Quantitative Determination of the Different Higher Saturated Fatty Acids in Fat from Blood, Chyle and Faeces, by Means of Partition Chromatography on Rubber

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(Received 24 December 1954)

In the study of fat metabolism, it is important to examine the behaviour of each constituent fatty acid of the ingested fat under investigation. For this purpose a method is necessary by which a quantitative determination of each of the different saturated and unsaturated fatty acids can be carried out, not only in the fat ingested but also in the fat of the blood, chyle and faeces. Reversed-phase partition chromatography according to Boldingh (1950, 1953) was found to be very satisfactory for the analysis of the higher saturated fatty acids. With this method it is possible to obtain an accurate quantitative analysis of a mixture of 10 mg. of each of the higher saturated fatty acids from hexanoic to tetracosanoic.

A description is given in this article of the quantitative determination of the different saturated fatty acids in blood serum, chyle and faeces. The blood-fat analysis has been carried out with blood fat from normal healthy adults. The other data have been chosen from an investigation of the fat in the chyle of a patient with a chylothorax after the consumption of various fats (Fernandes, Kamer & Weijers, 1955), and from an investigation of faecal fat in patients with coeliac disease and pancreatic fibrosis, whose diet contained different fats during different experimental periods (Weijers & Kamer, in preparation).

METHODS AND RESULTS

The procedure adopted for the estimation of the higher fatty acids in blood, chyle and faeces consists of the following steps: extraction of the fats; saponification and removal of unsaponifiable material; extraction of the total fatty acids with light petroleum; removal of the unsaturated fatty acids by oxidation with KMnO_4 ; separation and estimation of the saturated fatty acids by the chromatographic method of Boldingh (1950, 1953). Removal of the unsaturated acids was essential, since their presence interfered with the separation of the saturated fatty acids. An estimate of total unsaturated fatty acids present is obtained by determination of the iodine value of the fatty

acids present in the extract, using a separate sample of the extract. The widely differing nature of the materials to be analysed, i.e. blood, chyle and faeces, necessitated different methods of extraction.

Chromatographic method

Apparatus. Cylindrical tubes (total length about 45 cm.; internal diameter 10.75–10.85 mm.) with side tube, provided with separating funnel of 200 ml. (see Fig. 1).

Fig. 1. Cylindrical tube containing the rubber column according to Boldingh (1950, 1953) to separate the saturated fatty acids.

Chrome-nickel sieve plates, diameter about 10.8 mm., with about 250 holes per cm.² (a square perforation of 0.3 mm.). Cylindrical tubes of 50 ml. calibrated at 20 ml. Fig. 2 shows the arrangement for titration under a nitrogen atmosphere.

Fig. 2. Double arrangement for microtitration under a nitrogen atmosphere.

Solvents. Acetone, distilled over anhydrous K_2CO_3 . Acetone 30% (v/v); 600 ml. CO_2 -free distilled water was added to 300 ml. acetone in a 1 l. measuring cylinder, mixed, and made up to 1 l.

Acetone 45, 55, 60, 65, 70, 74, 77 and 80% (v/v) was prepared in the same way as acetone 30% (v/v).

Neutral arachis oil. Arachis oil was purified by chromatography on Al_2O_3 : the Al_2O_3 (Brockmann) was heated for 6 hr. at 600°. After cooling, 4% water (w/w) was added and the system thoroughly mixed and 80 g., suspended in light petroleum, was used for preparation of the column (diam. about 2.5 cm.), arachis oil (100 g.) was dissolved in 200 ml. light petroleum and this solution was allowed to run through the column. The light petroleum was completely removed from the eluate by distillation *in vacuo* at 100°, under a stream of nitrogen.

Sodium ethoxide solution, 0.005N, was prepared by dissolving metallic sodium in 96% purified ethanol.

Preparation of the rubber suspension. The rubber powder was prepared from 'Mealorub' ('Rubberstichting', Delft, The Netherlands) as follows: Rubber (150 g.) was washed well by rinsing in water and dried by suction through a Büchner filter. The rubber was then boiled for 1 hr. with 450 ml. acetone and 50 ml. 25% (w/w) HCl. Treatment of the rubber with HCl served to remove the ZnO added for vulcanization, which otherwise interferes with the separation of the fatty acids. The rubber was filtered, boiled again for 1 hr. after adding acetone and HCl, and this treatment was repeated twice more. Finally, it was boiled twice for 1 hr. with acetone only, after which it was washed with acetone until free from acid (litmus paper) and dried by suction. The rubber was then suspended in acetone and kept at least 2 days and at most 1 month. Before grinding, the acetone was filtered off from a portion of the rubber and light petroleum added until the acetone was displaced. Next, the rubber, just moist with light petroleum, was ground for 4 hr. in a ball mill half-filled with balls of different sizes; we used a vibratory Ball Mill (Griffin and Tatlock R.P.M. 1425) with a porcelain pot of 1 l. The mill must be tightly closed so that no light petroleum evaporates. The rubber powder so obtained was sieved, whilst stirring under light petroleum, through a sieve with pores of 0.3 mm. (British Standards Institution (1943) 410, mesh no. 52). It is important that the rubber should not be too fine (a quantity of rubber sufficient for 5.5 columns should pass the sieve in about 15 min.).

Sufficient of the milled and sieved rubber suspension in light petroleum is poured into a measuring cylinder, so that after settling for about 15 min., the volume of the rubber is 177 ml. (this quantity is sufficient for 5 columns, viz. 5×32 ml. with a surplus of half a column). The light petroleum is then decanted, the rubber transferred quantitatively to a Büchner funnel and washed with acetone until completely free from light petroleum. Neutral oil (19 ml.) is pipetted into an Erlenmeyer flask, and the rubber added with the aid of 126.5 ml. acetone and mixed well, after which 91 ml. water is added drop by drop whilst swirling. The rubber suspension thus obtained is allowed to stand overnight.

Preparation of the rubber column. The chromatographic tube, which was closed at the lower end by rubber tubing with screw clamp, was half filled with 65% acetone and a glass bead dropped in and covered with a layer (0.5 cm. thick) of finely cut glass wool which was pressed well down. The acetone was then run off until it was 1 cm. above the glass wool, and the screw clamp was closed again. The tube was then practically filled with the rubber suspension (see above) and the clamp opened and the tube rotated in as nearly vertical a plane as possible. In order to avoid warming the column with the hand, the constricted lower end of the tube was rotated between thumb and forefinger, while the uppermost part of the tube was held in place by a small metal ring in which it was able to revolve freely. Fresh rubber suspension was gradually added to the tube (which must never be allowed to run dry) until the rubber column was 21 cm. high. Care was taken that the level of the acetone was 5 cm. above the rubber. A chrome-nickel sieve plate was placed on the column. With the aid of a long glass rod flattened at the tip, the column of rubber was compressed to a height of 19 cm., the screw clamp was closed and the rubber particles floating in the acetone above the chrome-nickel sieve plate were removed by means of a pipette with rubber bulb. Next the column was

washed with about 50 ml. 65 % acetone, care being taken to prevent the column from running dry. The column was then washed with about 50 ml. 30 % acetone which caused it to shrink about 0.5-1.0 cm. A wet cloth was put round the tube in order to keep the temperature below that of the atmosphere, thus preventing the formation of gas bubbles in the column (cf. Pikaar, 1955).

Addition of acids to the column. The rubber tubing with the screw clamp was removed and the 30 % acetone was run off until the level was just beneath the sieve plate. Meanwhile, 1 ml. of the solution of fatty acids in acetone-oil (~5-10 mg. saturated fatty acids) was pipetted and transferred drop by drop to the sieve plate on the rubber column. Care was taken that after adding one drop, the liquid fell to just under the sieve plate before adding the following drop, regulating this by opening and closing the tube underneath with a finger, care being taken that the rubber did not run dry. After the fatty acid solution had been added, three 0.5 ml. portions of 30 % acetone were added, and when these had run in, the tube was filled with 30 % acetone. The tube was then closed with the rubber tubing and screw clamp, and the separating funnel, filled with 140 ml. 30 % acetone, was placed on the tube.

Elution and titration. The rate of flow was adjusted to about 20 ml./15 min. and seven 20 ml. fractions were collected. Nitrogen (purified by washing with alkali, acid and water) was passed through for 3 min. and each fraction was titrated with 0.005 N sodium ethoxide under a stream of nitrogen. The sum of the titrations is a_1 ml. Next the separating funnel was filled with 140 ml. 45 % acetone and seven fractions of 20 ml. each were collected and titrated, giving a total titration of a_2 ml. This procedure was repeated with 55, 60, 65, 70, 74, 77 and 80 % acetone. The blank titration of each of the acetone mixtures was determined by titrating 20 ml. samples with 0.005 N ethanolic alkali, giving $b_1, b_2, b_3, \dots$ ml.

To check the sum of the titrations of the different fractions obtained by elution, 1 ml. of the original fatty acid solution in acetone-oil was titrated, after 7 ml. acetone and 3 ml. 96 % ethanol had been added (c ml.). This value, corrected for a blank titration of 7 ml. acetone and 3 ml. 96 % ethanol (d ml.), has to be equivalent to

$$(a_1 + a_2 + a_3 + \dots) - 7(b_1 + b_2 + b_3 + \dots) \text{ ml.}$$

For calculation the following formula is used:

$$\text{mg. fatty acid per 140 ml.} = (a - 7b) \times N_{\text{alkali}} \times \text{mol. wt.}$$

The molecular weights of the fatty acids, corresponding with the different acetone concentrations are summarized in Table 1.

Table 1. *Molecular weights of fatty acids, eluted by different acetone concentrations*

Acetone (%, v/v)	Fatty acid	Mol. wt.
30	Hexanoic + octanoic	130
44	Decanoic	172
55	Dodecanoic	200
60	Tetradecanoic	228
65	Hexadecanoic	256
70	Octadecanoic	284
74	Eicosanoic	312
77	Docosanoic	340
80	Tetracosanoic	368

Sometimes 7 fractions are not sufficient for a complete elution; in such a case eight or nine times 20 ml. must be collected until the value of the blank titration is (approximately) reached.

In order to make the separation of the different fatty acids more complete, 10 ml. instead of 20 ml. can be collected at the beginning and end of an elution, if desired.

If the analysis has to be interrupted during the night, it is best to stop the analysis after a certain elution has been completed. The following day an elution is first carried out once with 20 ml. of the elution liquid last used. A considerable titration value is then found, especially in the case of the higher acetone concentrations, which, however, is not caused by higher fatty acids, so that it must be discarded. Next the elution is continued in the usual way with seven times 20 ml. of the next elution liquid.

The recovery of fatty acids from a mixture of pure materials is shown in Table 2.

Table 2. *Percentage recovery of saturated fatty acids from a known mixture*

Fatty acids	Amount weighed (mg.)	Recovery (mg.)	Recovery (%)
Hexanoic acid and } octanoic acid }	5.0 } 5.0 }	9.4	94
Decanoic acid	5.0	4.8	96
Dodecanoic acid	5.0	4.9	98
Tetradecanoic acid	5.0	4.75	95
Hexadecanoic acid	5.0	5.0	100
Octadecanoic acid	5.0	4.9	98

Analysis of blood

Reagents. Ethanol-ether mixture (3 vol. ethanol/1 vol. peroxide-free ether). N Sodium ethoxide was prepared by dissolving metallic Na in 96 % ethanol which had been purified by boiling for 1 hr. with solid NaOH, followed by distillation and storage in the refrigerator.

Quinol, 0.1 % in 96 % ethanol; this solution should be colourless. Light petroleum, b.p. 60-80°, was washed twice with 33 % NaOH, followed by washing twice with water; dried with $\text{MgSO}_4 \cdot 2.5 \text{H}_2\text{O}$ and then distilled after addition of about 5 g. neutral arachis oil/4 l. light petroleum. Magnesium sulphate, $\text{MgSO}_4 \cdot 2.5 \text{H}_2\text{O}$ ($\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ is gently heated until a loss of weight of 32-35 % has occurred). Ethanol, 96 % by volume; ethanol, 75 % by volume. Thymol blue solution, neutralized 0.2 % in 96 % ethanol.

Extraction, saponification and purification. The lipids from serum were extracted according to Bloor (1914), by adding 25 ml. serum to 750 ml. of an ethanol-ether mixture (3:1), drop by drop with continual swirling. The mixture is boiled for 1 min., filtered through defatted filter paper and washed quantitatively with hot ethanol-ether. N Sodium ethoxide (10 ml.) and 0.5 ml. quinol solution were added to the filtrate, the ethanol

and ether distilled off and the remainder evaporated to dryness on the water bath under nitrogen. The residue so obtained was dissolved in 10 ml. sodium ethoxide (care being taken that all was dissolved), 0.5 ml. of the quinol solution was then added and the mixture evaporated to dryness on the boiling-water bath while introducing nitrogen above the surface for 15 min. An absolutely dry white crust is left. This crust was taken up in about 10 ml. water, 36 ml. 96% ethanol was added and the mixture extracted three times with 25 ml. light petroleum; the light petroleum extracts were shaken once with 75% ethanol. The ethanolic phases were combined and about 12 ml. 4N-HCl (red to thymol blue) and 90 ml. water were added. This solution was extracted three times with 50 ml. light petroleum; most of the light petroleum was distilled off from the pooled extracts and sufficient 0.1N sodium ethoxide was added to give a blue colour, using thymol blue as indicator, and this solution was evaporated to dryness.

Removal of the unsaturated fatty acids. The dry residue obtained as described above was dissolved in 20 ml. water (the solution should be clear). To this was added 1 ml. 33% (w/w) KOH and mixed well, if necessary with gentle heating. After cooling, KMnO_4 solution was added until the colour of the solution remained just visibly purple after standing for 15 min.; normally 5–10 ml. were required. The solution was set aside for 1 hr. at

room temp. and then acidified with about 4 ml. 50% (w/v) H_2SO_4 and the excess KMnO_4 removed by the gradual addition of solid NaHSO_3 . After addition of 96% ethanol up to a concentration of about 40% the solution was extracted three times with light petroleum and the extract washed with 20 ml. weakly acidified (HCl) water in order to remove the sulphuric acid, and the light petroleum was evaporated quantitatively. The dry residue was then dissolved in exactly 5 ml. acetone containing 20% (v/v) neutral arachis oil. The saturated fatty acids in 1 ml. of this solution were determined according to the chromatographic procedure described above.

Results. Table 3 gives the analysis of the blood fat of a normal adult after fasting for 12 hr.

Hence, the blood fat of this subject contained 1% dodecanoic acid (C_{12}), 2% tetradecanoic acid (C_{14}), 20% hexadecanoic acid (C_{16}) and 6% octadecanoic acid (C_{18}); the remaining 72% was unsaturated fatty acids.

From the titrated values for dodecanoic, eicosanoic and docosanoic acid the corresponding amounts of fatty acid have not been calculated, because these single determinations are too small to allow a conclusion to be drawn as to the presence of these fatty acids.

Table 4 gives the analysis of the blood fat of a normal adult without and with addition of known quantities of fatty acids.

Table 3. *Saturated fatty acids in the blood of a normal adult*

Fatty acids were extracted as described in the text and unsaturated acids removed by oxidation with KMnO_4 ; 19.7 mg. of the total fatty acids were taken and after oxidation with KMnO_4 were equivalent to 5.91 ml. 0.005N-NaOH.

Fatty acids			Dode- canoic acid	Tetra- decanoic acid	Hexa- decanoic acid	Octa- decanoic acid	Eico- sanoic acid	Doco- sanoic acid
Concentration of acetone in the developing solvent (% v/v) ...	30*	45*	55	60	65	70	74	77
Total of titrations } ml. 0.005N-	1.18	0.39	0.39	0.62	3.28	1.07	0.38	0.40
Total of blanks } NaOH	0.21	0.20	0.24	0.28	0.32	0.28	0.28	0.21
Difference	0.97	0.19	0.15	0.34	2.96	0.79	0.10	0.19
Fatty acids in mg.	—	—	0.15	0.39	3.8	1.12	—	—
Fatty acids as % of fat in the blood	—	—	1	2	20	6	—	—

* These fractions contain the oxidation products of the unsaturated acids and the titrations cannot, in consequence, be equated with hexanoic, octanoic and decanoic acids, which are, in any case, attacked by KMnO_4 —see Table 5.

Table 4. *Percentage recovery of saturated fatty acids added to blood fat*

Fatty acids	Found in blood fat (mg.)	Added to blood fat (mg.)	Found in blood fat after addition (mg.)	Recovery of added fatty acids (mg.)	Recovery of added fatty acids (%)
Tetradecanoic acid	0.61	3.20	4.00	3.37	106
Hexadecanoic acid	5.61	2.79	8.60	2.99	107
Octadecanoic acid	1.44	4.40	5.84	4.40	100

Analysis of chyle

The fat is extracted from chyle, using the method described by Röse-Gottlieb (cf. Williams, 1950, p. 456) for the extraction of fat from milk, by adding ammonia and ethanol and extracting by shaking with a mixture of equal parts of ether and light petroleum.

Apparatus and reagents. Weighing bottles diam. 1.5 cm., height 1.5 cm.; sodium ethoxide, 0.1N. For other reagents, see Analysis of Blood.

Extraction, saponification and purification. To 100 g. chyle in a separating funnel were added 20 ml. 10% NH_4OH , 100 ml. 90% ethanol, 250 ml. ether and 250 ml. light petroleum in this order. The mixture was shaken after each addition and finally shaken vigorously for 1 min. After 1 hr. the ether-light petroleum layer is collected and the ammoniacal ethanol layer again shaken for 1 min. with a mixture of 250 ml. ether and 250 ml. light petroleum. The ether and the light petroleum were distilled off from the combined extracts. When fatty acids which are easily oxidized are present the work is carried out in an atmosphere of nitrogen. About 2 g. of the fat residue were weighed out and saponified by boiling for 30 min. under reflux with 25 ml. 0.5N potassium ethoxide. Water (7 ml.) was added and after cooling the mixture was shaken twice with 20 ml. light petroleum. The collected light petroleum extracts were shaken once with 5 ml. 75% ethanol. The combined ethanolic solutions were acidified with 10 ml. 4N-HCl and then 64 ml. water were added. This was shaken twice with 75 ml. light petroleum and the light petroleum was distilled off from the combined extracts.

Removal of unsaturated fatty acids. About 125 mg. of the fatty acids were weighed out, dissolved in 10 ml. 96% ethanol and made alkaline with about 4 ml. 0.1N ethanolic alkali. The ethanol from the alkaline soap solution was evaporated quantitatively. The residue was dissolved in 20 ml. water, 1 ml. 33% KOH was added and the solution treated as described for blood.

Besides the higher saturated fatty acids chyle fat, contrary to blood fat, may also contain octanoic and decanoic acids. When these acids are to

Table 5. *Oxidation of the saturated fatty acids by KMnO_4*

Percentage recovery of 100 mg. fatty acids after 1 hr. treatment with excess of KMnO_4 at 20°

Fatty acid	Recovery (%)
Octanoic acid	77
Decanoic acid	96
Dodecanoic acid	99
Tetradecanoic acid	100
Hexadecanoic acid	100
Octadecanoic acid	100

Table 6. *Estimation according to Boldingh's technique of the saturated fatty acids of chyle fat in a chylothorax patient after ingestion of coconut oil*

Fatty acids	...	...	...	Without oxidation with KMnO_4 (25.9 mg. of fatty acids, iodine value 37.5)			After oxidation of the unsaturated fatty acids with KMnO_4 (27.1 mg. of fatty acids ~21.0 ml. of 0.005N-NaOH)						
				Hexanoic + octanoic acid	Decanoic acid	Dodecanoic acid	—	—	Dodecanoic acid	Tetra- decanoic acid	Hexa- decanoic acid	Octa- decanoic acid	Eicosanoic acid
Concentration of acetone of the elution liquids in % (v/v)	...	...	...	30	45	55	30	45	55	60	65	70	74
Total of titrations in ml. 0.005N- NaOH	...	...	...	0.42	0.94	9.31	3.18	1.13	9.39	4.04	3.54	1.03	0.36
Total of blanks	...	...	...	0.35	0.35	0.35	0.35	0.35	0.35	0.21	0.21	0.21	0.21
Difference	...	...	...	0.07	0.59	8.96	2.83	0.78	9.04	3.83	3.33	0.82	0.15
Fatty acids in mg.	...	...	...	—	0.5	9.0	—	—	9.0	4.4	4.3	1.2	—
Fatty acids as % of fat in the chyle	...	...	...	—	2	35	—	—	33	16	16	4	—

be determined, the KMnO_4 oxidation for the removal of the unsaturated fatty acids cannot be applied since both octanoic and decanoic are attacked by this treatment, see Table 5.

It is a lucky circumstance that the unsaturated fatty acids do not interfere with elution of these two acids and hence they can be determined without KMnO_4 treatment.

In practice, a suitable amount of fatty acids is straight away dissolved in 5 ml. acetone-oil and, without oxidation, the chromatographic analysis is carried out with 1 ml.

Results. Table 6 gives the analysis of chyle fat from a chylothorax patient who had eaten a meal containing coconut oil.

Table 7 gives the analysis of chyle fat of the same patient after addition of known quantities of some fatty acids.

triglycerides present in the first light petroleum extract were saponified with potassium ethoxide. After the unsaponifiable substances had been removed by shaking with light petroleum in alkaline medium, the solution was acidified and the fatty acids derived from the original triglycerides present were extracted with light petroleum. For the rest the procedure was the same as that described for the analysis of the total fatty acids.

Reagents. Ethanol, 75%, alkaline to thymol blue ($\text{pH} > 9$); hydrochloric acid 1N in 25% ethanol. For other reagents, see Analysis of Blood.

Extraction, saponification and purification. Fresh faeces (20 g., corresponding to at least 200 mg. saturated fatty acids) were boiled with 160 ml. ethanol (containing 0.4% amyl alcohol) and 40 ml. 33% KOH for 20 min. under reflux. After cooling, about 50 ml. light petroleum were added and the

Table 7. *Percentage recovery of saturated fatty acids added to chyle fat*

Fatty acids	Found in chyle fat (mg.)	Added to chyle fat (mg.)	Found in chyle fat after addition (mg.)	Recovery of added fatty acids (mg.)	Recovery of added fatty acids (%)
Dodecanoic acid	2.17	3.98	6.43	4.26	107
Tetradecanoic acid	1.11	3.38	4.52	3.41	101
Hexadecanoic acid	1.00	3.36	4.46	3.46	103
Octadecanoic acid	0.32	3.58	3.68	3.36	94

Analysis of faeces

Faeces contain free fatty acids, soaps and triglycerides, and it is possible to determine either the total amount of each fatty acid present in these three forms or to determine the amounts present in each form separately.

For the estimation of the total fatty acids present as free acids, soaps and triglycerides, the faeces were boiled with potassium ethoxide and the ethanolic extract was treated with light petroleum to remove the unsaponifiable material. The fatty acids present in the ethanolic extract were liberated by acidification, extracted with light petroleum and purified by treating the light petroleum extract with 25% (w/v) aqueous ethanol (Kamer, Bokkel Huinink & Weijers, 1949). The unsaturated fatty acids were removed by oxidation and the saturated acids separated and estimated chromatographically as described above.

For the estimation of the free fatty acids and soaps and the triglycerides separately, the faeces were boiled with acid, made alkaline and, after addition of ethanol to a concentration of 75%, the triglycerides were extracted with light petroleum. The ethanolic lower layer was acidified and, the fatty acids derived from the free fatty acids and the soaps were extracted with light petroleum. The

mixture was shaken for 1 min. The clear light petroleum layer was separated from the ethanolic layer and the latter was shaken again for 1 min. with 50 ml. light petroleum. If the light petroleum extract had not immediately cleared, it was left to stand overnight, or filtered. The collected light petroleum extracts were shaken once with about 15 ml. 75% ethanol, and the light petroleum layers discarded. The ethanolic phases were acidified with 60 ml. 25% (w/v) HCl, and the ethanol concentration was brought to 60% (v/v) by adding water; after this the mixture was shaken three times for 1 min. with about 75, 50 and 50 ml. light petroleum respectively. The light petroleum extracts were purified by shaking once with about 85 ml. 25% ethanol in N-HCl (van de Kamer, 1953) and the light petroleum was distilled off.

Sometimes an extra purification of the faeces extract was advantageous. It is possible to combine this purification with the oxidation of the unsaturated fatty acids by oxidizing with KMnO_4 during the night instead of for 1 hr. However, it is necessary to take into account not only a loss of hexanoic, octanoic and decanoic acid, but also a slight loss of dodecanoic acid (3%).

Removal of unsaturated fatty acids. For the removal of the unsaturated fatty acids about 100 mg. of the mixed fatty acids were weighed out,

Table 8. *Estimation of the saturated fatty acids in the faeces of a patient with coeliac disease*

Unsaturated fatty acids removed by oxidation with KMnO_4 ; 34.5 mg. of fatty acids were equivalent to 22.7 ml. 0.005N-NaOH.

Fatty acids	...	...	...	Dodecanoic acid	Tetra-decanoic acid	Hexa-decanoic acid	Octa-decanoic acid	Eico-sanoic acid	Doco-sanoic acid
Concentration of acetone of the elution liquids in % (v/v)	...	30	45	55	60	65	70	74	77
Total of titrations	} ml. 0.005N-NaOH	1.47	0.52	0.45	2.31	11.09	7.32	0.44	0.48
Total of blanks		0.21	0.28	0.21	0.21	0.29	0.21	0.24	0.24
Difference		1.26	0.24	0.24	2.10	10.80	7.11	0.20	0.24
Fatty acids in mg.		—	—	—	2.4	13.8	10.1	—	—
Fatty acids as % of the fat in faeces		—	—	—	7	40	29	—	—

Table 9. *Percentage recovery of saturated fatty acids added to faecal fat*

Fatty acids	Found in faecal fat (mg.)	Added to faecal fat (mg.)	Found in faecal fat after addition (mg.)	Recovery of added fatty acids (mg.)	Recovery of added fatty acids (%)
Decanoic acid	—	3.88	3.88	3.88	100
Dodecanoic acid	0.77	2.19	2.88	2.11	96
Tetradecanoic acid	0.74	3.25	4.32	3.58	110
Hexadecanoic acid	3.84	2.13	6.05	2.21	104
Octadecanoic acid	2.47	4.12	6.77	4.30	104

dissolved in 96% ethanol, made alkaline, evaporated to dryness and taken up in about 20 ml. water (clear) and treated further as described for the analysis of blood.

Results. Table 8 gives the analysis of fat in faeces from a coeliac patient whose diet contained butter and rice flour.

Owing to the low fat content of faeces, viz. 4%, the faecal extract contained a relatively large amount of compounds other than fatty acids with the result that the separation of the fractions eluted with 60, 65, and 70% acetone respectively was not as precise as usual. Therefore only 0.14 ml. of the overnight increase of 0.38 ml. was neglected from the calculations and it was assumed that the remaining 0.24 ml. corresponded with tetradecanoic acid; but it is not impossible, however, that a portion of this corresponds with hexadecanoic acid. These uncertainties have only slight influence on the percentage composition of the original fatty acid mixture.

Altogether 14.45 ml. were titrated; the titration of the same amount of fatty acid before the analysis was 14.57, so that 99% was recovered.

Table 9 gives the analysis of faecal fat without and with addition of known quantities of some fatty acids.

SUMMARY

The extraction, purification and quantitative determination of the saturated fatty acids in blood,

chyle and faeces are described. In order to carry out the quantitative determination, it is necessary to remove the unsaturated fatty acids by oxidation with KMnO_4 in an alkaline medium. The quantitative determination of the saturated fatty acids hexanoic acid (C_6), octanoic acid (C_8) and decanoic acid (C_{10}) and of the unsaturated Δ^{13} -docosanoic acid (C_{22}) before oxidation with KMnO_4 , and of the saturated fatty acids dodecanoic acid (C_{12}), tetradecanoic acid (C_{14}), hexadecanoic acid (C_{16}), octadecanoic acid (C_{18}), eicosanoic acid (C_{20}) and docosanoic acid (C_{22}) after oxidation with KMnO_4 , is carried out by means of reversed-phase partition chromatography according to Boldingh (1950, 1953).

Some examples of the application are given.

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