VI. A HUMAN ENTEROLITH CONTAINING CHOLEIC ACID.

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THE enteroliths occurring in man which have been described previously have consisted largely of inorganic material, principally ammonio-magnesium phosphate, calcium phosphate and calcium carbonate. Concretions in the alimentary tract may also occur, however, which contain notable quantities of calcium or magnesium soaps [Williams, 1907], and yet others which contain gall stones as a nucleus.

The enterolith described in the present paper was found by Mr James Phillips, Honorary Surgeon to the Bradford Royal Infirmary, in a case operated on for intestinal obstruction and he kindly allowed me to examine it. The stone was found about the middle of the jejunum and was the cause of the obstruction. In the ordinary way it might have been mistaken for a gall stone but was more friable than gall stones usually are. It weighed 6.8 g., was about the size of a walnut and on section had no definite nucleus. When ground up it had a yellowish sand colour. From the ground material two small pieces of tissue resembling fruit skin were separated. On heating a small portion on platinum foil the material was seen to be almost entirely organic, only a trace of ash being left. An actual estimation of ash showed that only 1.19 % was present.

A preliminary extraction with solvents showed that a portion was easily soluble in ether and light petroleum; another portion was slightly soluble in ether, more so in acetone and very soluble in alcohol. After removing all the soluble matter with ether and alcohol a pigmented residue was left which gave bile pigment reactions and contained food debris (starch and protein colour reactions). A quantitative examination by extraction of a weighed amount of the material in a Soxhlet apparatus was next carried out in order to determine the amount of the various fractions. The amount of material used for the extractions was 1.8896 g.

Ether Extract. This extraction was not carried to completion but allowed to proceed until it was judged that all the easily soluble substance had been removed. After distilling off the ether the residue was extracted several times

with hot light petroleum to remove fatty acids, fat and cholesterol-like substances. The residue from the petroleum extract weighed 0.3094 g. and represented 16.37 % of the original material. It was dissolved in alcohol, the fatty acids neutralised with sodium hydroxide and the alcoholic solution diluted with water until it contained about 50 % alcohol. The fat and unsaponifiable matter were then extracted by light petroleum and weighed. 0.0561 g. was obtained, which is equal to 5.52 % of the original material. From the alcoholic solution of the soaps the fatty acids were recovered and their iodine value determined. 0.2233 g. absorbed 0.1022 g. I; iodine value, 45.8. That part of the ether extract which was insoluble in light petroleum was easily soluble in alcohol and was therefore added to the next extract, which was obtained by continuing the extraction in the Soxhlet apparatus with alcohol. The total alcohol-soluble substance thus obtained weighed 1.37 g. and represented 72.5 % of the original material. Its further examination is described later. The residue from the enterolith which was insoluble in alcohol or ether weighed 0.16 g., and formed therefore 8.4 % of the whole. It gave the usual colour reactions for proteins and on extracting with boiling water a solution was obtained which gave a good reaction for starch. On extracting some of the material with acid alcohol and applying Cole's modification of Huppert's test a strongly positive reaction for bile pigments was obtained. This residue therefore consisted of food debris mixed with bile pigment.

Alcohol Extract. As indicated above, this formed the main portion of the enterolith. It was insoluble in water, easily soluble in hot alcohol and moderately soluble in hot acetone. It crystallised easily from hot aqueous alcohol (75 %), and after recrystallisation melted at 185°–186° (corr.). It was decolorised by boiling with charcoal and by further recrystallisation was obtained in colourless rosettes of prisms melting at 186.5°–187.5°. This melting point did not change on further recrystallisation. The substance did not contain nitrogen, it was an acid, since it dissolved easily in aqueous alkali and it gave Pettenkofer's reaction for bile salts. It was therefore suspected to be an unconjugated bile acid. Further investigation confirmed this view and it was identified as choleic acid. The melting point of choleic acid is usually given as 185°–187° [Schryver, 1912; Fischer and Meyer, 1911; Wieland and Sorge, 1916], whereas that found in the present instance was slightly higher and more sharp. To confirm the identity of the acid a mixed melting point was determined. Dr Schryver kindly furnished me with a specimen of choleic acid prepared by him from ox bile and a portion of this, after recrystallisation until its M.P. was constant, was mixed with an equal amount of the acid from the enterolith. The two were fused, the mass ground up and the M.P. of the mixture determined. It was 186°–187°. The two acids were therefore identical.

The optical activity was determined in a 2.316 % solution in absolute alcohol. Length of tube 1 dcm., temp. 20°, rotation observed + 1.17° hence

$[\alpha]_D^{20} = + 50.52^\circ$. The values previously recorded for choleic acid are slightly lower than this, the highest being $+ 48.72^\circ$.

On combustion the following results were obtained:

	Found	Calc. for $C_{24}H_{40}O_4$	Calc. for $C_{24}H_{42}O_4$
C	72.92, 73.28	73.47	73.10
H	10.53, 10.78	10.20	10.66

Fischer and Meyer [1911] give $C_{24}H_{40}O_4$ as the formula for choleic acid but the analytical figures obtained in this case agree better with the formula $C_{24}H_{42}O_4$ as also do those of Fischer and Meyer.

The molecular weight was determined by titration with $N/10$ NaOH, the acid being dissolved in neutral alcohol, and was found to be 384.9. Calculated for $C_{24}H_{42}O_4$, 394.

According to Wieland choleic acid is a compound of eight molecules of desoxycholic acid and one molecule of a higher fatty acid. The figures obtained in the combustion analysis are not incompatible with this view except that the carbon figure is somewhat low, neither is the value obtained for the specific rotation.

The acid was tasteless like that isolated from ox gall stones by Fischer and Meyer. It was also observed that the choleic acid sent by Dr Schryver, when quite pure was also tasteless. Some observers have described choleic acid as having a bitter taste but small traces of cholic acid might have been responsible for this.

There appears to be no doubt that the acid from the enterolith was choleic acid. Being unassociated with other bile acids in any considerable quantity it was easily purified and probably on this account had a slightly higher melting point and specific rotation than other workers have observed.

After the choleic acid had been crystallised out, an examination of the mother liquors was made in order to determine whether desoxycholic acid was present. On evaporation of the mother liquor and crystallisation of the residue from glacial acetic acid a very small amount of an acid, M.P. 139.5° – 142.5° , was obtained. Desoxycholic acid melts at 145° and according to Wieland and Sorge [1916] choleic acid when heated with glacial acetic acid forms a compound melting at 144° – 146° ; it was not possible therefore with the small amount of acid obtained to establish its identity.

It may be concluded therefore that the enterolith had the following composition:

Fatty acids	...	...	...	...	...	10.85 %
Neutral fat and unsaponifiable substances	...	...	...	...	...	5.52 "
Unconjugated bile acids (almost wholly choleic acid)	...	...	...	...	...	72.5 "
Food residues	...	...	...	...	...	8.4 "

It was found later that the simplest method of getting the choleic acid from the calculus was to extract the latter with acetone in a Soxhlet apparatus. The acetone extract, after removal of the acetone, was boiled with light petroleum to remove fatty acids and fat. There was then left behind a residue,

insoluble in light petroleum, consisting largely of the bile acid. This was found to be practically pure after a single crystallisation from aqueous alcohol.

I believe this is the first recorded instance of the occurrence of an intestinal calculus composed largely of one of the unconjugated bile acids. Choleic acid has been detected in ox gall stones by Fischer and Meyer, but, so far as I am aware, it has not been found in the ordinary cholesterol stones of man. There is no evidence in the present case that the enterolith originated in the gall bladder. Although cholesterol was not definitely isolated from it the amount present must have been very small since the unsaponifiable matter soluble in light petroleum was only 5 %. Further, the bile acid found was unconjugated and had probably arisen from the conjugated acid by bacterial action in the small intestine. Since cholic, choleic and desoxycholic acids are all relatively insoluble in water it is difficult to understand how preferential deposition of one of these acids rather than the other two should take place unless it be assumed that the acid found was the only one being formed by the liver. The existing analyses of human bile from various individuals do not furnish any ground for such an assumption and it certainly seems very improbable. What appears more likely in the present case is that glycocholeic acid, which is more insoluble than glycocholic acid, was deposited in the first instance and subsequently decomposed by bacteria giving rise to choleic acid.

Cases of intestinal obstruction due to gall stones which have ulcerated through the gall bladder into the small intestine are well known. It seems possible however, as pointed out by Phillips [1921], that some of the cases of intestinal obstruction supposedly due to gall stones may be due to deposits of choleic acid similar to that described in this paper. In appearance, such deposits are not unlike gall stones.

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