The Effect of 'Essential Fatty Acid' Deficiency on the Fatty Acid Composition of the Total Lipid of the Intestine

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(Received 18 May 1962)

There have been few reports on the intestinal fatty acid composition of rats, although the effect of dietary fatty acids on other tissues has been studied (Sinclair, 1931, 1935). Coniglio & Cate (1958) found that in their rats 37% of the intestinal fatty acid was palmitic acid. This proportion remained in animals that had been starved for 72 hr. Similar amounts of saturated fatty acids were found in total intestinal lipids by Paoletti & Grossi (1960), who showed that two-thirds of the saturated acids were present as phospholipid. In the present study, the fatty acid composition of the total lipid of the intestinal wall has been determined in rats in four dietary states. Rats on a fat-deficient diet supplemented with 'essential fatty acids' were used to determine the basic fatty acid composition of the intestinal wall, and this was compared with the composition of intestinal wall from 'essential fatty acid'-deficient rats. The effect of the fatty acid composition of the diet on that of the intestinal wall was studied in fed rats and also starved rats, since the latter are often used for studies of intestinal fatty acid absorption.

Besides changes in the overall fatty acid composition of the intestine in response to dietary changes, this study shows that the muscle and mucosa differ in their fatty acid composition, which also varies along the length of the intestine. Widmer & Holman (1950) failed to observe any changes in intestinal polyenoic acids after feeding 'essential fatty acid'-deficient rats with a supplement of linoleic acid and linolenic acid, but we have found a fourfold difference in the concentration of arachidonic acid with a linseed oil supplement given to rats on a fat-free diet.

EXPERIMENTAL

Materials

Animals. Wistar strain rats, approx. 250 g. each, fed *ad lib.* on a diet of rat cubes (Oxo Ltd., London) are termed fed rats (diet *a*). After 24 hr. starvation, they are termed starved rats (diet *b*). Rats deficient in 'essential fatty acids' (diet *c*) were obtained by giving the following diet: 20 parts (by wt.) of casein (Genatosan, low in vitamins, extracted twice with 50% ethanol, once with 90% ethanol and once with diethyl ether), 70 parts (by wt.) of sucrose and 5 parts (by wt.) of mineral salts. The salt mixture contained

(parts by wt.): NaCl 104, anhydrous MgSO₄ 166, NaH₂PO₄ · 2H₂O 204, K₂HPO₄ 272, CaHPO₄ · 2H₂O 324, ferric citrate pentahydrate 70, calcium lactate pentahydrate 780, KI 0.1, CaSO₄ · 5H₂O 2, MnCl₂ · 4H₂O 5. The daily intake of vitamins (mg.) was thiamine (20), riboflavin (40), biotin (0.34), pyridoxine (40), nicotinamide (100), pantothenic acid (240), *p*-aminobenzoic acid (250), choline (2000), inositol (500), calciferol (0.8), vitamin A acetate (40), tocopherol (530). The last three were given in 0.15 ml. of olive oil and the rest in a thick aqueous paste. The olive oil used contained 8% of its total fatty acids as linoleic acid. Pair-fed controls (diet *d*) were also given a daily supplement of 100 mg. of linseed oil. This contained 60% of its total fatty acid as linolenic acid. The animals were kept singly and their weights recorded weekly. After 3 months the deficient animals ceased to grow and showed many of the symptoms of 'essential fatty acid' deficiency. They were torpid, had mild scaling of the tails and had lost patches of hair from the back (Burr & Burr, 1929, 1930). They also walked in a cramped attitude, not extending the hind limbs.

Chemicals. All solvents were of analytical grade. The light petroleum (boiling range 40–60°) was redistilled from solid sodium hydroxide to remove traces of fatty acids. Methyl stearate was obtained from the California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A. Palmityl dimethyl acetal was kindly supplied by Dr Marjorie G. Macfarlane and Dr G. M. Gray.

Methods

Analytical procedures. Dry weights were determined by drying the tissue overnight at 110°. Fatty acid esters were determined by the following modification of the method of Rapport & Alonzo (1955). After addition of ether and alkaline hydroxylamine, the samples and standards were placed as a batch in a water bath at 65° and left for 5 min. after the ether had evaporated. They were removed from the bath and left to cool for 2 min. before addition of the ferric perchlorate reagent. Aldehydes were estimated by the method of Gray & Macfarlane (1958).

Treatment of tissue preparations. Rats were killed by stunning and decapitation. The abdomen was opened and the contents of the small intestine were washed out with 0.9% sodium chloride. Lengths of 10 cm. were removed from the proximal and medial ileum by stripping away the mesentery, and placed in cold 0.9% sodium chloride. The colon contents were also washed out and the proximal part was removed. The segments of intestine were blotted dry and opened longitudinally, and the mucosa was scraped off with a glass slide. Portions of the muscle and mucosa were weighed and used for the determination of dry weights and the remainder was saponified with 5.0 ml. of a mixture consisting of methanolic (50%) 2N-potassium hydroxide

solution, containing 0.1% of quinol, for 3 days at 30°. The solution was diluted and washed three times with light petroleum (40–60°). No fatty acids or fatty aldehydes were detected in the washings after saponification. After acidification with sulphuric acid, the fatty acids and fatty aldehydes were removed by three extractions with light petroleum (40–60°). The extracts were neutralized by adding solid sodium hydrogen carbonate and dried over anhydrous sodium sulphate. Samples were removed for estimation of fatty aldehyde content before methylation. Methyl esters were prepared by treatment of the free acids with diazomethane in ether.

Gas-liquid chromatography. The apparatus used was an Argon Chromatograph (Pye Co. Ltd., Cambridge). The support was acid- and alkali-washed Celite (J.J.'s, Ewell, Surrey). Apiezon L high-vacuum grease (10%) was the non-polar stationary phase and polyethylene glycol adipate cross-linked with pentaerythritol (Cambridge Industries Ltd., Cambridge, Mass., U.S.A.; Lac 2R-446) (15%) was the polar stationary phase. Samples (0.1 μ l.) of dry ester were placed on the column. Peak areas were determined by triangulation according to Keulemans (1957). The limits of

accuracy and reproducibility in determining the quantities of fatty acids by this method are discussed by Biran & Bartley (1961). The chromatographic peaks were identified by (1) comparison of the retention volumes with known acids, (2) determination of carbon numbers (Woodford & van Gent, 1960) on polar and non-polar stationary phases and (3) hydrogenation and chromatography of the products.

RESULTS

Long-chain aldehydes in the lipids of the intestine.

The results of examination by gas chromatography of the methyl esters of fatty acids prepared from extracts of various segments of the intestine are shown in Table 1. The concentration of aldehydes (Table 2) was low relative to the fatty acid concentration (Table 3), and no attempt was made to separate the aldehydes from the acids before chromatography. However, they contributed to two peaks on the chromatograms. The free fatty

Table 1. *Identification and carbon number of compounds observed on gas chromatograms*

ald., Aldehyde; chain $n-x$ contains n C atoms and x double bonds. For explanation of carbon number see Woodford & van Gent (1960).

$n-x$	Description or trivial name	Carbon number	
		On apiezon L as stationary phase	On polyethylene glycol adipate as stationary phase
14-1	Myristoleic	13.80	14.20
14-0	Myristic	14.00	14.00
—	Unknown	14.20	14.20
15-1*	—	14.75	15.25
15-0	Pentadecanoic	15.00	15.00
16-ald.*	—	15.15	15.25
—	Unknown	15.35	15.35
16-1	Palmitoleic	15.70	16.35
16-0	Palmitic	16.00	16.00
17-2*	—	16.50	17.50
17-1*	—	16.70	17.25
—	Unknown	16.80	—
17-0*	Margaric	17.00	17.00
18-ald.*	—	17.15	17.30
—	Unknown	17.25	—
—	Unknown	—	17.60
18-2	Linoleic	17.50	18.80
18-3	Linolenic	17.50	19.15
18-1	Oleic	17.65	18.25
18-0	Stearic	18.00	18.00
—	Unknown	18.60	—
20-4	Arachidonic	18.95	21.35
19-0*	—	19.00	19.00
20-3*	—	19.05	21.10
20-2*	—	19.35	20.70
—	Unknown	19.55	—
—	Unknown	—	21.75
—	Unknown	19.75	—
20-1*	—	19.85	20.30
20-0*	Arachidic	20.00	20.00
22-6*	—	20.50	24.36
22-5*	—	20.65	24.07
22-4*	—	20.80	23.62

* Tentative identification.

Table 2. *Intestinal fatty aldehyde concentration*

Values (μ moles of aldehyde/g. dry wt.) are means $\pm$ S.D. between animals (with number of animals in parentheses).

Dietary procedure ...	Starved rats (b)	Fed rats (a)	Deficient rats (c)	Supplemented rats (d)
Intestinal segment				
Muscle				
Proximal ileum	6.48 $\pm$ 0.21 (6)	6.83 $\pm$ 1.43 (3)	7.41 $\pm$ 1.09 (7)	10.09 $\pm$ 3.42 (6)
Medial ileum	7.83 $\pm$ 2.17	6.40 $\pm$ 1.10	5.82 $\pm$ 2.44	8.12 $\pm$ 2.52
Colon	7.17 $\pm$ 1.13	4.45 $\pm$ 3.45	8.75 $\pm$ 3.70	10.35 $\pm$ 3.18
Mucosa				
Proximal ileum	3.46 $\pm$ 0.24	2.90 $\pm$ 0.90	2.25 $\pm$ 0.81	2.29 $\pm$ 1.02
Medial ileum	3.62 $\pm$ 0.72	3.33 $\pm$ 0.23	1.87 $\pm$ 0.45	2.05 $\pm$ 1.01
Colon	17.55 $\pm$ 0.15	7.31 $\pm$ 2.21	16.04 $\pm$ 7.70	16.19 $\pm$ 11.50

Table 3. *Amounts of fatty acid in the lipids*

Values (m-mole of acid/g. dry wt.) are means $\pm$ S.D. between animals (with numbers of animals in parentheses).

Dietary procedure ...	Starved rats (b)	Fed rats (a)	Deficient rats (c)	Supplemented rats (d)
Intestinal segment				
Muscle				
Proximal ileum	0.250 $\pm$ 0.036 (6)	0.209 $\pm$ 0.017 (3)	0.116 $\pm$ 0.072 (7)	0.130 $\pm$ 0.059 (6)
Medial ileum	0.270 $\pm$ 0.100	0.343 $\pm$ 0.142	0.129 $\pm$ 0.072	0.101 $\pm$ 0.031
Colon	0.231 $\pm$ 0.047	0.192 $\pm$ 0.068	0.192 $\pm$ 0.077	0.232 $\pm$ 0.132
Mucosa				
Proximal ileum	0.207 $\pm$ 0.009	0.371 $\pm$ 0.036	0.190 $\pm$ 0.071	0.183 $\pm$ 0.116
Medial ileum	0.294 $\pm$ 0.042	0.302 $\pm$ 0.040	0.188 $\pm$ 0.074	0.219 $\pm$ 0.178
Colon	0.208 $\pm$ 0.042	0.277 $\pm$ 0.153	0.277 $\pm$ 0.263	0.182 $\pm$ 0.094

acids could be extracted from light petroleum by treatment with 0.5 % potassium carbonate in 50 % methanol, leaving the aldehydes in the light petroleum. Concentration, methylation with diazomethane and chromatography of the original light petroleum residue showed the presence of 35 aldehyde peaks, of which two formed 80 % of the total and were visible on the original fatty acid chromatograms.

Fatty acids of the total lipids of the intestinal wall of the fed animals (diet a)

Mucosa. The total quantity of fatty acids decreased from the proximal to the distal end of the intestine (Table 3). In the proximal ileum (Fig. 4) the three main unsaturated fatty acids were linoleic (27 % of the total; 101 μ moles/g. dry wt.), oleic (25 %; 92 μ moles/g. dry wt.) and arachidonic (9 %; 34 μ moles/g. dry wt.). The main saturated fatty acids were palmitic (17 %; 64 μ moles/g. dry wt.) and stearic (10.5 %; 39 μ moles/g. dry wt.). About 72 % of the acids were therefore unsaturated. In the medial ileum (Fig. 5) the percentage of arachidonic acid was higher (14 %; 42.5 μ moles/g. dry wt.), and all of the other acids listed were lower (linoleic 21 %, 76 μ moles/g. dry wt.; oleic 23 %, 70.5 μ moles/g. dry wt.; palmitic 15 %, 45 μ moles/g. dry wt.; stearic 7.5 %, 23 μ moles/g. dry wt.). Neither of these actively absorbing mucosae contained fatty acids in the same proportion as in the

diet. According to Getz & Bartley (1961), the lipid of the rat cubes fed contained 40 % of linoleic acid, 29 % of oleic acid, 0.9 % of arachidonic acid, 18 % of palmitic acid and 1.5 % of stearic acid. Presumably the low content of linoleic acid in the mucosa is accounted for by conversion into arachidonic acid in the mucosa or oxidation.

In the mucosa (Fig. 6) of the colon, oleic acid was the predominant fatty acid (31 %; 71 μ moles/g. dry wt.). Linoleic acid (10 %; 37 μ moles/g. dry wt.) and arachidonic acid (9 %; 23 μ moles/g. dry wt.) were lower but palmitic acid was higher and comprised 19 % of the total (44 μ moles/g. dry wt.).

Muscle. Fatty acids were obtained in the greatest quantity from the medial ileum (Table 3). In the proximal ileum (Fig. 1), oleic acid replaced linoleic acid as the major fatty acid. In the medial ileum (Fig. 2) the percentage of oleic acid was 35 % (120 μ moles/g. dry wt.). The muscle of the colon (Fig. 3) resembled the muscle of the medial ileum in its fatty acid pattern but there was only about half the amount of lipid present in the muscle of the colon as there was in the muscle of the ileum (Table 3).

Effects of starvation for 24 hr. on the fatty acids of the total lipids of the intestinal wall

Mucosa. The total fatty acid content of the mucosa of the proximal ileum and of the colon was lower by 44 and 24 % respectively in the starved

animals (diet *b*) but the fatty acid content of the mucosa of the medial ileum remained unchanged. The main differences in the fatty acids of the mucosa of the proximal ileum were in palmitic acid, linoleic acid, oleic acid and stearic acid (57, 52, 39 and 55 % lower respectively). The other fatty acids showed little change (Fig. 4). In the mucosa of the medial ileum (Fig. 5) there were 8 μ moles/g. dry wt. more of palmitic acid and 22 μ moles/g. dry wt. more of oleic acid. The differences in the colon mucosa (Fig. 6) were similar to, but less than, the differences found in the mucosa of the proximal segment of the intestine. The colon mucosa maintained its content of linoleic acid whereas the other two segments did not.

Muscle. In contrast with the mucosa, the fatty acid content of the muscle of the proximal ileum (Fig. 1) and the colon (Fig. 3) was 20 % greater,

Fig. 1. Concentrations of fatty acids in rat proximal ileum muscle. Fatty acid C_{n-x} has n carbon atoms and x double bonds. ■, Fed rat (diet *a*); ▨, starved rat (*b*); □, 'essential fatty acid'-deficient rat (diet *c*); ▤, 'essential fatty acid'-supplemented rat (diet *d*). N.D., Not detected. Concentrations for acids C_{20-2} to C_{22-4} are multiplied by 5.

Fig. 2. Concentrations of fatty acids in rat medial ileum muscle. For details see Fig. 1.

Fig. 3. Concentrations of fatty acids in rat colon muscle. For details see Fig. 1.

Fig. 4. Concentrations of fatty acids in rat proximal ileum mucosa. For details see Fig. 1.

Fig. 5. Concentrations of fatty acids in rat medial ileum mucosa. For details see Fig. 1.

Fig. 6. Concentrations of fatty acids in rat colon mucosa. For details see Fig. 1.

whereas that of the medial ileum (Fig. 2) was 21 % less. In the proximal ileum oleic acid was 31 μ moles/g. dry wt. more and linoleic acid 11 μ moles/g. dry wt. more. By contrast, the difference in the colon muscle was mainly in the saturated fatty acids (palmitic acid 10 μ moles more, stearic 8 μ moles) but oleic acid was also 7 μ moles/g. dry wt. more. In the muscle of the medial ileum there were 29 μ moles/g. dry wt. less of oleic acid and 17 μ moles/g. dry wt. less of linoleic acid.

Effect of the synthetic diet on the fatty acids of the total lipids of the intestinal wall

Both deficient (diet c) and control rats (diet d) received 0.15 ml. of olive oil daily as the vehicle for administering the vitamin supplement. In addition, the control animals (diet d) received a further 0.15 ml. of linseed oil. Thus the maximum intake of fat by the animals was about 0.27 g./day compared with that of the stock-fed rats (diet a) of 0.43 g./day, which contained about 0.16 g. of linoleic acid (for analysis of the fatty acids of the rat diet see Getz & Bartley, 1961) with about 0.12 g. of oleic acid. The other major fatty acid found in the stock diet was palmitic acid, contributing about 0.07 g. daily. Thus the intake of polyunsaturated acid in the control animals was about 0.13 g. daily, some 80 % of that of the free-fed animals (diet a). The intake of oleic acid was almost the same in the rats fed on the stock diet as in the rats on the deficient (c) or supplemented diet (d). The linseed oil used as the supplement contained about 60 % of its fatty acid as linolenic acid with the rest linoleic acid. The rats (diet d) supplemented with linseed oil thus had an intake of about 0.05 g. of linoleic acid/day.

Fatty acids of the total lipid of the mucosa of the supplemented rats. The total amount of fatty acid found in the mucosa from the supplemented rats

(diet d) was less in all three sections of the mucosa than in the mucosa of the free-fed animals (diet a) (50% in proximal ileum; 27 % in medial ileum; 34 % in the colon; Table 3). The overall fatty acid intake was about 37 % less and thus the changes in fatty acid content of the mucosa are roughly parallel to the change in the amount of fatty acid fed. However, the composition of the fatty acids of the mucosa did not reflect the composition of the diet. Although the intake of linoleic acid was about one-third of that of the free-fed animals (diet a) the amount of this acid found in the mucosa (Figs. 4, 5, 6) was only about one-tenth of that in the free-fed animals (diet a). Also, although the synthetic diet contained no palmitic acid or stearic acid, the gut mucosa contained from 39 to 67 % of the former and from 36 to 70 % of the latter compared with free-fed animals. The animals (diet d) had a plentiful supply of linolenic acid in the diet but this acid was not detected in the gut mucosa. The oleic acid content of the gut mucosa did not change appreciably, which is consistent with the relatively stable intake of this acid in all the groups of rats. The amount of arachidonic acid was about half that found in the free-fed animals (diet a) and since the diet contained no arachidonic acid this must represent mucosal synthesis from linoleic acid or linolenic acid. A striking feature was that the amount of eicosatrienoic acid was about the same as that of linoleic acid. Only the docosahexaenoic acid content of the ileum mucosa (Figs. 4, 5) reached the concentration found in the starved rats (diet b). There were also lower concentrations of eicosadienoic acid, eicosatrienoic acid and docosatetraenoic acid, compared with the muscle from 'essential fatty acid'-deficient animals (diet c), but none was as low as in the starved animals (diet b).

Fatty acids of the total lipids of the muscle of the supplemented rats. As with the mucosa of proximal and medial ileum the total quantity of fatty acids in muscle was lower than in the fed animals (diet a) (Table 3): 47 % less in the proximal ileum and 70 % in the medial ileum. In contrast with the colon mucosa the colon muscle had a fat content 20 % higher than the free-fed animals (diet a). The differences in the individual fatty acids of the muscle were, with the exception of oleic acid, similar to but rather greater than those found in the mucosa. In the proximal ileum (Fig. 1) the oleic acid content was slightly lower than in stock fed animals (diet a), as it was in the corresponding mucosa (Fig. 4). In the medial ileum (Fig. 2), the oleic acid content was 120 μ moles/g. dry wt. in the stock fed animals (diet a) but only 38 μ moles/g. dry wt. in the supplemented (diet d), whereas in the colon muscle (Fig. 3) the values were 67 and 111 μ moles/g. dry wt.

Effect of linoleic acid deficiency on the fatty acids of the total lipids of the intestinal wall

Mucosa. As with the supplemented control rats (diet *d*) the fatty acid content of the mucosa was lower in the rats (diet *c*) deprived of the polyunsaturated fatty acid supplement than in free-fed rats (diet *a*) (Table 3). The two ileum segments showed the same differences compared with the free-fed animals (diet *a*) as did the supplemented animals (diet *d*) but the colon mucosa had the same amount of fatty acid as in the free-fed animals (diet *a*).

As might be expected, the linoleic acid content of the ileum mucosa from the deficient rats (diet *c*) (Figs. 4, 5) was very low, about one-quarter of the amount in the supplemented animals (diet *d*) and about one-fiftieth of that in the free-fed animals (diet *a*). The colon mucosa (Fig. 6) contained even less linoleic acid ($0.72 \mu\text{mole/g. dry wt.}$) than the ileum mucosa. The arachidonic acid content of the 'deficient' mucosa (diet *c*) was less than that of the supplemented animals (diet *d*). The most striking effect of the deficiency was that the amount of eicosatrienoic acid was about seven times as great as that found in the free-fed animals (diet *a*) and between two and three times that of the supplemented animals (diet *d*). This acid thus became the second or third most abundant acid in the intestinal mucosa. More oleic acid and palmitoleic acid were found in the colon and proximal ileum mucosa of the deficient animals (diet *d*) than in the free-fed animals (diet *a*), but more palmitoleic acid was found only in the medial ileum mucosa. There were also higher concentrations of eicosadienoic acid and docosatetraenoic acid in all segments of the intestine.

Muscle. The total fatty acid content of the ileum muscle was about 50% of that in the free-fed animals (diet *a*) (Table 3). As with the mucosa, the muscle of the colon had the same fatty acid content as in the free-fed animals but had 17% less fatty acid than the supplemented animals (diet *d*).

The linoleic acid content of the colon muscle (Fig. 3) was the same as in the colon mucosa (Fig. 6), but the ileum muscle (Figs. 1, 2) had only about one-fifth to one-quarter of the linoleic acid content of the corresponding mucosa (Figs. 4, 5). Since the supplemented animals (diet *d*) also contained less linoleic acid in the muscle than in the corresponding mucosa this difference is to be expected. The differences in the amount of eicosatrienoic acid compared with the free-fed animals were the same as those found in the mucosa but somewhat less in magnitude.

DISCUSSION

The changes in the fatty acids of the total lipid of the intestine in 'essential fatty acid'-deficient

rats were similar to those observed in the adipose tissues and pooled organs of rats by Mead (1957), in chicks by Dam, Engel & Nielson (1956) and in the fat of eggs from 'essential fatty acid'-deficient fowls by Reiser (1951). The appearance of a docosatetraenoic acid in 'essential fatty acid'-deficiency has not previously been reported. Under the chromatographic conditions used, which would detect 0.5% of a fatty acid of this type occurring in the mixture, none was visible on the chromatographs of the fatty acids from starved (diet *b*) and fed rats (diet *a*). Although the amount of this acid was small, it would contribute considerably to the amount of tetraenoic acid measured in deficient animals by alkali-isomerization techniques, when it might be presumed to be arachidonic acid. On the basis of studies by Steinberg, Slaton, Howton & Mead (1956, 1957) and Fulco & Mead (1959), this acid has been tentatively given the structure of docosa-6,9,12,15-tetraenoic acid, assuming synthesis from eicosa-7,10,13-trienoic acid found by Fulco & Mead (1959) in deficient animals and supposedly derived from palmitoleic acid. The increase in an eicosadienoic acid has not previously been reported, but Fulco & Mead (1959) found 8,11-eicosadienoic acid in deficient rats, presumably the precursor of 5, 8, 11-eicosatrienoic acid, and this may be the dienoic acid which was observed to increase in this study.

The effect of 'essential fatty acid' deficiency on fatty acid composition varies from organ to organ (Rieckehoff, Holman & Burr, 1949; Widmer & Holman, 1950), and variation was also observed in different parts of the intestine. The eicosatrienoic acid, first observed by Nunn & Smedley-MacLean (1938) and believed to be eicosa-5,8,11-trienoic acid by Mead & Slaton (1956), was found in highest concentrations in the middle ileum and least in the colon. Conversely, arachidonic acid and docosapentaenoic acid were at a very low concentration in the colon and differed from the normal least in the middle ileum. The greatest differences between supplemented (diet *d*) and deficient (diet *c*) animals in the concentration of linoleic acid, arachidonic acid, docosapentaenoic acid and docosahexaenoic acid are in the ileum mucosa; the smallest differences are in the colon mucosa. The docosahexaenoic acid concentration in the mucosa of the supplemented animals (diet *d*) was even greater than the concentration in the starved animals (diet *b*). These facts suggest a preferential requirement for essential fatty acids in the ileum compared with the colon.

The fatty acid composition of the intestine from the 'essential fatty acid'-supplemented animals (diet *d*), although markedly different from the 'essential fatty acid'-deficient animals (diet *c*), was not the same as the starved animals (diet *b*).

Since the total amount of fat in the supplemented rats (diet *d*) is on average 27 % below that in the starved animals (diet *b*) a direct comparison of the amounts of individual fatty acid does not indicate whether any differences are caused by a general decrease in the total lipid content or whether concentration changes have occurred in specific fatty acids or whether the proportions of the various lipid classes have changed. The stearic acid content of the mucosa of the supplemented rats (diet *d*) and the total lipid content are both lower than those found in the starved animals (diet *b*) by the same amount. Both deficient (diet *c*) and supplemented (diet *d*) animals have the same stearic acid concentration and, since stearic acid is readily synthesized by the animals, its concentration may be used as a standard against which the differences in the concentration of other acids may be judged. Thus the ratio of concentrations of stearic acid in proximal ileum muscle of supplemented and starved rats (supplemented/starved) is 0.55. The same ratio (0.55) is found if the concentrations of arachidonic acid in the proximal ileum muscle (supplemented/starved) are compared. Thus although the absolute concentration of arachidonic acid in the supplemented animals (diet *d*) is only about half that found in the starved animals (diet *b*), the relative proportion of the two acids is unchanged. Most fatty acids behave in the same way as arachidonic acid, or the ratio shows only small changes. For palmitoleic acid, however, the ratio of concentrations in proximal ileum muscle (supplemented/starved) is 1.02. Thus the absolute concentration of palmitoleic acid is almost the same in both types of animals in the proximal ileum muscle, but the concentration relative to stearate is 50 % greater. This shows that the proximal ileum muscle resembles the rest of the intestine where not only the ratio is high, but the absolute palmitoleic acid concentration is greater.

Docosahexaenoic acid is the only essential fatty acid that has the same concentration in the supplemented (diet *d*) and starved animals (diet *b*). The relatively high concentration of this acid in the supplemented animals may be due to the presence of 60 % of linolenic acid in the linseed oil supplement. Widmer & Holman (1950) observed that feeding with linolenic acid caused an increase in hexaenoic acids in the organs of 'essential fatty acid'-deficient rats.

The 'essential fatty acid'-deficient animals (diet *c*) and the supplemented animals (diet *d*) have a high absolute concentration of palmitoleic acid in the intestine. Since the diet in both cases is low in its content of total unsaturated fatty acids compared with the diet of the free-fed animals, the unsaturated fatty acids required to maintain a constant degree of unsaturation in the phospho-

lipids of the body can be made available only by synthesis in the body. Both palmitoleic acid and oleic acid can be readily synthesized. Thus the high concentration of these acids in both types of animal may reflect the low content of unsaturated fatty acids in the diet rather than being a specific sign of 'essential fatty acid'-deficiency.

The selective retention of arachidonic acid in 'essential fatty acid'-deficient rats (diet *c*) suggests that the major 'essential fatty acid' requirement is for this acid. However, the arachidonic acid peak may contain eicosa-8,11,14,17-tetraenoic acid derived from linolenic acid (Steinberg *et al.* 1957). The concentration ratios for arachidonic acid are similar to those for stearic acid in the supplemented animals (diet *d*), although the total concentration is half that of the starved animals (diet *b*). However, the relative and absolute concentrations of linoleic acid in the supplemented animals remain very low, as found by Mead (1957) in shorter-term experiments. Turpeinen (1938) found arachidonic acid to be a better growth-promoting agent for 'essential fatty acid'-deficient rats than linoleic acid, possibly because the latter is oxidized rapidly before it can be converted into arachidonic acid (Steinberg *et al.* 1956). Some linoleic acid, however, appears to be necessary, since small amounts of it persist in 'essential fatty acid'-deficient animals (Christensen, Dam & Engel, 1957). The linoleic acid remaining that was found in this study of the 'essential fatty acid'-deficient rats (diet *c*) supports this idea, although it may have been derived from the 8 % present in the olive oil in which the vitamins were given. Holman & Taylor (1950) observed that arachidonic acid did not alleviate the dermal symptoms of 'essential fatty acid'-deficiency.

The similarity in the fatty acid composition of the intestinal wall of fed (diet *a*) and starved rats may be explained by the similarity between the tissue fatty acids and those of the diet (Getz & Bartley, 1961). However, linoleic acid is at a higher concentration in the fed rat (diet *a*). This acid formed 40 % of the dietary fatty acid. Coniglio & Cate (1958) found no change in the intestinal palmitic acid but the disappearance of some unsaturated fatty acids not present in the diet suggests that these are preferentially oxidized during starvation. The concentration of the major fatty acids of the fed rats (diet *a*) are similar to those obtained by Paoletti & Grossi (1960).

The significance of the concentration of aldehydes in the muscle (and markedly in colon muscle and mucosa) is not known.

SUMMARY

1. The quantity and composition of the fatty acids of the total lipid of the rat intestinal muscle

and mucosa were determined at different sites in the intestinal tract in rats in four nutritional states: (a) normal diet of rat cubes; (b) normal diet of rat cubes followed by starvation for 24 hr.; (c) 'essential fatty acid'-deficiency; (d) fat-free diet supplemented by linseed oil.

2. The arachidonic acid and docosahexaenoic acid concentrations were 50 % higher in mucosa than in muscle.

3. Compared with the supplemented animals the 'essential fatty acid'-deficient rats contained 1 % of linoleic acid, 10 % of arachidonic acid, 8 % of docosapentaenoic acid and 20 % of docosahexaenoic acid. They also contained eicosadienoic acid and docosatetraenoic acid, which were not detected in starved or fed rats.

4. Both 'essential fatty acid'-deficient and supplemented rats had 50 % more palmitoleic acid than rats on a normal diet of rat cubes.

5. Essential fatty acids were lost more readily from the colon in deficient animals and replaced to a lesser extent in the supplemented animals.

6. The 'essential fatty acid'-deficient and supplemented animals have 35 % less fat in the intestine than the normal and starved rats.

7. Long-chain aldehydes were also estimated and found to be concentrated in the colon muscle.

We thank Professor Sir Hans Krebs, F.R.S., for his interest and advice and Dr C. W. Carter for providing a supply of rats deficient in 'essential fatty acids'. M.E. thanks the Medical Research Council for a training scholarship. This investigation was supported in part by the National Institutes of Health, United States Public Health Service (Grant no. A. 3369), and the Rockefeller Foundation.

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The Neutral Carbohydrate of Bovine Nasal Cartilage

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(Received 25 April 1962)

Besides chondroitin sulphate there exists in cartilage other carbohydrate material that contains hexose and seems to be far less negatively charged. A fraction containing such material was separated by Glegg, Eidinger & Leblond (1954) from an alkaline extract of cartilage by first precipitating the chondroitin sulphate with ethanol (67 %, v/v) and then precipitating the carbohydrate containing the

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neutral hexose by further addition of ethanol (to 84 %, v/v). So little is known about the hexose-containing carbohydrate that other approaches to its isolation and characterization are desirable. Extraction of bovine nasal cartilage with water in a high-speed homogenizer gives a water-soluble product that contains a large part, but not all, of the chondroitin sulphate of the cartilage in a form combined with protein which was called chondromucoprotein (Malawista & Schubert, 1958). Since