# Fatty Acid Composition of Feces and Fecaliths.* (26052)

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The presence of appendiceal concretions, or fecaliths, is an important factor in the genesis of acute distinctive appendicitis(1,2). Early reports of appendicitis often refer to the presence of a fecalith or a foreign body (3,4). The fact that many fecaliths were mistaken for fruit stones led to the popular misconception that fruit stones were the cause of appendicitis. As early as 1813, the fact that fecaliths contained lipid material was noted by Wegeler(5). The first detailed study of fecalith composition was by Williams(6), who considered that they were formed largely of calcium soaps of palmitic and stearic acids. Maver and Wells(7) found 50% of the dry weight of fecaliths to be soluble in ether or hot amyl alcohol. They concluded that there was a high proportion of lipids present as insoluble soaps. Bowers(2) compared the composition of fecaliths and fecal pellets and concluded that the former were of a specific composition, and were not simply inspissated feces. He suggested that fecaliths were formed in the lumen of the appendix by a deposition of layers upon a central nucleus of undigested material.

Whether the material that goes to form the layers of a fecalith comes from the feces or is excreted by the appendix is not known. However, Williams suggested that fatty acids are excreted by the wall of the appendix in the form of calcium soaps(8). Sperry *et al.* (9,10) showed that fatty acids, largely palmitic and stearic acids, are excreted by the intestinal mucosa in the dog. If the pattern of fatty acids in fecaliths is the same as that of the acids excreted by the intestinal mucosa and different from that of fecal fatty acids, Williams' theory of appendiceal fatty acid excretion would be supported.

The purpose of this study was to compare the relative proportions, in fecalith and fecal lipids, of the 4 fatty acids most abundant in nature and the human body, namely palmitic, stearic, oleic and linoleic acids, to determine the types of lipids present in fecaliths and feces and to make some comparisons.

*Materials.* A total of 52 fecaliths, stored in 10% formalin solution, were obtained from the collection made by the University Hospital during the past 5 years. Feces were obtained from normal adults, with no symptoms of bowel disturbance, who were on an "average" North American diet.

*Methods.* Methyl esters of fatty acids were prepared from a petroleum ether extract of 6 groups of fecaliths, and from 5 fecal samples. It was initially assumed from previous reports(6,7,8) that the fatty acids would be present largely in the form of soaps. The samples, therefore, were homogenized and acidified with HCl (pH 1) to liberate free acids. Lipids were extracted with petroleum ether (b.p. 35-45°C) and after removal of the solvent, saponified with excess ethanolic potasium hydroxide. The non-saponifiable material was removed by ether extraction of the hydrolysate, the aqueous phase was acidified and the fatty acids extracted with ether. These acids were converted to methyl esters with methanolic hydrogen chloride. In addition, 3 fresh fecalith samples and 3 samples of feces were extracted with chloroform-methanol (2:1, v/v) for analysis of their total lipids.

It is appreciated that all the lipids would not have been extracted from the first group of fecalith and fecal samples using petroleum ether. However detailed analysis of the fatty acid content of the various fractions of a total lipid extract has since shown that the relative proportion of the 4 fatty acids under consideration is not significantly altered by excluding the lipids insoluble in petroleum ether. This analysis will be reported later.

*Gas-liquid chromatography* (*GLC*) was carried out with a 6 ft. x ¼ in. I.D., U-

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TABLE I. Relative Proportions of 4 Major Naturally Occurring Fatty Acids in Fecaliths and Feces as Determined by GLC and Expressed as % of Their Total Area.

| | Fecalith samples | | | | | | | Fecal samples | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acids | 1 | 2 | 3 | 4 | 5 | 6 | Mean | 1 | 2 | 3 | 4 | 5 | Mean |
| Palmitic | 39 | 42 | 36 | 44 | 45 | 43 | 41 | 26 | 26 | 29 | 27 | 17 | 25 |
| Stearic | 53 | 46 | 55 | 47 | 45 | 41 | 48 | 31 | 26 | 33 | 26 | 44 | 32 |
| Oleic | 8 | 12 | 9 | 9 | 10 | 16 | 11 | 35 | 36 | 31 | 33 | 33 | 33.5 |
| Linoleic | — | — | — | — | — | — | — | 8 | 12 | 7 | 14 | 6 | 9.5 |

shaped copper column having Craig polyester,‡ as stationary phase, coated on acid-washed Chromasorb W (25:75 by weight). The column was operated at 212°C and helium was used as the carrier gas flowing at 130 ml/min at a head presure of 22 p.s.i. Detection was by means of a thermal conductivity cell.

*Thin-layer chromatography.* This elegant method developed by Stahl(11,12) and applied to analysis of lipids by Mangold and Malins(13,14) was used to study the total lipid extract from fecaliths and feces. Samples of 0.01 to 1.0 mg, in solution, were spotted along one edge of the plate and separated by ascending elution. A suitable solvent for separation of these samples was found to be a mixture of petroleum ether (b.p. 35-45°C), diethyl ether and acetic acid (80:20:1, v/v/v) and spots were made visible by spraying the plates with 50% sulphuric acid and heating to char the organic material (16). The fluorescence of many of the spots or bands under ultraviolet light enabled their positions to be determined without their destruction so that individual fractions could be scraped off the plate, extracted from the adsorbent and weighed. To obtain large enough amounts of each component for weighing, 20 to 25 spots, each containing 1-2 mg of sample, were run on a single plate and each whole row of spots (or band) corresponding to a component was then scraped off.

*Results.* Gas-liquid chromatographic analysis of methyl esters derived from fecalith lipids demonstrated the presence of as many as 30 fatty acid esters in the range of C-10 to C-24. Tentative identification and possible significance of the other fatty acids present will be reported later.

‡ Butanediol-pentaerythritol succinate polyester. (Obtained from Wilkens Instrument & Research, Inc., California).

The proportions of the 4 major fatty acids were calculated as % of their total area under the GLC curves, other minor components being excluded from the calculations (Table I).

From the relative proportions of these 4 fatty acids in fecaliths and feces, it is obvious that fecalith lipids differ markedly from fecal lipids in containing no apparent linoleic acid and a much smaller proportion of oleic acid.

Chloroform-methanol extraction of fecaliths removed 20-25% of the dry weight of the samples. Acidification of the residue with HCl to liberate fatty acids from any insoluble soaps enabled a further 3-4% of the dry weight to be dissolved in chloroform-methanol. This fraction, however, did not consist of fatty acids because it did not migrate like a standard fatty acid sample on a thin-layer chromatogram, but remained at the starting point. It seems, therefore, that none of the lipids in fecaliths exist in the form of soaps.

Thin-layer chromatography of the total lipid extract resulted in separation of 7 distinct lipid classes. These types were compared with known standards run simultaneously on the same plate (Fig. 1). Additional information about the nature of the components of individual spots was obtained by watching the development of colors on heating, after spraying with sulphuric acid(16). For example, cholesterol and cholesteryl esters are among the first to show color, the spots being a characteristic bright pink. Thus, band 7 of the fecalith lipids (Fig. 1) showed early coloration coincident with the cholesteryl ester standard but later was covered with a darker brown spot due to another component, as yet unidentified.

To conduct a quantitative analysis of the lipids 25-50 mg of these were spotted on a single plate and separated. After localization

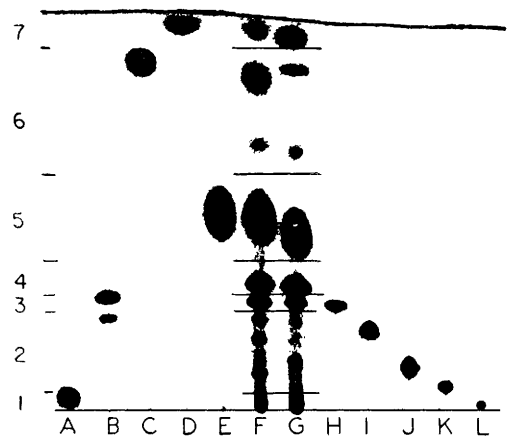


FIG. 1. Thin-layer chromatogram of fecalith and fecal lipids and standards. Plate was eluted with a mixture of ethyl ether, petroleum ether and acetic acid (20:80:1, v/v/v), the spots developed by charring after spraying with 50% sulphuric acid and reproduction made by photo-copying. Samples were: A, monoolein; B, 1,2- and 1,3-diolein; C, triolein; D, cholesteryl palmitate; E, oleic acid; F, fecal lipids; G, fecalith lipids; H, cholesterol; I, 14-hydroxystearic acid; J, 2-hydroxystearic acid; K, selachyl alcohol; L, yeast lecithin.

under ultraviolet light the individual bands were extracted from the adsorbent and weighed. Table II shows the proportion of the lipids found in the bands from feces and from fecaliths. Approximate figures only are given, as the relative inaccuracy of this technic is appreciated. Tentative identification of some of the lipid classes was made from comparison with standards. The presence of cholesterol in bands 3 and 7 and of phosphorous in band 1 was verified chemically.

*Discussion.* Fecaliths have been shown to have a lipid content of 20-25% of their dry weight, the remainder being inorganic calcium salts plus undigested fecal debris. Contrary to previous opinion, the lipid fraction of fecaliths does not appear to exist in the form of soaps but approximately one-third is present as free fatty acids. Compared with the lipids from feces, fecalith total lipids contain much less triglyceride and more free fatty acids and the component fatty acid composition is significantly different from that of feces. These observations suggest that fecalith lipids are not simply a quantitative deposition of fecal lipids.

TABLE II. Proportion of Total Lipid Extract, by Weight, Recovered from the Bands Separated by Thin-Layer Chromatography.

| Band No. | Identification | Fecaliths % | Feces % |
|---|---|---|---|
| 7 | Cholesteryl esters and unknown | 10-25 | 5-10 |
| 6 | Triglycerides | 2- 5 | 10-15 |
| 5 | Free fatty acids | 25-36 | 12-20 |
| 4 | Unknown | 24-29 | 22-32 |
| 3 | Cholesterol and unknown | 4-10 | 10-15 |
| 2 | Unknown | 3- 8 | 10-15 |
| 1 | Phospholipids and unknown | 5-10 | 8-10 |
| % of original sample recovered | | ca. 95 | ca. 90 |

If it is assumed that fecaliths form within the appendix, then the possible sources of the fecalith lipids are either the secretions of the appendix or the feces that pass in and out of the appendix. If deposition from feces occurs, there must either be a selective deposition of certain lipids, particularly fatty acids, or there must be some alteration of the lipids when once deposited.

Williams' theory of appendiceal secretion of lipids would appear to be substantiated by the finding of a predominance of palmitic and stearic acids, but no convincing proof of the secretion of lipids by the human appendix has been found. Indeed the appendiceal secretion of the rabbit appendix has been found to contain no significant lipids even after forced fat feeding(17). There may be, however, considerable difference in function of the appendix in man and the rabbit.

Differential deposition of free fatty acids can occur only in an acid medium. The pH of obstructed human appendices has been shown to be in the range 6.0-8.3(18). The laminated appearance of many, but not all, fecaliths suggests an alternating deposition of lipids and inorganic calcium salts, possibly resulting from variations between acid and alkaline conditions.

The alteration of deposited lipids could result from bacterial action and the large number of peaks obtained by GLC analysis of the methyl esters derived from fecaliths, many of which are due to esters of branched chain acids, suggest that such bacterial action in the appendix may be important. Chipault *et al.* (19) have shown that incubation of triolein with a culture of stool resulted in conversion of unsaturated to saturated acids. The small proportion of unsaturated acids in fecalith

lipids may be the result of similar bacterial action within the appendix. Further information about the nature and fate of lipids secreted into the bowel should help to determine the reasons for the characteristic and relatively constant lipid pattern of the fecalith.

*Summary.* The relative proportions of palmitic, stearic, oleic, and linoleic acids in lipids extracted from fecaliths and feces are compared by means of gas-liquid chromatography. Fecaliths, in contrast to feces, contain little oleic acid and no apparent linoleic acid. Thin-layer chromatography was used to determine the types of lipids found in fecaliths and feces. The former contain less triglyceride and more free fatty acids than the latter. Fatty acid soaps were not found in fecaliths.

1. Wangensteen, O. H., Bowers, W. F., *Arch. Surg.*, 1937, v34, 496.
2. Bowers, W. F., *ibid.*, 1939, v39, 362.
3. Hancock, H., *Lancet* (London), 1848, v2, 380.
4. Kelly, H. A., Hurdon, E., *The Vermiform Appendix and Its Diseases,* W. B. Saunders, Philadelphia, 1905.
5. Wegler, quoted by Kelly and Hurdon(4).
6. Williams, O. T., *Biochem. J.,* 1906-7, v2, 395.
7. Maver, M. E., Wells, H. G., *Arch. Surg.,* 1921, v3, 439.
8. Williams, O. T., *Biochem. J.,* 1908, v3, 391.
9. Sperry, W. M., *J. Biol. Chem.,* 1926, v68, 357.
10. Sperry, W. M., Angevine, R. W., *ibid.*, 1932, v96, 769.
11. Stahl, E., *Pharmazie,* 1956, v11, 633.
12. Stahl, E., *Chemiker-Ztg.,* 1958, v82, 323.
13. Mangold, H. K., *Fette Seifen Anstrichmittel,* 1959, v61, 877.
14. Mangold, H. K., Malins, D. C., *J. Am. Oil Chemists' Soc.,* 1960, v37, 383.
15. Malins, D. C., Mangold, H. K., *ibid.,* in press.
16. Morris, L. J., Holman, R. T., Fontell, K., *J. Lipid Res.,* in press.
17. Williams, J. A., unpublished data.
18. Wangensteen, O. H., *Lancet* (Lond.), 1939, v59, 491.
19. Chipault, J. R., Lundberg, W. O., Wollaeger, E E., *Gastroenerology,* 1953, v24, 422.

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## Electrocardiogram in Hamsters after Large Fat Meals.* (26053)

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It has been shown in hamsters that the red blood cells aggregate and the circulation slows after large fat meals(1,2). Availability of oxygen in cerebral tissues is also significantly decreased(3) and convulsions occur(4) after meals of fat. These changes are either absent or much less marked after meals of oil. This paper reports changes in the electrocardiograms which also occur after fat, but not after oil meals.

*Material and methods.* Hamsters weighing from 80 to 120 g were tube fed butterfat as cream, or oil and synthetic fat mixtures emulsified in skim milk under very light ether anesthesia. Amounts of the lipid meals varied from 1.6 to 10.0 g/kg body weight. The volume of each feeding was the same, 3 ml/100 g body weight; concentration of the lipid in the meals varied. Standard 3 lead electrocardiograms were determined 0, 3, 6, 9, 24, 48, and 72 hours after each feeding. The animals were restrained by leg ties for recording of the E.K.G.s. E.K.G. potentials were amplified by a Tektronic polygraph and recorded by an Offner dynograph.

*Results.* The electrocardiographic changes after fat meals consisted of elongation of QT and ST intervals. The QT interval was measured from the beginning of the QRS complex to the end of the T wave (where the T wave returns to the isoelectric line). The ST segment was measured from the end of the QRS complex to the beginning rise of the T wave. In 55 animals before lipid feeding RR, QT, and ST intervals were measured in the stand-

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