Biochem. J. (1974) **140**, 509–516
Printed in Great Britain

Involvement of Binding Lipoproteins in the Absorption and Transport of α-Tocopherol in the Rat

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(*Received* 3 *December* 1973)

1. Specific lipoproteins binding α-tocopherol but not its known metabolites have been isolated and identified from cytosol of rat intestinal mucosa and from serum. 2. A time-study of the appearance of the orally administered α-[^{3}H]tocopherol with these lipoproteins indicates that very-low-density lipoprotein of serum acts as a carrier of the vitamin. 3. The involvement of the mucosal lipoprotein in the absorption of the vitamin from the intestine has been inferred from observations on the amounts of α-tocopherol in serum of orotic acid-fed rats where release of lipoproteins from the liver to serum is completely inhibited. A considerable decrease in the association of α-tocopherol with serum very-low-density lipoprotein under this condition is interpreted to mean that serum lipoproteins are limiting factors for the transport of the vitamin across the intestine and that this is possibly effected by exchange of α-tocopherol between serum very-low-density lipoprotein and mucosal lipoprotein.

The occurrence of an α-tocopherol-binding lipoprotein in rat liver cytosol, its isolation and purification have been reported in a previous communication (Rajaram *et al.*, 1973). Since little is known about the mechanism of absorption and mode of transport of this vitamin, the present studies were designed to find out whether similar lipoproteins exist in the cytosol of intestinal mucosa and in serum and, if so, their possible involvement in the processes of absorption and transport of the orally fed vitamin.

Materials and Methods

Chemicals

DL-α-[5-*Me*-^{3}H]Tocopherol (4.6mCi/mg) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Acrylamide, *NN'*-methylenebisacrylamide and *NNN'N'*-tetramethylethylenediamine were purchased from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K. Sephadex G-200 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. PPO (2,5-diphenyloxazole), dimethyl-POPOP [1,4-bis(4-methyl-5-phenyloxazol-2-yl)benzene] and orotic acid monohydrate were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. All other chemicals used were either AnalaR grade from BDH Chemicals Ltd., Poole, Dorset, U.K., or Proanalyst quality from E. Merck A.G., Darmstadt, Germany.

Animals and treatment with orotic acid

Male albino rats of the Wistar strain, housed in individual cages, were fed normal laboratory stock diet and used when they attained a weight of about 200g. The normal diet contained vitamin-free casein (18%), glucose (68%), cellulose (5%), peanut oil (5%) and salt mixture (USP XIV; 4%). The following vitamins were added to this diet (mg/kg): thiamine hydrochloride, 2; riboflavin, 4; pyridoxine, 4; choline, 1000; inositol, 1000; *p*-aminobenzoic acid, 300; nicotinamide, 100; folic acid, 2.5; vitamin B_{12}, 0.05; biotin, 0.1; ergocalciferol, 0.042; vitamin K, 10; α-tocopherol, 50; vitamin A, 1.72; calcium pantothenate, 10 (Roels *et al.*, 1964). Orotic acid, when fed, was mixed into this diet at a concentration of 1% and animals were maintained on this diet for 1 week.

Methods

Oral administration of α-[^{3}H]tocopherol. DL-α-[5-*Me*-^{3}H]Tocopherol (100μCi) was mixed with 1.2mg of unlabelled DL-α-tocopherol in 95% (v/v) ethanolic solution. The mixture, after evaporation of the solvent, was dissolved in 0.5ml of olive oil and administered orally by stomach tube under light ether anaesthesia to rats weighing 200g that had been starved overnight.

Preparation of liver and small-intestinal mucosal cytosol. Rats under light ether anaesthesia had their

livers perfused via the hepatic portal vein with chilled 0.9% NaCl. After perfusion, which took about 5min, the liver was quickly excised, weighed and chilled in 0.25M-sucrose containing 10mM-Tris–HCl buffer, pH7.4, and 1mM-EDTA. A 50% liver homogenate was prepared in the same medium by using a Potter–Elvehjem-type glass homogenizer with a Teflon pestle and giving two strokes at a low speed of 1000–2000rev./min. The homogenate was centrifuged at 105000*g* for 60min in a Beckman L265B ultracentrifuge to give clear cytosol which was withdrawn with a syringe without disturbing the top fatty layer.

The lumen of the entire small intestine was washed by flushing several times with 0.25M-sucrose containing 10mM-Tris–HCl buffer, pH7.4, and 1mM-EDTA to remove the food particles, and the washings were checked for counts until free of radioactivity. The intestine was cut into short lengths, split longitudinally to expose the mucosa and scraped. The mucosal scrapings were homogenized in 9vol. of a solution containing 0.25M-sucrose, 10mM-Tris–HCl (pH7.4), 1mM-EDTA and 0.1% $BaSO_4$ as described by Hübscher *et al.* (1965). After an initial centrifugation at 10000*g* for 10min to sediment cell debris, mucus and mitochondria, the supernatant was spun at 105000*g* for 60min to obtain clear cytosol. This was concentrated by reverse dialysis against Polywax (polyethylene glycol) 6000 (Koppikar *et al.*, 1971).

Serum was obtained from the blood drawn through the posterior vena cava.

Sephadex chromatography. A column (1.8cm× 50cm) uniformly packed with Sephadex G-200 gel and equilibrated with 0.05M-sodium phosphate buffer (pH7.4) was employed for the isolation and initial purification of α-tocopherol-binding protein. A sample of cytosol or serum containing 70–100mg of protein was loaded on the gel and elution carried out in the cold (4°C) at a flow rate of 20ml/h with the phosphate buffer mentioned above, and fractions (2.5ml) were collected (Koppikar *et al.*, 1971). Extinction of the various fractions was measured in a Shimadzu QV 50 spectrophotometer at 280nm in a 1cm light-path cell. Suitable dilutions of the samples were made wherever necessary to measure *E* within the working range of the instrument.

Thin-layer chromatography. The fractions comprising peak 1 from the Sephadex column were extracted three times with acetone–light petroleum (b.p. 40–60°C) (1:2, v/v) (7.5ml of acetone+15ml of light petroleum each time) and the extracts were evaporated to dryness in the dark under a stream of N_2. The residue was dissolved in 0.5ml of ethanol containing 300μg of carrier α-tocopherol. α-Tocopherol was separated from interfering lipids by t.l.c. on silica-gel G with benzene–hexane (19:1, v/v) as developing solvent. The completed chromatogram was viewed under u.v. light to mark the α-tocopherol spots which were quickly scraped and eluted with 2×1.5ml of absolute ethanol. The u.v.-absorption spectra of the eluates were recorded in a Perkin–Elmer–Hitachi double beam spectrophotometer. Additionally, the eluates were also tested by the Emmerie–Engel (1938) reaction. The chromatographic plate was exposed to I_2 vapour to identify other spots, if any.

Polyacrylamide-gel electrophoresis. Polyacrylamide-gel electrophoresis was carried out in a 3.5% running gel. The spacer gel of 2.5% polyacrylamide, as modified by Koppikar *et al.* (1971), was used. All other details of the electrophoretic system were essentially as described by Narayan *et al.* (1966*a*). Prestaining of the lipoprotein was carried out with Sudan Black B in ethylene glycol as described by McDonald & Ribiro (1959). Amido Black was used as a protein stain.

Uptake in vitro of α-[^{3}H]tocopherol by serum from mucosal cells of orotic acid-fed rats. α-[5-*Me*-^{3}H]-Tocopherol (100μCi) was fed orally to orotic acid-fed rats and 4h later the mucosal scrapings were collected. A portion (1g) of the mucosal cell suspension in 2ml of 0.25M-sucrose, buffered with 10mM-Tris–HCl, pH7.4, and 1mM-EDTA and containing a total radioactivity of about 389750c.p.m., was incubated with 1ml of unlabelled serum either from normal rat or from orotic acid-fed rat with or without 1ml of lipoprotein fraction I isolated by Sephadex chromatography of normal serum. The incubations were carried out in test tubes at 37°C for 30min in a Dubonoff metabolic shaker water bath, and then were chilled immediately and centrifuged quickly to sediment the mucosal cells. The radioactivity incorporated into the supernatant was determined.

Analytical methods. Protein determinations were carried out by the method of Lowry *et al.* (1951) with crystalline bovine albumin as standard. Lipids were extracted by chloroform–methanol (2:1, v/v) and portions were used for lipid analysis by the method of Bragdon (1951).

Radioactivity determinations. Portions of the various samples were spotted on strips (7cm×1.8cm) of Whatman 3MM filter papers, dried and placed in counting vials containing 0.3% PPO and 0.01% dimethyl-POPOP in 10ml of toluene as scintillator (Roodyn *et al.*, 1965). Radioactivity was counted in a Beckman LS-100 liquid-scintillation spectrometer with 59% instrument efficiency. A comparison of the effect of filter-paper strips on counting efficiency was made by measuring the radioactivity of 0.01ml of ethanolic DL-α-[5-*Me*-^{3}H]tocopherol of known radioactivity in the presence and absence of Whatman 3MM filter-paper strips. In the presence of paper strips the counting efficiency was 12%. All values reported are actual c.p.m. measured without any correction being applied. For determining the radioactivity in the polyacrylamide gel, the gels were sliced

with a thin razor to isolate the individual bands and the segments placed in loosely capped vials containing 0.5ml of 30% H_2O_2 and incubated at 60°C for 5h as described by Young & Fulhorst (1965). Portions of this solution were spotted on Whatman 3MM strips and counted as described above.

Portions of ethanolic extracts from the silica-gel t.l.c. plates were also counted for ^3H radioactivity.

Results

Association of α-tocopherol with proteins of mucosal and liver cytosol and serum

A possible binding of orally fed α-[^{3}H]tocopherol with proteins of mucosal cytosol and serum was investigated to determine its significance in the absorption and transport of the vitamin. Rats fed with 100μCi of α-[5-*Me*-^{3}H]tocopherol were killed after 4h. This time-interval was chosen because it is known that lipids are maximally absorbed at 3–4h after oral administration (Fatterpaker, 1952). Sephadex G-200 gel filtration of mucosal cytosol (Fig. 1*a*) gave three distinct protein fractions of which the first protein peak, eluting between fractions 15 and 20, contained about 87% of the total cytosol α-[^{3}H]tocopherol. This particular fraction also showed the presence of lipids when analysed by the method of Bragdon (1951), indicating that the binding protein is probably lipoprotein in nature. Vitamin E was not associated with any other protein fractions of the cytosol.

The serum proteins were separated into five fractions (Fig. 1*b*). Vitamin E was maximally (82%) associated with the first protein fraction which had a specific radioactivity (i.e. c.p.m. in a portion of protein solution that gives $E_{280} = 1$ in a 1cm-light-path cell) of 5080 c.p.m./*E*. This fraction, on the basis of lipid composition, has been shown to contain a mixture of chylomicrons, VLD lipoprotein* and LD lipoprotein (S. V. Koppikar, P. Fatterpaker & A. Sreenivasan, unpublished work). No measurable radioactivity was found in other fractions.

A simultaneous study of the liver cytosol proteins (Fig. 1*c*) was done for comparison. As has been shown previously (Rajaram *et al.*, 1973), vitamin E is carried by a lipoprotein fraction of liver cytosol that comes immediately after the void volume. The lipoprotein fractions of liver and mucosal cytosol that carry vitamin E, as well as that of serum, resemble each other in their elution pattern.

It was also ascertained that the radioactivity associated with the lipoprotein fractions of mucosa, serum and liver was not due to the metabolic products of α-tocopherol. For this, the first protein fractions obtained after Sephadex chromatography of these samples were extracted with acetone–light petroleum (b.p. 40–60°C) (1:2, v/v) and the concentrated extract was subjected to t.l.c. with benzene–hexane (19:1, v/v) as developing solvent. The completed chromatogram in each case revealed a major spot with R_F value 0.71 corresponding to that of standard α-tocopherol; this spot also gave a u.v.-absorption spectrum characteristic of α-tocopherol (Fig. 2), besides showing a positive Emmerie–Engel (1938) reaction. In addition, the chromatogram also showed a minor spot with R_F value 0.11, which corresponds to α-tocopheryl-quinone. The distribution of radioactivity among these spots is represented in Table 1. About 75–80% of the radioactivity in the various fractions could be

* Abbreviations: VLD lipoprotein, very low-density lipoprotein; LD lipoprotein, low-density lipoprotein.

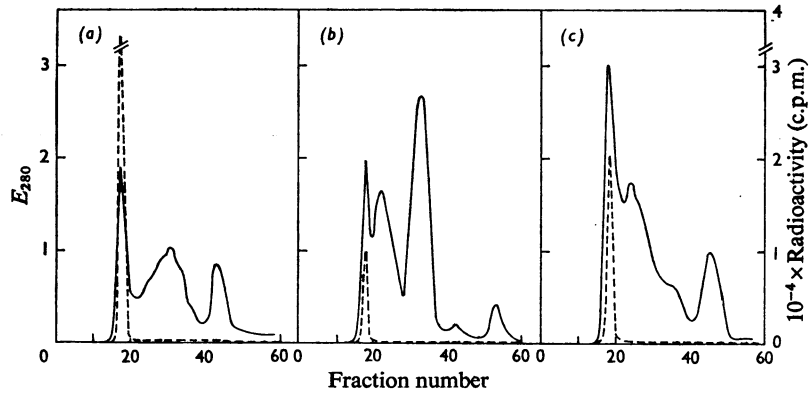


Fig. 1. *Gel filtration of intestinal mucosal cytosol (a), serum (b) and liver cytosol (c)*

A Sephadex G-200 column was equilibrated with 0.05M-sodium phosphate buffer (pH7.4). Portions (2ml) of the respective samples were loaded on the columns and 2.5ml fractions collected. Other details are given in the text. ——, E_{280}; – – – –, radioactivity.

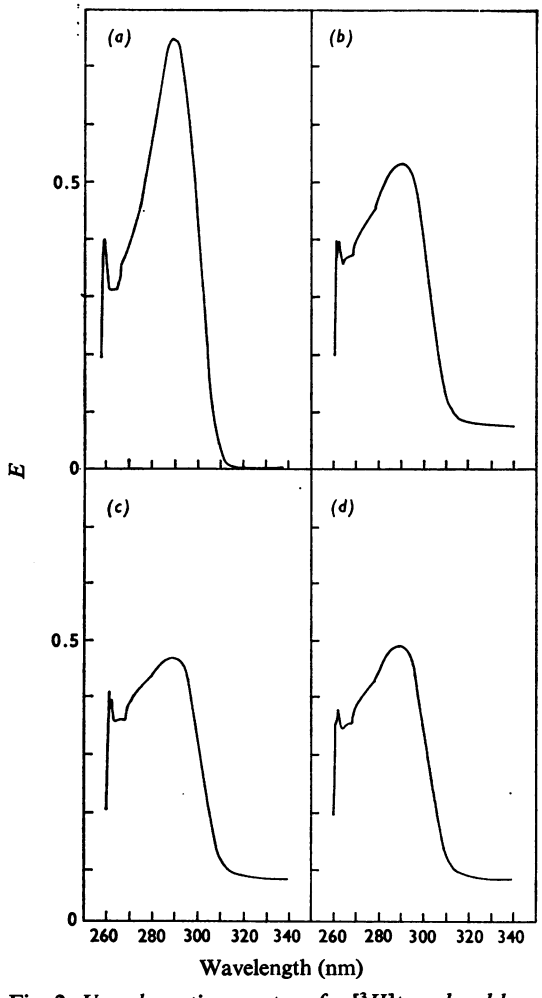


Fig. 2. *U.v.-absorption spectra of α-[^3H]tocopherol bound to lipoprotein fractions*

U.v. spectra, showing absorption maxima at 292nm of α-tocopherol separated by t.l.c. from cytosol of mucosa (*b*), serum (*c*) and liver cytosol (*d*) respectively. For details see the Materials and Methods section. The spectrum for standard α-tocopherol (*a*) is included for comparison.

recovered as α-tocopherol and only 2–3% as α-tocopherylquinone.

In the second experiment (Table 2), the association of α-[^{3}H]tocopherol with serum, liver and mucosal cytosol lipoproteins was studied at various time-intervals after oral administration of the vitamin. Orally administered vitamin appears in the mucosal cytosol and blood serum only at 3h after administration and not at earlier time-intervals studied, whereas at 3h the liver cytosol contained no measurable radioactivity. The association of α-tocopherol

Table 1. *Percentage recovery of α-[^3H]tocopherol from lipoprotein fractions*

The first lipoprotein fractions obtained after Sephadex chromatography of cytosol of mucosa and liver and of serum samples were each extracted with acetone–light petroleum (b.p. 40–60°C) (1:2, v/v) and α-tocopherol was separated from the concentrated extract by t.l.c. as described in the text. R_F values of α-tocopherol and α-tocopherylquinone were 0.71 and 0.11 respectively. Values in parentheses represent the total radioactivity (c.p.m.) in the sample used for extraction. Results are typical of three independent experiments.

	α-Tocopherol		α-Tocopherylquinone	
Sample	(c.p.m.)	(% recovery)	(c.p.m.)	(% recovery)
Mucosa (54260)	41695	75	780	2.0
Serum (13415)	10730	80	340	3.0
Liver (24590)	19180	78	425	2.5

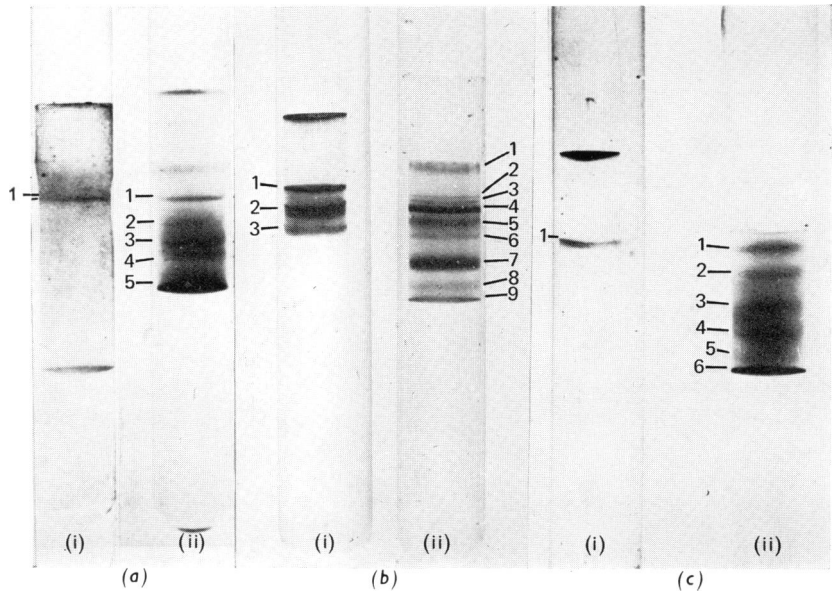
Table 2. *Association of α-[H^3]tocopherol with serum, liver and mucosal lipoproteins*

α-[5-*Me*-^{3}H]Tocopherol (100μCi/200g body wt.) was administered orally to rats, and its association with the first lipoprotein fraction obtained by Sephadex chromatography of mucosal cytosol, serum and liver cytosol was studied at various time-intervals. Other details are given in the text. Values are expressed as specific radioactivities (i.e. c.p.m. present in a portion of a protein solution that gives $E_{280} = 1$ in a 1cm light-path cell). Results are means of five independent determinations ±S.E.M.

	α-[^{3}H]Tocopherol (c.p.m./*E*)		
Time (h)	Mucosal cytosol	Serum	Liver cytosol
3	4160± 321	1700±151	—
4	19030±1365	5080±540	8160±620
6	9275± 715	1600±125	3388±280
8	1240± 95	845± 60	1970±165
18	105± 12	212± 20	479± 30

with these lipoprotein fractions increased with time, reaching a maximum at 4h after administration and declining thereafter. The serum showed no further increase in radioactivity up to 18h.

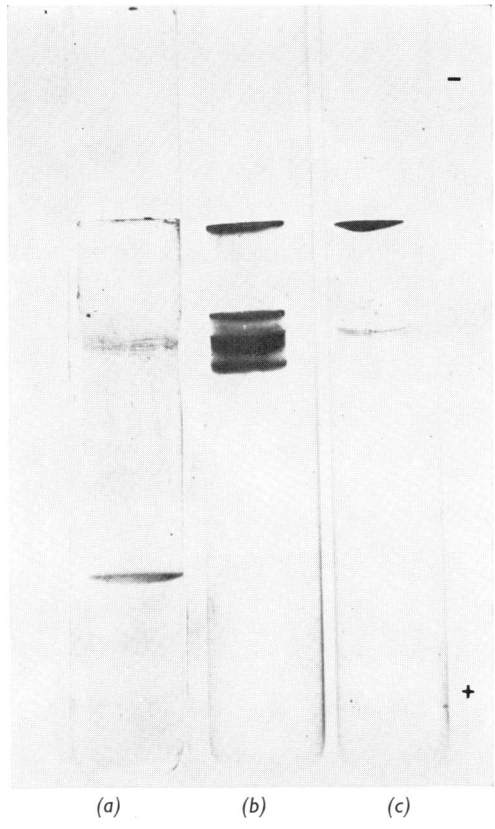
Further purification of vitamin E-binding lipoprotein fraction of mucosal and liver cytosol and serum has been achieved by polyacrylamide-gel electrophoresis. The first protein peak obtained after Sephadex chromatography of these samples was concentrated by reverse dialysis against Polywax 6000 and used for electrophoresis. The proteins of the mucosal cytosol fraction resolved into five bands of which the first protein band was a lipoprotein (Plate



EXPLANATION OF PLATE 1

Polyacrylamide-gel electrophoretogram of mucosal cytosol (a), serum (b) and liver cytosol (c) protein fractions

The first protein peak obtained after Sephadex chromatography of these samples was concentrated by reverse dialysis against Polywax and used for gel electrophoresis. Protein (350μg) was loaded on the column. For lipoprotein separation a prestained sample of 1 mg equiv. of protein was used. Other details are given in the text. (i) Lipoprotein; (ii) protein.



EXPLANATION OF PLATE 2

Electrophoretic mobilities of the lipoprotein carrying α-[^3H]tocopherol

(*a*) Mucosal cytosol; (*b*) serum; (*c*) liver cytosol. For details see the text.

Table 3. *Relative radioactivity of the different lipoprotein/protein bands separable by polyacrylamide-gel electrophoresis*

Details of electrophoresis are given in the text and in the legend to Plate 1. The individual bands were sliced, treated with 30% H_2O_2 and radioactivity determinations were made as described in the text. Values in the bands are expressed as percentage of the total radioactive (c.p.m.) of all protein fractions (minus blanks) in a given gel. Remaining bands contain negligible radioactivity. Results are typical of four independent experiments. Maximum radioactivity was observed in the same fraction in each experiment.

	% recovery of α-[^{3}H]tocopherol					
	Mucosal cytosol		Serum		Liver cytosol	
Band no.	Proteins	Lipoproteins	Proteins	Lipoproteins	Proteins	Lipoproteins
Origin	4.2	8.0	7.2	5.6	6.0	8.3
1	81.5	79.8	9.4	4.5	82.9	84.5
2	6.7	—	77.3	84.0	5.5	—
3	3.2	—	—	4.6	3.0	—
4	—	—	6.1	—	—	—

1*a*), which stained with Sudan Black B. The maximum recovery of radioactivity from this lipoprotein band (Table 3) indicated the specific association of vitamin E with this lipoprotein. The proteins of the serum fraction resolved into nine well-defined bands of which three were lipoproteins (Plate 1*b*). Vitamin E was associated with the lipoprotein band 2. Narayan *et al.* (1966*b*) have measured the electrophoretic mobilities of the lipoproteins of various density classes. By using this system it has been shown in our laboratory (S. V. Koppikar, P. Fatterpaker & A. Sreenivasan, unpublished work) that fraction I from Sephadex chromatography of serum could be resolved into chylomicrons, VLD lipoprotein and LD lipoprotein. The association of α-tocopherol with the lipoprotein band 2 strongly indicates that VLD lipoprotein is a carrier of orally fed vitamin. The protein and lipoprotein bands separable from the liver cytosol fraction are represented for comparison in Plate 1(*c*). The presence of a specific α-tocopherol-binding lipoprotein in this fraction has already been discussed in our previous communication (Rajaram *et al.*, 1973). Plate 2 compares the electrophoretic mobilities of lipoproteins from mucosal cytosol, serum and liver cytosol carrying α-tocopherol.

The radioactivity in the protein and lipoprotein bands obtained after electrophoresing the mucosal and liver cytosol and serum fractions has been represented in Table 3. In the small-intestinal mucosal supernatant, protein band 1 (Table 3) and the corresponding lipoprotein band 1 contained about 80% of the total radioactivity as against negligible values in the remaining bands. Protein bands 2 and 3 (Table 3) of serum together account for about 77% of the total radioactivity. These two bands had very similar electrophoretic mobility, hence they could not be individually sliced. They correspond to the lipoprotein band 2 from which 84% of the radioactivity could be recovered. For the liver cytosol fraction, protein band 1 contained the highest radioactivity and this corresponds to the lipoprotein band from which 84% radioactivity could be recovered.

Effect of orotic acid feeding on the absorption and binding of α-tocopherol with serum lipoproteins

To understand the part played by the lipoprotein of intestinal mucosa in the transfer of the vitamin across the intestine, absorption of α-[^{3}H]tocopherol was studied in orotic acid-fed rats. It is known that addition of as little as 1% orotic acid in the diet completely inhibits the production of lipoproteins by the liver (Windmueller & Levy, 1967). With this as a basis, Windmueller & Levy (1968) have tested the capacity of the intestine to synthesize lipoproteins. Their results indicate that intestinal lipoprotein synthesis is not affected by orotic acid feeding. Hence, we have utilized this treatment as a means of ascertaining whether α-tocopherol is transported directly into serum in association with mucosal lipoprotein or not. Orotic acid feeding resulted in a 75–80%

Table 4. *Effect of orotic acid feeding on the association of α-[^{3}H]tocopherol with lipoprotein fractions of serum and mucosal cytosol*

Orotic acid (1%) in the diet was fed to rats for 7 days. Lipoproteins from serum and intestinal mucosal cytosol were isolated by Sephadex G-200 gel chromatography as described in the text. Results are averages of four experiments ±S.E.M.

	α-[^{3}H]Tocopherol (c.p.m./*E*)	
Group	Mucosal lipoprotein	Serum lipoprotein
Orotic acid-fed	18120±1150	1215±110
Control	19030±1365	5080±540

decrease in the radioactivity of serum lipoprotein fraction as compared with controls (Table 4). In the case of mucosal lipoprotein, however, there was no change in radioactivity. A very low specific radioactivity of α-[^{3}H]tocopherol in the serum lipoprotein of orotic acid-fed rats with no change in the radioactivity of the mucosal lipoprotein probably indicates that α-tocopherol is not carried *in toto* into serum bound with mucosal lipoprotein and that the concentration of serum VLD lipoprotein may be important for optimum transfer of the vitamin across the intestine.

Uptake of α-tocopherol by serum from the mucosal cells *in vitro* was examined to gain further information. Table 5 shows the uptake of α-[^{3}H]tocopherol by serum from the small-intestinal mucosal cells of orotic acid-fed rats. Serum isolated from orotic acid-treated rats takes up 11% of the label and this is enhanced to 38% on the addition of VLD lipoprotein-containing fraction of normal serum to the incubation medium. Control serum takes up to 33% of the label which is three times the value for serum from orotic acid-fed rats. The results thus indicate that there is enhancement in the uptake of α-tocopherol by serum from orotic acid-fed rats upon addition of VLD lipoprotein fraction of normal serum.

Table 5. *Uptake in vitro of α-[^{3}H]tocopherol by serum from small intestinal mucosal cells of orotic acid-fed rats*

Orotic acid-fed rats weighing about 200g received an oral dose of 100μCi of α-[5-*Me*-^{3}H]tocopherol and were killed 4h later. Mucosal cells (1g) suspended in 2ml of the homogenizing medium and containing a total radioactivity of 389750c.p.m. were incubated with 1ml of serum for 30min at 37°C. The first lipoprotein fraction (VLD lipoprotein) isolated by Sephadex chromatography of normal serum (Fig. 1*b*) was concentrated by reverse dialysis over polyethylene glycol 6000, and 1ml of concentrate containing 20–25mg of protein was added to the incubation system. Other details are described in the text. Results are averages of four experiments each with a different batch of mucosal cells ±S.E.M. *P* values for significance of increase from A to B and A to C are less than 0.01.

System	Uptake in serum (total c.p.m.)	(%)
A. Mucosal cells+serum from orotic acid-fed rats	45500±1960	11.7
B. Mucosal cells+serum from normal rats	128360±9471	33.0
C. Mucosal cells+serum from orotic acid-fed rats+lipoprotein fraction of normal serum	147800±12400	38.0

Discussion

Despite the numerous studies on the absorption of α-tocopherol in animals (Simon *et al.*, 1956*a*; Krishnamoorthy & Bieri, 1963; Pearson & Barnes, 1968; Kelleher *et al.*, 1969) and a few in man (Klatskin & Molander, 1952; Rosenkrantz *et al.*, 1953; Kelleher & Losowsky, 1970), the mechanism of absorption is still obscure. Unlike vitamin A, as reported by Ganguly (1969), vitamin E is not re-esterified during absorption. This is inferred from several studies (Simon *et al.*, 1956*a*,*b*; Wiss *et al.*, 1962) that have shown an increase in the amount of free α-tocopherol in plasma even after oral dosing with α-tocopherol acetate or α-tocopheryl phosphate. Blomstrand & Forsgren (1968) have suggested that the route of absorption is lymphatic and that most of the absorbed vitamin is probably located in the chylomicrons (Johnson & Pover, 1962). It is known that orally administered α-tocopherol is absorbed to the extent of about 20–25% only, the remainder being excreted in faeces (Simon *et al.*, 1956*a*).

In our preliminary experiments on the intracellular distribution of the vitamin, we observed that the soluble portion of the small-intestinal mucosa contained appreciable radioactivity 4h after oral administration of α-[^{3}H]tocopherol. About 40% of the total radioactivity in the mucosa was associated with cytosol and the remainder with the particulate material. The results of the present experiments indicate that the absorbed α-tocopherol is partly associated with a lipoprotein fraction of the intestinal mucosal cytosol separable by Sephadex gel filtration and polyacrylamide-disc electrophoresis. This is accompanied by a simultaneous appearance of the vitamin in the VLD lipoprotein of serum and a lipoprotein fraction of liver cytosol. The association of the vitamin with these lipoproteins reaches a maximum at 4h after administration and declines thereafter. Unlike vitamin A, there is no further increase in the vitamin content of serum implying that the transported vitamin is not released back from the liver into the serum. This suggests that VLD lipoprotein of serum may be the carrier of orally fed vitamin.

A variety of metabolic products, chiefly α-tocopherylquinone, dimers and trimers, are known to be formed after administration of the vitamin (Csallany *et al.*, 1962; Csallany & Draper, 1963; Draper *et al.*, 1967). Csallany *et al.* (1970) have successfully separated these products by t.l.c. with benzene–hexane (19:1, v/v) as developing solvent. In the present investigation, the same solvent system has been employed for the purification of α-tocopherol. The results indicate that over 75% of radioactivity associated with the lipoprotein fraction is due to α-tocopherol alone. The recovery of radioactivity as α-tocopherylquinone was negligible and it is possible that this product could have been formed

during the isolation procedures. Dimers or trimers, detectable by this procedure, were absent in the protein-bound fractions. The results thus rule out the possibility of any of these metabolic products being associated with the lipoprotein fraction.

The lipoproteins of the mucosal and liver cytosol are identical with VLD lipoprotein of serum in their elution pattern from the Sephadex column (Fig. 1) and mobility on polyacrylamide gel (Plate 2). The electrophoretic mobility of these lipoproteins in a sufficiently large-pore gel of 3.5% polyacrylamide was rather slow and at higher concentrations (5% and 7.5% polyacrylamide) these bands could not penetrate the running gel. The Sephadex gel-filtration pattern and the electrophoretic behaviour of these lipoproteins of mucosal and liver cytosol that carry α-tocopherol strongly indicate that the molecular size of the binding protein is very high. Of the several protein bands obtained, vitamin E is associated with a lipoprotein band in all the cases as seen by the recovery of 80% radioactivity in these bands. The results incidentally give a direct proof for the presence of VLD lipoprotein-like lipoprotein in the mucosal cells of small intestine, though its presence had been implicated by other workers (Windmueller & Levy, 1968; Kessler *et al.*, 1970). The observed association of α-tocopherol with the soluble lipoproteins of the intestinal mucosa is of significance from the point of view of its possible involvement in the transfer of α-tocopherol across the intestinal wall. The results of orotic acid feeding experiments suggests that α-tocopherol is not secreted directly into serum in association with mucosal lipoproteins. These studies also suggested that the concentration of serum VLD lipoprotein is a limiting factor for optimum transfer of the vitamin across the intestine, and that this is possibly effected by an exchange of α-tocopherol between mucosal cytosol lipoprotein and serum VLD lipoprotein, analogous to the exchange of phospholipids among the various plasma lipoproteins (Minari & Zilversmit, 1963). No increase in the radioactivity of the mucosal lipoprotein was observed (Table 4) as would be expected for a feedback mechanism. It is probable that such an increase may occur in the mucosal subcellular fractions which may reflect an increase, perhaps, in the free α-tocopherol. This possibility needs further investigation. The present observations are in accordance with the suggestions of Ganguly *et al.* (1959) and Glover & Green (1957) regarding occurrence of specific lipoproteins in the intestinal mucosa, which help in the transport of fat-soluble substances like carotenoids, vitamin A, sterols etc. across the intestine. A probable involvement of α-tocopherol-carrying lipoprotein of liver cell-sap in the intracellular transport and distribution of the vitamin has been reported in our previous communication (Rajaram *et al.*, 1973).

Thus the present observations point to the existence of specific lipoproteins in rat tissues, i.e. intestinal mucosa, blood and liver cells. The mucosal lipoprotein that carries the vitamin probably aids in the absorption of α-tocopherol. The serum VLD lipoprotein, which represents the transport form is involved in the carriage of the vitamin to the various tissues in addition to its important role in the transfer of the absorbed vitamin across the intestine. Lastly, the transported vitamin is distributed among the intracellular organelles, in association with a lipoprotein of the cytosol probably specific to various organs.

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