BBA 76075

UPTAKE OF VITAMIN E BY RAT SMALL INTESTINAL SLICES

C. K. PEARSON AND ALISON M. LEGGE

Department of Biochemistry, The University of Aberdeen, Marischal College, Aberdeen, AB9 1AS (Great Britain)

(Received May 15th, 1972)

SUMMARY

1. The uptake of vitamin E (α-tocopherol) by rat small intestinal slices from micellar incubation media of varying composition has been examined.

2. About 82 % of the radioactivity recovered from tissue lipids was associated with the mucosal layer, and was shown by thin-layer chromatography to be mostly unchanged α-tocopherol.

3. Uptake of α-tocopherol (30 min, 37 °C) was not substantially affected by: adding 5 mM monoolein, with or without 5 mM oleic acid, to 10 mM sodium taurodeoxycholate; increasing sodium taurodeoxycholate from 10 to 30 mM, or changing pH from 5.3 to 7.2. Uptake was greatly reduced by addition of an oil phase (triolein) to the micellar solution or by absence of a micellar phase.

4. When the concentration of α-tocopherol in the incubation medium was increased beyond the turbidity point a substantial change in uptake occurred only in one-quarter of the experiments.

INTRODUCTION

The molecular mechanisms operating during the intestinal absorption of lipids have come under considerable scrutiny throughout the last decade. However, relatively little work has been reported concerning the mechanism of absorption of the fat-soluble vitamins. Previous reports[1,2] have suggested that vitamin E is absorbed more efficiently from a micellar dispersion than from an emulsion. It has also been shown that the presence of bile salts in the intestinal lumen is necessary for absorption[3]. Bile salts are required for the formation of mixed micelles with the products of fat digestion, namely β-monoglycerides and free fatty acids[4]. These observations imply a need for a micellar dispersion of vitamin E prior to its absorption from the intestinal lumen.

This paper establishes the basic properties concerning the uptake of vitamin E by rat small intestinal slices when the composition of the incubation medium was systematically varied, in order to gain further knowledge about the molecular events which are important during its intestinal absorption.

MATERIALS AND METHODS

^{3}H-labelled vitamin E, DL-α-[5-*Me*-^{3}H]tocopherol (2000 mCi/mmole, 1 mCi/ml), was purchased from the Radiochemical Centre, Amersham, and its purity was checked by thin-layer chromatography on silica gel G using two solvent systems, chloroform or diethyl ether–light petroleum, b.p. 40–60 °C (15:85, by vol.) as developing solvents. The purity was checked at monthly intervals and it was repurified by preparative thin-layer chromatography if it fell below 90%. Unlabelled DL-α-tocopherol (Calbiochemicals, London) was used as supplied. It ran mainly as one spot when 100 μg were chromatographed on thin layers of silica gel G. A small amount of material was observed at the solvent front.

Sodium taurodeoxycholate was prepared using the method of Normann[5] as modified by Hofmann[6]. It ran as one spot when 100 μg was examined by thin-layer chromatography using the solvent system *n*-butanol–acetic acid–water (100:10:10, by vol.)[7]. Oleic acid (Sigma Chemical Co.) was used as purchased at a stated purity of 99%. Glycerol trioleate (British Drug Houses, Poole) was used as purchased. Glycerol monoolein (Calbiochemicals, London) was about 90% pure and was freed from the main impurities, diglyceride and triglyceride, by chromatography on columns of silicic acid–celite (2:1, w/w). About 3 g of glycerol monooleate was applied to a column of dimension 6 cm × 14 cm containing 250 g silicic acid–celite prepared in light petroleum, b.p. 40–60 °C. Diglyceride and triglyceride were removed completely by elution with 2 l diethyl ether–light petroleum, b.p. 40–60° (30:70, by vol.) followed by 50 ml diethyl ether–light petroleum (50:50, by vol.). The monoolein was then eluted from the column with 1 l diethyl ether and ran as one spot when 100 μg were examined by thin layer chromatography on boric acid-impregnated silica gel G in the solvent system chloroform–acetone–acetic acid (95.9:4.0:0.5, by vol.)[8].

Preparation of media

All dispersions of lipids were prepared in Krebs–Ringer phosphate buffer containing glucose (1 mg/ml) and the pH was usually 6.3 unless stated otherwise.

Lipids to be solubilised in a micellar form were pipetted into a flask in organic solvent which was then removed by evaporation under a stream of nitrogen. The required volume of sodium taurodeoxycholate in Krebs–Ringer phosphate buffer was added and the flask was then incubated at 37 °C in a shaking water bath until a clear micellar dispersion, as judged by the naked eye, was obtained. This method was found to be appropriate for solubilising glycerol monooleate[9] and oleic acid[10]. The α-tocopherol was added in a small volume of ethanol (usually 25 μl) to aqueous dispersions (usually 15 ml). This was to facilitate solubilisation particularly in the absence of swelling amphiphiles.

Preparation of intestinal slices

Female hooded rats of the Norwegian strain which weighed about 200–220 g were used throughout this work. Animals were starved overnight prior to an experiment, but were allowed water *ad libitum*. They were killed by a blow on the head and the small intestine was removed immediately into ice-cold Krebs–Ringer phosphate buffer usually at pH 6.3. The first 10 cm of small intestine (from the pyloric end) were discarded and the next 25 cm were taken and everted over a chilled glass rod

into Krebs–Ringer buffer. The everted tissue was slit lengthways and then cut into 4-cm lengths (about 400–600 mg tissue) to yield open sheets or segments. This was to prevent trapping of media inside the tissue during the washing procedure which followed incubation.

Experimental procedure

Intestinal slices were added to 2.5 ml prewarmed medium at 37 °C contained in screw cap phials. The medium had first been oxygenated by passing 100 % oxygen through it for about 1 h prior to the incubations, and the phials were flushed with oxygen when the slices were added. Incubations were carried out at 37 °C in a shaking water bath operating at about 80 cycles/min. At the required time intervals slices were removed and immediately washed in three separate 10-ml aliquots of 0.9 % saline, followed by a quick rinse in 10 ml ethanol.

The latter rinse was to remove remaining traces of adsorbed α-tocopherol. It is possible, however, that this wash might contain some tocopherol from the cell membrane or from the whole cell. There was no correlation between the proportion of ethanol-extractable label and the incubation conditions. Radioactivity recovered in this wash was usually about 10–30 % of that recovered in the tissue lipid extracts. The slices were blotted dry and weighed. Total lipid was extracted by homogenising the tissue in 10 ml chloroform–methanol (2:1, v/v). The homogenate was allowed to stand for 30 min and was then filtered through Whatman No. 1 filter paper. The residue and filter paper were re-extracted with 10 ml chloroform–methanol, filtered and the filtrate combined with the first extract. An equal volume of water was carefully layered on to the lipid extract and allowed to stand at 4 °C overnight. The upper aqueous phase was removed and the lower chloroform layer was evaporated to dryness under a stream of nitrogen[11]. Lipids were redissolved in 2 ml chloroform for radioactivity determination.

Liquid scintillation counting

All counting was carried out in an Intertechnique liquid scintillation counter (model SL 30). Two scintillation fluids were used. The first of these consisted of 5.0 g PPO and 0.1 g POPOP in 1.0 l toluene and was used for counting radioactivity in lipid samples. The second fluid contained 3.25 g PPO, 0.065 g POPOP, 52 g naphthalene, 250 ml toluene, 250 ml dioxane and 150 ml methanol, and was used for determining radioactivity in aqueous samples.

For radioactivity determinations samples were taken as follows: 0.1 ml from the medium before and after incubation with the tissue, 0.5 ml from the combined saline washes of the slices, 1 ml from the ethanol wash, 0.2 ml from the total lipid extract and 0.5 ml from the upper aqueous phase formed during lipid extraction. Radioactivity recovered in this latter fraction was negligible (0.2 % or less). Recovered radioactivity was always better than 93 % unless otherwise stated.

To correct for quenching the efficiency of counting was determined by internal standardisation. All results were then calculated as disintegrations per minute.

Calculations

Values for the uptake of α-[^{3}H]tocopherol are expressed as nmoles per g wet weight of tissue. Where appropriate (Table III) statistical analysis of results has been applied using the Student's *t* test.

RESULTS

Effect of incubation time on uptake

Uptake of α-[^{3}H]tocopherol was still proceeding after 60 min incubation, although the rate was slower than that of the first 15 min (Fig. 1). It was hoped to compare uptake from media of different composition but it was found in subsequent experiments that reproducible results were obtained only when comparisons were made at the same time, on the same source of intestinal segments. All experiments illustrated in Fig. 1 were performed independently, and comparisons of the effect of composition of the media on uptake are not therefore valid. The results do show however, that the tissue does not become saturated within a 60-min incubation

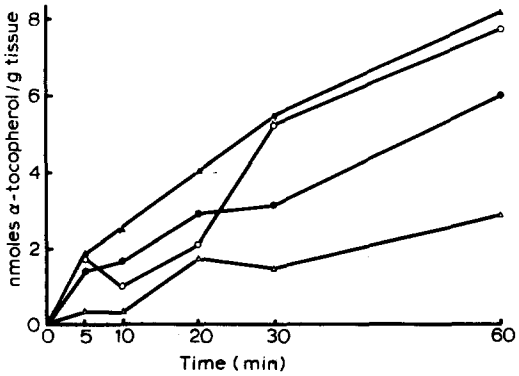

Fig. 1. Effect of incubation time on the uptake of α-tocopherol by slices of rat proximal small intestine. Incubations were carried out in 2.5 ml oxygenated Krebs–Ringer phosphate buffer (pH 6.3), containing glucose at 1 mg/ml and dispersed lipids. Each flask contained one 4-cm segment of tissue, weighing 300–600 mg, and incubations were carried out in duplicate. All flasks contained 0.17 μCi DL-α-[5-*Me*-^{3}H]tocopherol (12 μM). In addition dispersions had the following composition: ●—●, 10 mM sodium taurodeoxycholate and 5 mM monoolein; ○—○, 10 mM sodium taurodeoxycholate; ▲—▲, 10 mM sodium taurodeoxycholate, 5 mM monoolein and 0.5 mM glycerol trioleate; △—△, 0.5 mM glycerol trioleate. At appropriate times slices were removed and total lipid was extracted and radioactivity determined. The results are expressed as nmoles α-tocopherol taken up per g wet wt of tissue.

period at the concentration of α-tocopherol we were using in these experiments, 12 μM. In subsequent experiments incubation times were always less than 60 min, and comparisons of uptake of α-tocopherol from different media were carried out within the same experiment.

Distribution of radioactivity in slices

When slices were incubated for different times up to 60 min in a micellar dispersion containing α-[^{3}H]tocopherol, about 82 % of the recovered radioactivity was associated with the mucosal layer (Table I). The time of incubation did not appear to affect this distribution.

Characterisation of radioactivity in tissue total lipid (Table II)

Most of the α-tocopherol was recovered unchanged after thin-layer chromatography of tissue total lipid. Most of the remaining radioactivity was recovered from the origin in both solvent systems. Using chloroform as solvent most was sometimes

TABLE I

DISTRIBUTION OF RADIOACTIVITY BETWEEN THE MUCOSAL LAYER AND THE SEROSAL LAYER OF SLICES OF RAT PROXIMAL SMALL INTESTINE AFTER INCUBATION FOR 5, 10, 20, 30 OR 60 min IN A DISPERSION CONTAINING α-[^{3}H]TOCOPHEROL

The conditions of incubation were the same as those described for Fig. 1 except that all incubation flasks contained identical dispersions, *i.e.* 0.17 μCi α-[^{3}H]tocopherol (12 μM), 10 mM sodium taurodeoxycholate and 5 mM monoolein. At appropriate time intervals slices were removed from the flasks, rinsed and dried. The mucosal layer was separated from the muscle layer (serosal aspect) of each slice by scraping with a microscope slide. The two aspects were treated separately for extraction of total lipid and for radioactivity determination. The radioactivity recovered from the mucosal and muscle layer lipids was summated, and the results for the amounts of activity present in each aspect are expressed as a percentage of this.

Tissue	*Incubation time (min)*:				
	5	*10*	*20*	*30*	*60*
Mucosal	81.2	81.7	83.9	82.8	85.7
Serosal	18.8	18.3	16.1	17.2	14.3

TABLE II

CHARACTERISATION, BY THIN-LAYER CHROMATOGRAPHY, OF THE RADIOACTIVITY RECOVERED AFTER INCUBATION OF RAT PROXIMAL SMALL INTESTINAL SLICES FOR 5, 10, 20, 30 OR 60 min IN A MICELLAR DISPERSION CONTAINING α-[^{3}H]TOCOPHEROL

All incubation flasks contained media of the same composition as that described for Table I. After incubation the slices were removed, rinsed and dried, and total lipid was extracted. The lipid obtained from each slice was dissolved in 0.1–0.2 ml chloroform and aliquots were applied to thin layers of silica gel G together with 80 μg unlabelled α-tocopherol as carrier. About 1000–4000 cpm, representing roughly one tenth of the total lipid, was applied to the chromatograms. Samples of the stock α-[^{3}H]tocopherol (about 5000 cpm), together with 80 μg unlabelled α-tocopherol, were also applied to the thin layers. Chromatograms were developed either in chloroform or in diethyl ether–light petroleum, b.p. 40–60 °C (15:85, by vol.). After development the plates were removed from the tanks, the solvent was allowed to evaporate, and each plate was sprayed with an ethanolic solution containing 0.2% $FeCl_3$ and 0.5% α,α-dipyridyl[12]. The α-tocopherol stained as a red/pink spot. Areas of silica gel from the origin to the solvent front, were scraped into scintillation phials for determination of radioactivity. Areas of silica gel where no lipids had run were also taken to represent background values. All samples were counted for 4 min. The results are expressed as the percentage of the total recovered in the α-tocopherol spot.

Expt	*Solvent system*	% *radioactivity in the α-tocopherol spot*					% *purity of stock α-[^{3}H]tocopherol*
		Incubation time (min):					
		5	*10*	*20*	*30*	*60*	
1	Chloroform	91.5	92.7	89.5	83.2	93.2	*95.3 (93.7–97.5)
2		95.0	94.1	94.3	—	90.2	
1	Diethyl ether in light petroleum	80.5	80.0	81.5	79.0	73.0	*90.6 (90.2–92.4)
2		85.0	66.6	81.0	90.6	82.7	

* Mean of six determinations (range).

recovered from an area with an R_F of 0.4, which corresponds to that of α-tocopherol quinone, an oxidation product of α-tocopherol. α-Tocopherol quinone has an R_F of 0.06 in the second solvent system. Recoveries of radioactivity were usually about 97 %. The small decrease in purity is believed to be due to destruction of the molecule

during the experimental procedure, and not due to tissue metabolism, because the time of incubation did not have any significant effect on the value. If the tissue had metabolised the molecule to any extent, then the percentage recoveries of radioactivity in the α-tocopherol spot would have been expected to fall with increasing time of incubation.

We are unable to explain the differences obtained in the purity of the stock α-[^{3}H]tocopherol by the two solvent systems, since in both systems the α-tocopherol does not have the same R_F value as the usual oxidation product α-tocopherol quinone, or the R_F of dimers and trimers of α-tocopherol. The diethyl ether and the light petroleum were dried and distilled immediately prior to use so the presence of damaging peroxides was unlikely.

Effect of composition of incubation medium on uptake (Table III)

Uptake of α-tocopherol was not substantially affected by: adding 5 mM monoolein, with or without 5 mM oleic acid, to 10 mM sodium taurodeoxycholate; increasing sodium taurodeoxycholate from 10 mM to 30 mM, or changing pH from 5.3 to 7.2. The α-tocopherol molecule contains a phenolic hydroxyl group at position 6 on the chroman ring and therefore presumably possesses acidic properties. It was of interest to see whether changes in pH of the medium, such as might be found in the small intestine during digestion, would affect uptake due to varying degrees of ionisation of this group.

TABLE III

EFFECT OF VARYING THE COMPOSITION OF THE INCUBATION MEDIUM ON THE UPTAKE OF α-[^{3}H]-TOCOPHEROL BY RAT PROXIMAL SMALL INTESTINAL SLICES

Incubations (30 min) were carried out as described for Fig. 1. In addition to α-tocopherol each flask contained components as illustrated in this table. Each result represents the mean ± S.D. of 5 incubations. *P* refers to the differences between adjacent results. Results are expressed as nmoles α-tocopherol taken up per g wet wt of tissue. N.S., not significant ($P > 0.05$).

Expt	*Composition of incubation medium*				*pH*	*Uptake ± S.D.*	*P*
	Sodium taurodeoxy-cholate (mM)	*Monoolein (mM)*	*Oleic acid (mM)*	*Triolein (mM)*			
3a	10	—	—	—	6.3	11.3 ± 2.6	<0.02
	10	5	—	—	6.3	8.8 ± 0.2	
3b	10	5	—	—	7.0	6.9 ± 1.2	N.S.
	10	5	5	—	7.0	6.5 ± 1.4	
3c	10	—	—	—	6.3	8.1 ± 2.3	N.S.
	30	—	—	—	6.3	8.6 ± 1.9	
3d	10	5	—	—	5.3	7.3 ± 1.4	N.S.
	10	5	—	—	7.2	7.1 ± 1.0	
3e	10	5	—	—	6.3	9.0 ± 1.8	<0.01
	10	5	—	5	6.3	3.9 ± 0.5	
3f	—	—	—	5	6.3	2.17 ± 0.6	<0.001
	10	5	—	—	6.3	6.48 ± 1.8	

Uptake was greatly reduced by addition of an oil phase (triolein) to the micellar solution or by absence of a micellar phase.

In Expts 3e and 3f recoveries of radioactivity were 70 and 53 %, respectively. This was due to low recoveries of radioactivity from the media after incubation, and was probably due to aggregation of the ultrasonically dispersed lipid droplets. This was less extensive in Expt 3e because bile salt was present in the medium to stabilise the emulsion, but it was more marked in Expt 3f where no such stabilising detergent was present. This could reflect what happens in the intestinal lumen in the absence of bile salts. Such an aggregation of oil phase particles would presumably take place thus effectively removing molecules, which are dissolved in the oil phase, from the absorptive surface of the intestine and thereby decreasing absorption. Bile salts are known to be necessary for the absorption of vitamin E[1,3,13], and it has been noted that in the absence of bile salts the products of pancreatic lipolysis do not form micelles, and therefore remain with other lipids in the oil phase[14].

Effect of increasing the concentration of α-tocopherol in the incubation medium (Figs 2a and 2b)

Only in one out of four experiments was there a substantial change in slope at the onset of turbidity (T), although in two/three cases there was an inflection at this

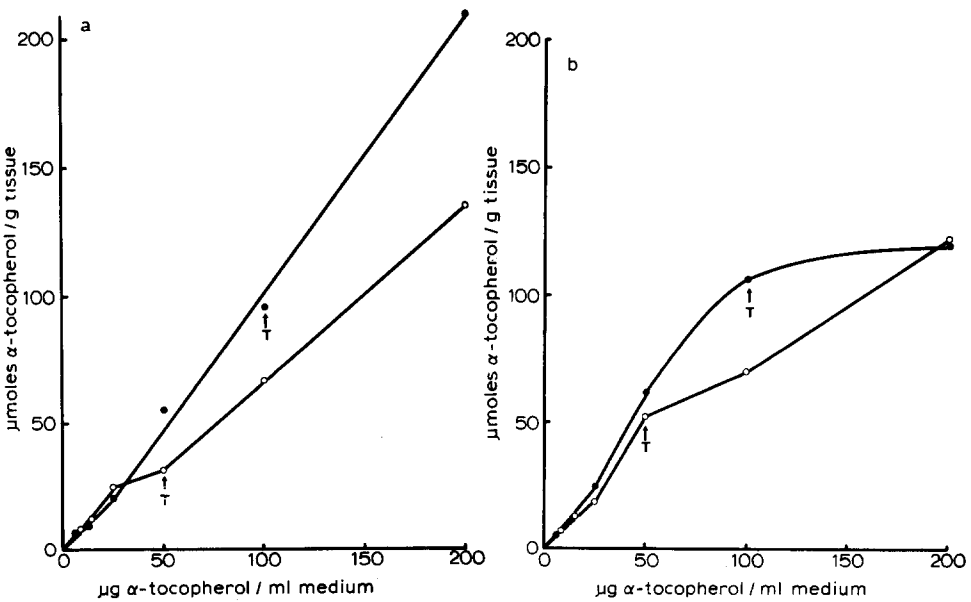

Fig. 2. Effect on uptake of increasing the concentration of α-tocopherol in the incubation medium. a. A series of dispersions (6-ml samples) of either 2.5 mM sodium taurodeoxycholate (○—○), or 1.25 mM monoolein and 2.5 mM sodium taurodeoxycholate (●—●), were prepared at 37 °C in Krebs–Ringer phosphate buffer (pH 6.3) containing glucose at 1 mg/ml. Different amounts of α-tocopherol in 10 µl ethanol were added to each sample to give concentrations of 6.25, 12.5, 25, 50, 100 and 200 µg/ml. 1 µCi DL-α-[5-*Me*-^{3}H]tocopherol was also added in 10 µl ethanol to each sample. 2.5-ml aliquots of these dispersions were taken for incubation with slices. One slice (300–600 mg) was present in each flask and incubations were for 30 min. Aliquots were also taken for absorbance measurements at 550 nm. Solutions were kept at 37 °C at all times. b. Repeat experiment of a.

point. Uptake was thus almost as effective in the presence of an emulsified phase, that is, after turbidity.

DISCUSSION

It is now well established that during intestinal digestion of fats the intestinal lumen contains an emulsified phase, consisting of triglycerides and diglycerides, and a micellar phase consisting of conjugated bile salts with 2-monoglycerides and free fatty acids, products of pancreatic lipolysis of triglycerides. Non-polar lipids, such as cholesterol and the fat soluble vitamins, will be partitioned between the two phases[15].

Table III (Expt 3e) shows the effect of the presence of an oil phase, represented by glycerol trioleate, in equilibrium with a micellar phase consisting of 10 mM sodium taurodeoxycholate and 5 mM monoolein. The α-tocopherol would be expected to be partitioned between the two phases. The uptake of the α-tocopherol compared with that from a micellar phase only was significantly lower ($P < 0.01$). Similar results have been reported for fatty acid uptake by rat everted jejunal sacs[16], whereby increasing amounts of oil phase in the media reduced fatty acid uptake in a manner corresponding to decreased fatty acid concentration in the micellar phase, that is decreased uptake of fatty acid was the result of increased partition in favour of the oil phase. Complete absence of a micellar phase (Expt 3f) reduced uptake even further.

In the presence of a finely dispersed emulsified phase (Figs 2a and 2b) uptake was not substantially affected (contrast Expt 3e). This may reflect the importance of the emulsifying properties of bile salts.

The hypothesis that the plasma membrane of the intestinal epithelial cell functions as an oil phase, and that the first stage of lipid absorption depends on a partitioning of lipids between the micellar phase and the cell membrane, has been considered for fatty acids and for the non-polar lipid oleyl alcohol[17]. Our results can be considered with such a hypothesis in mind. We found on increasing the complexity of the micellar composition that no substantial differences in uptake of α-tocopherol occurred (Table III). It would be expected that 10 mM sodium taurodeoxycholate would have a lower solubilising capacity for α-tocopherol than 10 mM sodium taurodeoxycholate and 5 mM monoolein, and on the above hypothesis the partition between cell membrane and micellar phase might be towards the cell membrane, *i.e.* favouring uptake. There was an increase in uptake of about 20 % ($P < 0.02$) (Expt 3a). However, further increase in micellar complexity, when oleic acid was also incorporated into the micelles, had no additional effect on uptake, neither did an increase in bile salt concentration from 10 to 30 mM sodium taurodeoxycholate (Table III, Expts 3b and 3c). This latter result can be contrasted with that of Webb *et al.*[17] who found a decreased uptake of the non-polar lipid oleyl alcohol with increasing bile salt concentration.

It would appear from these results that as long as the α-tocopherol was in a micellar dispersion, then the actual composition of the mixed micelles did not affect uptake. It is possible that the α-tocopherol could diffuse across the membrane at similar rates from different mixed micelles. The results could also be explained if the micelles were taken up intact. Conclusive data supporting such explanations are not available, however, at present.

The effect of pH using a range (pH 5.3–7.2) such as might be found in the small intestine, was found to have no affect on uptake (Table III, Expt 3d). As the pK_a of phenol and of poly alkyl-substituted phenols is about 10 it is probable that little, if any, ionisation of the phenolic hydroxyl group of α-tocopherol occurs. There would thus be a negligible difference in partition of the molecule between an oil and a micellar phase at the pH values used.

It does not appear, at least in the system we were using, that the epithelial cell membrane behaves merely as a lipid phase (*cf* Clark[18]).

ACKNOWLEDGEMENTS

We are grateful to Dr A. K. Lough of the Rowett Research Institute, Aberdeen, and to Professor H. M. Keir of this department for their valuable suggestions during the preparations of this manuscript.

REFERENCES

1 G. R. Thompson, *Gut*, 10 (1969) 1054.
2 M. T. MacMahon and G. R. Thompson, *Eur. J. Clin. Invest.*, 1 (1970) 161.
3 H. E. Gallo-Torres, *Lipids*, 5 (1970) 379.
4 A. F. Hofmann and B. Borgström, *Fed. Proc.*, 21 (1962) 43.
5 A. Norman, *Ark. Kem.*, 8 (1955–56) 331.
6 A. F. Hofmann, *Acta Chem. Scand.*, 17 (1963) 173.
7 H. Gänshirt, F. W. Koss and K. Morianz, *Arzneimittelforschung*, 10 (1960) 943.
8 A. E. Thomas III, J. E. Scharoun and H. Ralston, *J. Am. Oil Chem. Soc.*, 42 (1965) 789.
9 A. F. Hofmann, *Biochem. J.*, 89 (1963) 57.
10 A. F. Hofmann, *Biochim. Biophys. Acta*, 70 (1963) 306.
11 M. J. Albrink, *J. Lipid Res.*, 1 (1960) 53.
12 A. Emmerie and C. Engel, *Nature*, 142 (1938) 873.
13 M. T. MacMahon and G. Neale, *Clin. Sci.*, 38 (1970) 197.
14 A. F. Hofmann, *Gastroenterology*, 50 (1966) 56.
15 B. Borgstrom, *J. Lipid Res.*, 8 (1967) 598.
16 K. Y. Lee, W. J. Simmonds and N. E. Hofmann, *Biochim. Biophys. Acta*, 249 (1971) 548.
17 J. P. W. Webb, J. D. Hamilton and A. M. Dawson, *Biochim. Biophys. Acta*, 187 (1969) 42.
18 S. B. Clark, *J. Lipid Res.*, 12 (1971) 43.