

# The origin of faecal fat

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It has previously been shown (Webb, James, and Kellock, 1963) that, while the fatty acid composition of faecal fat varies considerably from individual to individual in normal subjects, it is only minimally affected by marked changes in the nature of the dietary fat. Theoretically this could be attributable to (1) differential absorption of different fatty acids in the small intestine; (2) a non-dietary origin for all the faecal fat; or (3) alteration of the unabsorbed fat during transit through the colon (probably by bacterial action). Preliminary observations in two patients with ileostomies (Bouchier, Kellock, and Manousos, 1963) seemed to favour the third possibility since the composition of the fat in the dejecta from the terminal ileum closely resembled the composition of the dietary fat.

In a further attempt to determine which of these possible mechanisms is operative, we have made more detailed studies of the effect of changes in the dietary fat on the fatty acid composition of ileostomy excreta. A preliminary report has appeared (Wiggins, Howell, Kellock, and Stalder, 1966).

## EXPERIMENTAL SUBJECTS

These were four volunteers, all of whom had undergone total colectomy and ileostomy for ulcerative colitis several years previously. All were in good general health and in none was there any suggestion of small bowel disease or ileostomy dysfunction.

## DIETS

These patients were given:

1 A very low fat diet consisting of dishes prepared from vitamin-free casein (Nutritional Biochemicals Ltd) with added rice, arrowroot, sugar, and flavourings. Cellulose powder, 10 g a day, was added to provide a residue. This diet provided a daily intake of 1,000 calories with only 0.3 g of fat.

2 A butter diet consisting of normal low fat foods supplemented by 50 g of butter.

3 A corn oil diet consisting of normal low fat food supplemented by 50 g of corn oil. These diets are identical with those previously used (Webb *et al*, 1963) and at least 80% of the fat was derived from butter or corn oil.

## DESIGN OF STUDY

The standard schedule is illustrated in Figure 1. On admission the subject was given a carmine marker and put on the 'very low fat diet' for the first 24 hours. As soon as all the marker had been eliminated, the first collection of ileostomy dejecta was started and continued for the remainder of the 24 hours. This provided a sample in which the ileostomy fat could be considered 'non-dietary'.

At the end of the first 24 hours, test diet A was started and a second collection begun; after 24 hours on this diet another carmine marker was given and the subject again put on the very low fat diet for a further 24 hours.

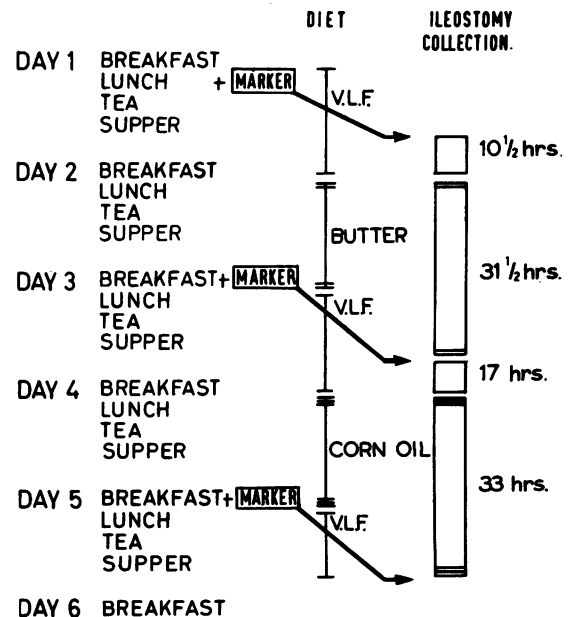


FIG. 1. Experimental schedule. This figure illustrates the plan of the study for subject A.L. The vertical lines denote the duration of the diet and the vertical boxes the duration of the collections. The horizontal lines at the top and bottom of the vertical lines and boxes denote the relationship between the dietary periods and the collection periods.

The collection from the ileostomy was ended when all the marker had been eliminated. Thus the second collection contained all the residual fat from diet A with the smallest possible non-dietary component from the few hours on the very low fat diet.

A third collection was made until the end of the 24 hours on the very low fat diet, providing a further sample containing only non-dietary fat.

Test diet B was then given for 24 hours and a fourth collection made in the same way. Two subjects received the corn oil diet as test diet A followed by the butter diet as test diet B and in the other two the order was reversed.

#### LABORATORY METHODS

Each collection consisted of a number of ileostomy bags and each bag was frozen to  $-20^{\circ}$  as soon as practicable and the collection remained frozen until analysed. The contents of the bags were pooled appropriately and homogenised.

Total fatty acids were estimated after hydrolysis of aliquots by the method of van de Kamer, Huinink, and Weyers (1949), extraction into heptane, and titration.

To determine the partition of fatty acids between lipid and non-lipid molecules further aliquots were freeze-dried and the residue (3 to 10 g) extracted by homogenizing with 100 ml portions of 2:1 chloroform-methanol (Folch, Lees, and Sloane Stanley, 1957). Glacial acetic acid (1 ml) was added to the first extraction. The homogenate was centrifuged and the clear supernatant decanted into a separating funnel and washed with water. This procedure was repeated twice. The solvent extracts were pooled and the fatty acid content estimated by alkaline hydrolysis, followed by extraction into heptane and titration. The washings and the extracted residue were combined and the fatty acids similarly estimated.

Portions of the heptane extracts obtained by both the van de Kamer and by the Folch procedures were dried under nitrogen and the fatty acids treated with 2% sulphuric acid in methanol for 16 hours at  $60^{\circ}$  in ampoules sealed under nitrogen. The methyl esters so formed were extracted and analysed by gas liquid chromatography on a Pye Argon chromatograph using 10% Apiezon L as the stationary phase at  $197^{\circ}$ . The relative areas under the peaks were calculated by triangulation, planimetry, and peak height multiplied by retention time. As all three methods gave essentially the same results, both on the initial specimens and also on standards obtained from Applied Science Laboratories, the peak height multiplied by retention time was adopted for all calculations.

#### RESULTS

The time for the elimination of the carmine markers varied from 10 to 15 hours with a mean time of 12.5 hours.

The total fat outputs during the three and a half-day periods, including both test diets and one very

low fat diet period, were all less than 4% of the intake, indicating normal fat absorption in these subjects. There was no consistent difference between the amount of fat excreted on the butter or corn oil diets (Table I).

The fatty acid composition of the test diets and the corresponding ileostomy dejecta are shown in

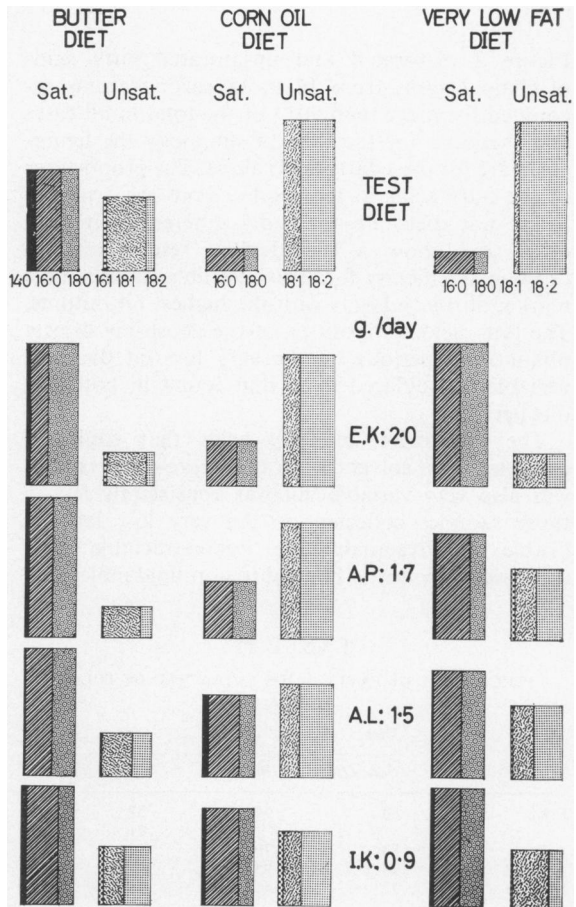


FIG. 2. This figure illustrates the change in the pattern of fatty acids in ileostomy dejecta when the dietary fatty acids are changed. The heights of the blocks represent the percentages of total unsaturated and total saturated fatty acids. The distance between the vertical divisions gives the proportion of the individual acids in each class and thus the area of the rectangle is proportional to the amount of the individual acid in the total mixture. The acids from right to left are for the saturated, stearic, palmitic, myristic, and for the unsaturated linoleic, oleic, and palmitoleic. The oleic acid in subjects E.K., A.P., A.L. on the butter diet includes isomers of oleic acid which were not found in any of the other specimens.

TABLE I

FAT OUTPUT (g) DURING THE EXPERIMENTAL PERIODS<sup>1</sup>

Subject	Diet		
	Corn Oil	Butter	Very Low Fat
E.K.	4.08	1.63	0.29
I.K.	0.96	1.29	0.36
A.L.	1.59	2.83	0.11
A.P.	3.13	1.36	0.54

<sup>1</sup>The very low fat period is the one between the test diets (collection three).

Figure 2. Saturated and unsaturated fatty acids of chain lengths from 14 to 18 carbon atoms accounted for more than 90% of the total in all cases and therefore for the sake of simplicity the figures given are for these fatty acids alone. The proportions of the fatty acids in the residue from the two different test diets are markedly different from each other and show a considerable resemblance to those in the dietary fat. This resemblance was most marked in the subjects with the highest fat outputs. The fatty acid composition of the ileostomy dejecta obtained in periods on the very low fat diet was variable but differed from that found in both test diet periods.

The proportion of fatty acids that could be extracted by a solvent from the freeze-dried residue was also very variable but was consistently less in those samples collected on the very low fat diet (Table II). Presumably the 'non-extractable' fatty acids were covalently bonded to non-lipid molecules.

TABLE II

PERCENTAGE OF FATTY ACIDS EXTRACTED BY FOLCH REAGENT

Subject	Diet		
	Corn Oil	Butter	Very Low Fat
E.K.	88	94	57
A.P.	84	70	10
A.L.	94	95	
I.K.	72	61	16
			43

## DISCUSSION

It is open to question whether the bowel contents produced from an ileostomy are identical with those passing through the ileo-caecal valve in a normal subject. It would seem reasonable to suppose, however, that they bear a very much closer resemblance to terminal ileal contents than to the faeces and hence to draw conclusions about the changes produced during the passage of the ileal contents through the colon by comparing the dejecta of

those without a colon and the faeces of normal subjects.

In addition to our previous study (Webb *et al*, 1963), other authors (Krakower, 1934; Annegers, Boutwell, and Ivy, 1948) have drawn attention to the difference between the proportion of various fatty acids in the faeces and those in the diet. Three possible alternative explanations have been outlined above and need to be examined in the light of the present study.

The first theory depended on the fact that different fatty acids are absorbed to differing degrees, depending on such factors as chain length and degree of saturation (Gompertz and Sammons, 1961; Fernandes, van de Kamer, and Weyers, 1962). If this were to account for the composition of faecal fat to any significant degree, however, and it is accepted that fat absorption is a small bowel function, it would be expected that the composition of ileostomy dejecta would resemble that of the faeces and differ from that of the diet while the present study shows the reverse.

The second theory was that all the faecal fat was non-dietary in origin. The fact that studies with labelled fats (Isley, Sanders, Baylis, Sharpe, Hymans, Ruffin, Shingleton, and Wilson, 1957; Blomstrand, 1955) have shown an absorption of 98% or more whereas balance studies show an 'absorption' of only 95% suggests that some of the faecal fat is non-dietary. Bergström and Blomstrand (1956) in a study on one normal subject have shown that only 50% of faecal fat is dietary in origin. Similarly in the present study the fat excreted in the periods on the very low fat diet was in excess of the intake and of a very different composition. Although it is not possible from the present study to calculate the rate of excretion of non-dietary fat, the findings are consistent with those of Bouchier *et al* (1963) who found a mean fat output of 0.7 g a day in five fasting ileostomy subjects and also with those of Bergström and Blomstrand (1956) who concluded that about 1 g of non-dietary fat was excreted per day in the faeces.

It follows that the non-dietary fat cannot account for all the difference between ileostomy fat and faecal fat. Moreover in the present study the increasing resemblance of the ileostomy fat to the dietary fat with increasing fat output suggests that the non-dietary fat is not handled in the same way as dietary fat. The explanation of this might be incomplete mixing of the two. Bolt, Napier, Howell, and Pollard (1965) have reported the fatty acid composition of human small intestinal mucosal specimens obtained by biopsy. The resemblance between the mean values reported by them and the mean values for the ileostomy fluid collected on the

TABLE III

COMPARISON OF MEAN FATTY ACID COMPOSITION (BOLT *et al.*, 1965) OF THE HUMAN SMALL INTESTINAL MUCOSA WITH MEAN FATTY ACID COMPOSITION OF ILEOSTOMY DEJECTA OBTAINED ON A VERY LOW FAT DIET

	Intestinal Mucosa (%) (n = 12)	Ileostomy Dejecta (%) (n = 4)
14 : 0 (myristic)	2.3	2.9
16 : 0 (palmitic)	33	30
16 : 1 (palmitoleic)	0.9	1.5
18 : 0 (stearic)	25.8	31.8
18 : 1 (oleic)	21.4	20.8
18 : 2 (linoleic)	16.6	13.1
Saturated	60	65
Unsaturated	40	35

very low fat diet is very close (Table III). This suggests that the non-dietary fat is derived from desquamation of the small intestinal epithelium. If this is so the ileostomy fat will sometimes consist of the residue from a mixture of dietary and non-dietary fat and sometimes of that from non-dietary fat alone, depending on the time of meals. Moreover, the fatty acids derived from the non-dietary fat might be less easily absorbed than those from the diet. This is supported by the finding that on the very low fat diet a greater proportion of the fatty acids excreted was not extracted by the Folch reagent.

The previous dietary study (Webb *et al.*, 1963) in intact subjects showed that the composition of the dietary fat made little difference to the composition of the faecal fat; the present study has shown that in the small intestine only minor changes occur in the nature of the dietary fat, mostly attributable to the addition of non-dietary fat, possibly from desquamation of intestinal epithelium. It follows that the major alteration in the composition of the unabsorbed dietary fat must occur in the colon. Further analysis of the results of the previous study shows that the composition of the faecal fat is not totally independent of that of the dietary fat: if the fatty acids are grouped by chain length alone, it is seen that in three of the studies changes in the

TABLE IV

COMPARISON OF CHANGES IN PROPORTION OF TOTAL C<sub>16</sub> AND TOTAL C<sub>18</sub> FATTY ACIDS IN FAECES WITH CHANGE OF DIET<sup>1</sup>

Diet	Butter Diet		Corn Oil Diet	
	C <sub>16</sub>	C <sub>18</sub>	C <sub>16</sub>	C <sub>18</sub>
	21	50	11	89
Faecal fat				
Wa	33	55	27	69
Co	29	46	15	74
Br	44	41	41	49
Cl	29	53	31	53

<sup>1</sup>Webb *et al.* (1963)

proportion of C<sub>16</sub> and C<sub>18</sub> fatty acids in the diet are reflected by similar changes in the faecal fat (Table IV). It would seem likely that the composition of faecal fat is largely determined by the saturation of unsaturated fatty acids of dietary and non-dietary origin by bacteria in the colon.

## SUMMARY

Four healthy subjects with well established ileostomies were fed test diets containing a high proportion of butter or corn oil and the proportions of the different fatty acids in the ileostomy dejecta were estimated. In contrast to previous studies on faeces in normal subjects, *ie.*, with a colon, the composition of the ileostomy fat was greatly affected by changes in the dietary fat. Samples of non-dietary fat in the ileostomy dejecta were shown to differ significantly from dietary fat.

It is suggested that the quality of faecal fat in the normal subject is largely determined by the addition of non-dietary fat derived from intestinal desquamation and bacterial reduction of unsaturated fatty acids in the colon.

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