidence for cleavage of conjugation in the manner suggested is conflicting (for references see Sobotka, 1937*a*); and lastly the pH required for the deposition of the free conjugated acids is definitely lower than that found in the intestine.

There are, however, other and more likely explanations why in some cases the choleic acid of the bile may be higher than is suggested by the figures already quoted. In the first place, the number of published differential bile acid analyses of human bile is small, and they give no indication of the variations which might occur normally in the amount and proportion of the different acids; in this connexion it has also to be remembered that the methods of analysis available for this purpose leave much to be desired. In the second place, there is some evidence that the proportion of conjugated to unconjugated bile acid, and of cholic acid to deoxycholic acid, etc., may be altered by disease. The liver is presumably the site of conjugation, and an increase in unconjugated bile acid has been noted in disease of that organ. (For references see Sobotka, 1937*b*.) An increase in the proportion of deoxycholic acid to cholic acid has also been noted, mainly in pathological conditions, but Sobotka thinks this is more likely to be due to a decrease in production of cholic acid than an increase of deoxycholic acid. Disease of the gall bladder, by altering its capacity to absorb various substances from bile, may also alter the bile acid composition of this fluid.

It will be seen, therefore, that the formation of choleic acid stones requires the concurrence of a number of factors, viz. a mechanical condition capable of allowing the retention of precipitated acid, bile which contains more deoxycholic acid than is usually present, and, also, bile which contains a considerable proportion of deoxycholic acid in the unconjugated form; whether these bile changes represent the extreme of normal variation, or are the result of disease cannot be decided on present knowledge of the bile acid composition of bile. Hyperchlorhydria may be an additional factor. The presence of retained indigestible matter in the intestine is also of some importance.

One other fact has not yet been mentioned, namely that all the reported cases have occurred in women.

Why this should be so is not clear, and can only be a matter for speculation. The fact that the production of choleic acid enteroliths requires the concurrence of a number of factors explains their rarity. Nevertheless, certain considerations suggest that they may not be so rare as the small number of reported cases would indicate. Of the ten previously reported cases, five have been recorded by one man (Mörner, 1939) and three by another (Helström, 1936). That one keen observer should have identified half the known cases does seem to show that others must have been missed; there is in fact much evidence in the literature that some enteroliths have not been examined at all, while some have received insufficient examination to identify their constituents with certainty. Any real knowledge of the incidence of bile-acid enteroliths—or indeed of any type of enterolith—can only be obtained if all stones found in the intestine are submitted to careful chemical analysis.

SUMMARY

1. Two enteroliths removed from the intestine of a woman have been analyzed, and it has been shown that they contained over 90% of bile acids. Of these

acids approximately 90% was choleic acid, and 10% was cholic acid.

2. The pH at which certain bile acids are precipitated from solutions of their salts has been determined, and it has been shown that, of the bile acids usually present, choleic acid is much the most likely one to be precipitated at the reaction normally found at the site of formation of these enteroliths. This is believed to explain why all the bile acid enteroliths so far reported, except one, consist mainly of choleic acid.

3. The factors which might be responsible for the formation of choleic acid enteroliths have been discussed and some deficiencies in our knowledge of the bile acid composition of human bile have been pointed out.

4. While bile acid enteroliths are undoubtedly of rare occurrence, it is considered that they are probably commoner than the number of reported cases indicates. This is believed to be due to insufficient examination of intestinal stones in some cases, and lack of any examination in others. Careful chemical examination of all intestinal stones is recommended.

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The Bence-Jones Protein of Multiple Myelomatosis: its Methionine Content and its Possible Significance in Relation to the Aetiology of the Disease

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Few chemical investigations have been made on Bence-Jones protein in recent years. Reports on the methionine content of the protein are particularly scanty since, at the time of the earlier work (Hopkins & Savory, 1911; Abderhalden & Rostoski, 1905), this amino-acid was unknown. The values reported for methionine (Calvery & Freyberg, 1935; Devine, 1941; Harvier & Rangier, 1943) show considerable

variation, but agree upon a low figure. As a patient with multiple myelomatosis, who was excreting large quantities of Bence-Jones protein, had come to our notice, it was decided to investigate again the content of methionine and other amino-acids. The quantities excreted by the patient permitted purity tests on the intact protein, and the use of five independent methods for determining the methionine content.