Studies on the in vivo absorption of micellar solutions of tocopherol and tocopheryl acetate in the rat: demonstration and partial characterization of a mucosal esterase localized to the endoplasmic reticulum of the enterocyte

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Abstract The in vivo absorption of α-tocopherol from micellar solubilized solutions of free α-tocopherol and α-tocopheryl acetate was investigated using isolated loops of rat jejunum and found to be similar. Although analysis of the fluid remaining in the loop following the absorptive period with tocopheryl acetate showed that esterase activity and free tocopherol were present, calculations suggested that luminal hydrolysis of the ester could not have accounted for the similar rates of absorption of the free and esterified tocopherols. A mucosal source of the esterase activity was postulated and subsequently identified and characterized with respect to pH optimum, enzyme kinetics, and activation by bile salts, and shown to be distinct from pancreatic esterase. Analytical subcellular fractionation studies on homogenates of isolated jejunal enterocytes demonstrated that esterase activity against both *p*-nitrophenyl acetate and tocopheryl acetate was localized to the endoplasmic reticulum. These studies suggest that although the bulk of tocopheryl esters are normally hydrolyzed in the intestinal lumen by pancreatic esterase prior to uptake, some of the esters may be hydrolyzed intraluminally by the mucosal enzyme and some taken up intact by the jejunal mucosa and hydrolyzed intracellularly. This mucosal esterase may be of particular importance in conditions of pancreatic insufficiency.—**Mathias, P. M., J. T. Harries, T. J. Peters, and D. P. R. Muller.** Studies on the in vivo absorption of micellar solutions of tocopherol and tocopheryl acetate in the rat: demonstration and partial characterization of a mucosal esterase localized to the endoplasmic reticulum of the enterocyte. *J. Lipid Res.* 1981. **22:** 829–837.

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Serum concentrations of vitamin E are frequently reduced in patients with fat malabsorption (1–4) and supplements of the vitamin are often administered to correct the deficiency. The vitamin is usually given orally in an esterified form (e.g., DL α-tocopheryl acetate), as the ester is less susceptible to oxidation than the free form. There have, however, been relatively few studies on the in vivo absorption of tocopheryl esters.

By cannulating the thoracic duct in man, Blomstrand and Forsgren (5) showed that DL α-tocopheryl [^{14}C]acetate given orally was hydrolyzed before being transported in lymph as free α-tocopherol. Gallo Torres (6) studied the absorption of radiolabeled tocopheryl acetate in the rat and found that most of the radiolabel recovered in lymph was in the free unesterified form and that almost no labeled α-tocopheryl acetate could be detected; moreover both bile and pancreatic juice were found to be "a requisite for the intestinal absorption and the lymphatic transport of vitamin E". Essentially similar findings were reported in studies on the absorption of [^{3}H]α-tocopheryl nicotinate (7). Nakamura et al. (8) studied the in vitro hydrolysis and in vivo absorption of a number of different tocopheryl esters in the rat. These workers found that the esters that were more easily hydrolyzed were better absorbed and were recovered in lymph mostly in the free form, whereas the esters that resisted hydrolysis were relatively poorly absorbed and were recovered unchanged in the lymph.

In previous in vitro studies (9) we have shown that pancreatic esterase rather than pancreatic lipase is the principal hydrolytic enzyme for tocopheryl acetate and that bile salts served two important functions in this process. First, together with the lipolytic products of dietary triglyceride, they formed mixed micelles that were able to solubilize highly nonpolar

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lipids such as tocopheryl acetate and thus provide the correct physico-chemical form of the substrate for esterase activity (10). Second, bile salts were important cofactors for optimal esterase activity. The aim of the present study was to investigate further the absorption of free tocopherol and tocopheryl acetate from mixed micellar solutions instilled into isolated jejunal closed loops of the rat in vivo, in order to gain a clearer understanding of the role and importance of pancreatic esterase and bile salts in the hydrolysis and absorption of tocopheryl acetate. During the course of the study, esterase activity was also demonstrated in the jejunal mucosa and studies were established to localize and partially characterize this mucosal esterase.

MATERIALS AND METHODS

Materials

Oleic acid, DL α-tocopherol, DL α-tocopheryl acetate, cholesteryl oleate, and *p*-nitrophenyl acetate were obtained from the Sigma Chemical Company Limited, and were all 99% pure. Sodium taurocholate was prepared by the method of Norman (11) as modified by Hofmann (12). The purity of the bile salt was determined by the enzymatic method of Iwata and Yamasaki (13) with 3α-hydroxy steroid dehydrogenase (Cambrian Chemicals Limited) following thin-layer chromatography using the solvent system, amyl acetate–n-propanol–propionic acid–water 4:3:2:1 (v/v) described by Hofmann (14). The sodium taurocholate was found to be 94% pure with sodium taurodeoxycholate being the major impurity. D-(5-methyl-^{3}H)tocopherol, and cholesterol [1-^{14}C]oleate were obtained from the Radiochemical Centre, Amersham. The tritium-labeled tocopheryl acetate was prepared from the labeled free tocopherol by the procedure of Wilson, Kodicek, and Booth (15) and was found to be more than 95% pure following thin-layer chromatography in cyclohexane–chloroform–glacial acetic acid 6:3:1 (v/v). The esterified cholesterol was 99% pure after thin-layer chromatography (cyclohexane–ethyl acetate 6:4 (v/v). Glycerol tributyrate was supplied by British Drug Houses Limited and *p*-tosyl-arginine methyl ester by the Sigma Chemical Company Limited. Scintillation counting was performed on a LKB Wallac 1210 betacounter with external standardization using a dioxane based scintillation cocktail of 70 g naphthalene, 7.0 g PPO, and 0.526 g POPOP per liter, of 1,4 dioxane. These materials were obtained from Hopkin and Williams Limited with the exception of PPO and dioxane, which were purchased from British Drug Houses Limited. Ultrafiltration was carried out using a 25 mm stirred ultrafiltration cell and filters supplied by Millipore (U.K.) Limited. Automatic titration was performed using a system supplied by Radiometer consisting of a pH meter 25, titrator 11, and autoburette ABU 11 linked to a recorder.

Methods

Preparation of mixed micellar solutions for in vivo experiments. Mixed micellar solutions of α-tocopherol and α-tocopheryl acetate were prepared as described previously (16). A dispersion containing tocopherol or tocopheryl acetate, 0.5 mmol/l (plus tracer amounts of radiolabel); oleic acid, 4 mmol/l; and sodium taurocholate, 10 mmol/l, in phosphate buffer (pH 7.4, NaH_2PO_4, 2.88 mmol/l; Na_2HPO_4, 12.12 mmol/l) containing KCl (4 mmol/l), glucose (20 mmol/l), and NaCl to bring the osmolarity to 280–290 mosmol/l was mixed on a vortex mixer, sonicated, and filtered through a 220-nm millipore filter. The maximal amount of tocopheryl acetate that could be solubilized was 0.16–0.18 mmol/l, compared to approximately twice that concentration for the free tocopherol. It was important that the micellar concentrations of the tocopherol and tocopheryl acetate solutions should be similar for the in vivo studies and so the concentration of the free tocopherol was adjusted to that of the ester by diluting with the isotonic buffer containing the sodium taurocholate, oleic acid, and glucose.

In vivo experiments. The in vivo jejunal closed loop experimental model was routinely used as described by Harries and Sladen (17). Preliminary experiments with micellar solubilized free tocopherol (0.17 mmol/l) showed that luminal disappearance (from now on referred to as absorption) increased rapidly during the first 10 min to reach a maximum of 100 mmol/g wet weight of jejunum by about 20 min; an absorptive period of 20 min was used in subsequent studies. Following the absorptive period, the animal was killed by cardiac puncture, the loop was dissected out, and the remaining intraluminal contents (i.e., postincubation fluid) was collected over solid carbon dioxide and stored at −20°C until analyzed. Where necessary, the small intestinal mucosa was scraped and handled as described below.

The luminal disappearance of α-tocopherol or its acetate ester was estimated radiochemically by taking 0.25-ml aliquots of the pre- and postincubation fluids in duplicate into 10 ml of scintillant. Net absorptive rates were calculated and expressed as nmol/g wet weight of jejunum/min.

On one occasion, postincubation fluid was collected after a number of sequential absorptive periods. An

outlet tube (Portex 4/80) was inserted into the distal end of the loop and clamped. At the end of the absorptive period, the clamp was removed and the postincubation fluid was drained by gently passing 5 ml of air through the proximal end of the loop.

In a further experiment, the proximal jejunum was perfused at a rate of 0.5 ml/min as described by Sladen and Harries (18), and effluent was collected as pooled 5-min aliquots over a 35-min period prior to a closed-loop absorption study.

In vitro assays for esterase activity. Esterase activity (EC 3.1.1.6) was estimated using mixed micellar solutions of tocopheryl acetate and cholesteryl oleate or the water-soluble substrate *p*-nitrophenyl acetate. The two micellar substrates were prepared as described above for the in vivo absorption studies and the assays were performed as described in detail elsewhere (16). Esterase activity against *p*-nitrophenyl acetate was estimated by the method of Erlanson (19). Activities were expressed as milliunits/ml fluid or milliunits/mg protein where 1 milliunit corresponds to the hydrolysis of 1 nmol of the substrate/min under the defined conditions.

Pancreatic and jejunal mucosal homogenates. A 20–30 cm portion of proximal jejunum was removed from a fasted male Wistar rat (250–300g) under ether anesthesia, washed with 20 ml of ice-cold isotonic saline, and placed on a glass plate resting on a block of ice. It was then cut along its antimesenteric border and the mucosa was carefully scraped off using the end of a glass slide. The mucosal scrapings were weighed and homogenized using an electrically operated variable speed stirrer with a P.T.F.E. plunger and glass vessel in 10 ml of potassium chloride solution (0.15 mmol/l). Homogenates of rat pancreas were also prepared in 10 ml of 0.15 mmol/l potassium chloride solution.

Analytical subcellular fractionation of enterocytes from the jejunum of the rat. Whole enterocytes were isolated from a 25–30-cm portion of rat proximal jejunum by the method of Batt and Peters (20) and a post-nuclear supernatant was prepared following homogenization and low speed centrifugation (21). Analytical subcellular fractionation of the post-nuclear supernatant was performed in a Beaufay automatic zonal rotar (22) as described by Peters (23). Following centrifugation, 15 fractions were collected, weighed, and stored at −20°C prior to assay. After thorough mixing of the tubes, the sucrose density was determined indirectly with an Abbe refractometer (24). Protein was assayed by a modification (25) of the fluorimetric method of Hiraoka and Glick (26) with bovine serum albumin (Armour Pharmaceutical Company) as a standard. The activity of α-glucosidase (EC 3.2.1.20) estimated at the appropriate pH was used as a marker enzyme for both the brush border and endoplasmic reticulum (27) and was assayed with a 4-methyl umbelliferyl derivative supplied by Koch Light Limited (23, 25). The brush border enzyme activity was estimated at pH 6.0 in the presence of zinc ions (an inhibitor of the endoplasmic reticulum enzyme), and the activity of the endoplasmic reticulum enzyme was measured at pH 8.0 in the presence of Tris, which is an inhibitor of the brush border enzyme (28). Esterase activities against tocopheryl acetate and *p*-nitrophenyl acetate were estimated as indicated above but with minor modifications to increase sensitivity.

Results are expressed in the form of frequency density histograms with the pooling and averaging of the distribution being performed by computer (29).

Miscellaneous estimations. Free tocopherol was estimated colorimetrically by a modification of the Emmerie and Engel reaction (30) as previously described (4). Tocopheryl acetate concentrations were determined following hydrolysis of the ester as described by Bieri and Prival (31); the liberated free vitamin was extracted and quantitated as above. To estimate mixtures of free and esterified vitamin E, tocopherol concentrations were assayed before and after alkaline hydrolysis.

Pancreatic lipase was estimated titrimetrically at pH 8.0 with glycerol tributyrate as substrate (32). Tryptic activity was also measured titrimetrically at pH 8.0 (33) but with *p*-tosyl-arginine methyl ester as substrate. Activities of lipase and trypsin are expressed as units/mg protein, where one unit corresponds to the conversion of 1 μmol of the substrate/min under the defined conditions. Protein was measured routinely by the method of Lowry et al. (34) with bovine serum albumin (grade V, Sigma Chemical Co) as a standard. Bile salts were quantitated by the enzymatic method of Iwata and Yamasaki (13) following chromatography on Amberlite XAD-2 resin (35) supplied by British Drug Houses Limited.

Results are expressed throughout as mean ± 1 SEM and the significance of differences between mean values was assessed by the Student *t* test.

RESULTS

In vivo studies

Absorption of α-tocopherol and α-tocopheryl acetate from mixed micellar solutions was compared in two groups, each of six rats. There was no difference between the two groups; the mean (±1

TABLE 1. Activity of postincubation fluids against tocopheryl acetate and *p*-nitrophenyl acetate

Test Solution	Volume of Postincubation Fluid	Substrate	
		Tocopheryl Acetate	*p*-Nitrophenyl Acetate
	ml	*milliunits/ml fluid*	
Tocopherol (6)[a]	2.11 ± 0.06[b]	12.4 ± 1.6	1200 ± 100
Tocopheryl acetate (6)	2.10 ± 0.12	9.0 ± 0.6	700 ± 50

[a] Figures in parentheses, number of animals studied.
[b] Mean ± 1 S.E.M.

SEM) absorption rates being 3.60 ± 0.08 and 3.45 ± 0.12 nmol/g wet weight/min for the free vitamin and its ester, respectively, which corresponded to an absorption of approximately 40%.

This finding was unexpected, as pancreatico-biliary secretions had apparently been excluded by this closed-loop model, and no esterase, lipase, or tryptic activity or bile salts could be detected in the saline wash. When, however, the residual post-incubation fluids from the study with tocopheryl acetate were analyzed, significant amounts of the vitamin were in the free form (16.5 ± 2.5%) and esterase activity against both tocopheryl acetate and *p*-nitrophenyl acetate was present in all loop contents (**Table 1**). Studies were therefore established to determine the origin of this esterase activity.

The effectiveness of the routine 20-ml saline wash to remove pancreatico-biliary secretions was re-assessed by washing jejunal loops with either one, three, five, or seven separate 3-ml volumes of isotonic saline and assaying the last 3-ml aliquot for esterase activity against *p*-nitrophenyl acetate and tocopheryl acetate (**Fig. 1**). Esterase activity was detectable against *p*-nitrophenyl acetate after a single 3-ml wash procedure, but no esterase activity could be detected against either substrate after more thorough washings. Following the last 3-ml wash, the loops were instilled with the isotonic buffer solution for 20 min followed by the mixed micellar tocopheryl acetate solution for a further 20 min period, and the postincubation fluids were assayed for esterase activity (Fig. 1). Activity against both substrates was present in all samples but incubation with the mixed micellar solution resulted in higher (approximately 5-fold) enzyme activities than with buffer alone. Similar levels of esterase activity were obtained after 20-min absorptive periods with a pure solution of 10 mmol/l sodium taurocholate in buffer (1.2×10^3 and 8 milliunits/ml against *p*-nitrophenyl acetate and tocopheryl acetate, respectively). On no occasion was there any detectable lipase or tryptic activity.

Esterase activity against *p*-nitrophenyl acetate was also estimated both during and after a perfusion with an isotonic buffer solution containing 10 mmol/l sodium taurocholate. Activity increased during the 35-min perfusion period with no activity being detectable in the first 5-minute period but an activity of 130 milliunits/ml being present in the last period. The total activity in a total volume of 13.9 ml was 820 milliunits (i.e., 59 milliunits/ml). Following the per-

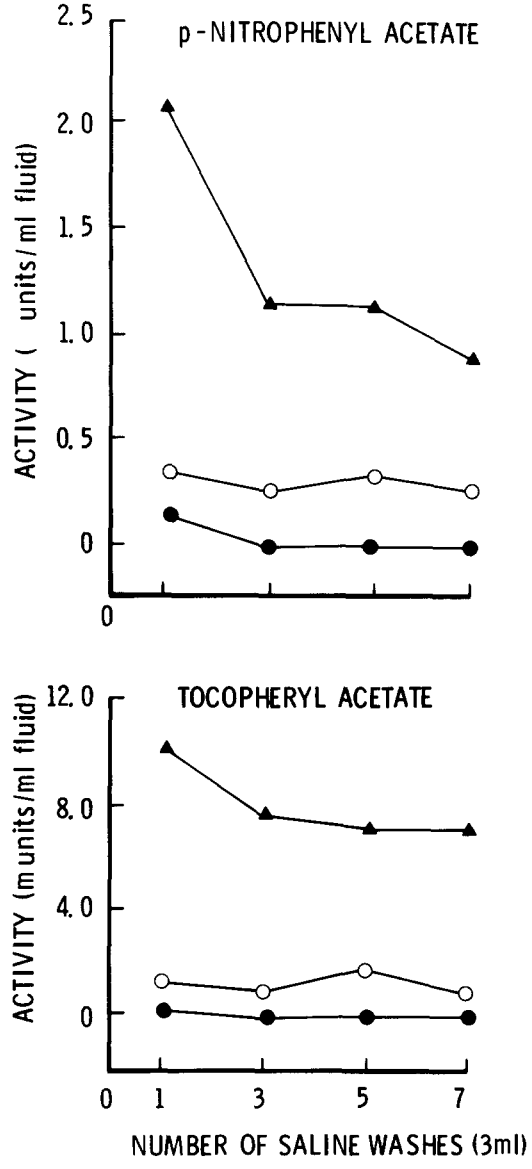

Fig. 1. Enzyme activities against *p*-nitrophenyl acetate and tocopheryl acetate following an increasing number of saline washes. Activity present in the last 3-ml saline wash (●), and activities present in post-incubation fluids following instillation of buffer (○) and micellar solubilized tocopheryl acetate (▲) in the jejunal loop for 20 min after the last 3-ml saline wash.

fusion, the perfused loop was closed and 2 ml of the same solution was instilled for a further 20-min absorptive period. The postincubation fluid was then assayed against *p*-nitrophenyl acetate and tocopheryl acetate and gave activities of 1.03×10^3 and 8.1 milliunits/ml, respectively.

In vitro studies on mucosal homogenate of rat mucosa

The in vivo studies suggested the presence of a mucosal esterase and this was confirmed by assaying crude mucosal homogenates for esterase activity. Mucosal homogenates were prepared from the jejunal loop following the perfusion experiment and when assayed against *p*-nitrophenyl acetate and tocopheryl acetate gave activities of 12.1×10^3 and 196 milliunits/g wet weight, respectively, which was approximately 7 and 15 times that present (on a weight basis) in the postincubation fluid (1.7×10^3 and 13.4 milliunits/g, respectively).

Characterization of mucosal and pancreatic esterases from crude homogenates

Homogenates of pancreas and jejunal mucosa of six rats were prepared and a comparison was made of the specific activities of esterase (using three substrates), lipase, and trypsin (**Table 2**). With *p*-nitrophenyl acetate and tocopheryl acetate as substrates, the activity of the mucosal esterase was significantly greater than the pancreatic activity ($P < 0.001$ and <0.002, respectively). The mucosal preparation, however, showed no activity against cholesteryl oleate over the pH range 6.0 to 8.5 despite increasing the bile salt concentration. The mucosal homogenate had only trace amounts of lipolytic and tryptic activity. The two enzyme activities had different pH optima

TABLE 2. Specific activities of esterase, lipase, and trypsin in mucosal and pancreatic homogenates

	Mucosa (6)[a]	Pancreas (6)	
	milliunits/mg protein		
Esterase			
p-Nitrophenyl acetate	687 ± 117[b]	140 ± 12.2	$P < 0.001$
Tocopheryl acetate	5.8 ± 0.5	3.2 ± 0.4	$P < 0.002$
Cholesteryl oleate	undetectable	15.5 ± 2.2	
	units/mg protein		
Trypsin	0.23 ± 0.04	3.54 ± 0.41	
	units/mg protein		
Lipase	4.5 ± 0.8	110 ± 8.2	

[a] Figures in parentheses, number of animals studied.
[b] Mean ± 1 S.E.M.

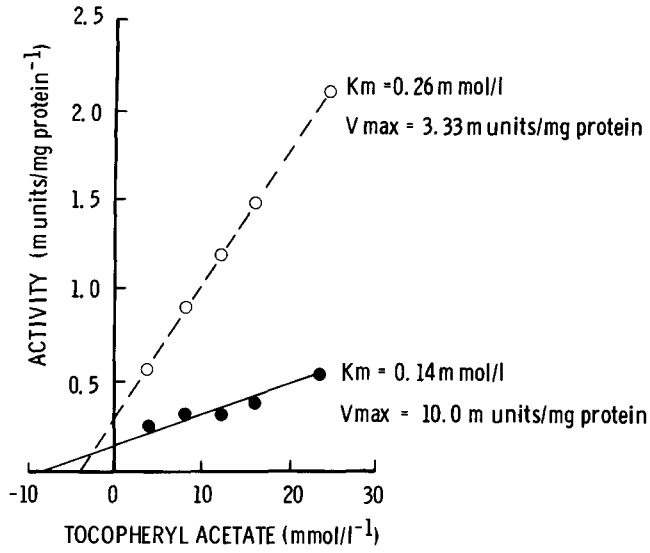

Fig. 2. Lineweaver-Burk plots of enzyme activity against various concentrations of micellar solubilized tocopheryl acetate. Homogenates of rat jejunal mucosa (●) and pancreas (○).

against tocopheryl acetate; they were pH 6.5 and 8.5 or greater for the mucosal and pancreatic enzymes, respectively. The kinetics of the two enzyme preparations using tocopheryl acetate as substrate also varied. The activity of the pancreatic enzyme was linear for at least 30 min and up to 0.6 mg protein, whereas the activity of the mucosal enzyme was only linear for 10 min and up to 0.15 mg protein. The maximal hydrolysis rates and substrate affinities were also different for the two enzyme sources (**Fig. 2**). The addition of increasing concentrations of sodium taurocholate to the mixed micellar tocopheryl acetate system resulted in increased activities of both enzyme sources (**Fig. 3**) but, at concentrations greater than 10 mmol/l, the mucosal enzyme showed the greater activity.

When stored at either −20 or −70°C, the esterase activities of both enzyme sources were stable against tocopheryl acetate for at least 7 months. At 4°C the pancreatic enzyme was more labile, with loss of more than 50% of its activity by the 1st week and more than 95% by the 5th week. The mucosal activity, however, decreased by 30% within 3 weeks but thereafter (up to 7 months) did not change.

To rule out bacteria as the source of the esterase activity, homogenates of jejunal mucosa from two germ-free rats (kindly donated by Dr. A. Watts, Department of Conservative Dentistry, Guy's Hospital, London) were assayed for esterase activity. Mean specific activities of 4.1 and 610 milliunits/mg protein were obtained against tocopheryl acetate and *p*-nitrophenyl acetate, respectively, which were similar

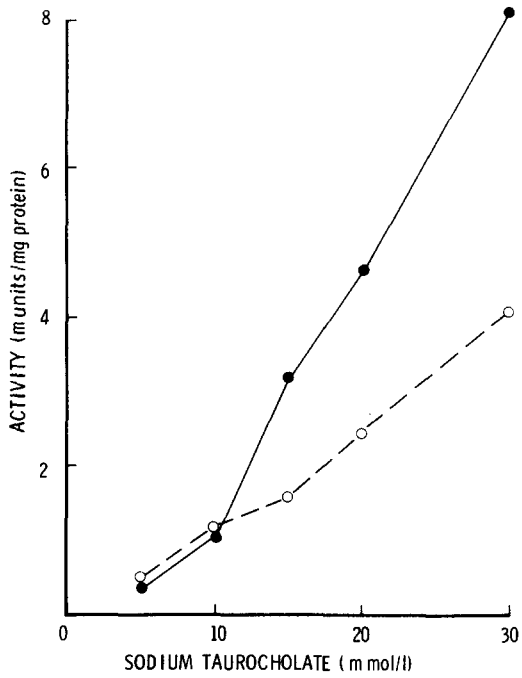

Fig. 3. Effect of increasing concentrations of sodium taurocholate on enzyme activities against micellar solubilized tocopheryl acetate. Homogenates of rat jejunal mucosa (●) and pancreas (○).

to those obtained using the conventionally reared animals.

Subcellular localization of the mucosal esterase activity

The sucrose density distributions of the marker enzymes for the brush border (zinc-resistant α-glucosidase) and endoplasmic reticulum (Tris-resistant α-glucosidase) of the jejunal enterocyte homogenate after isopycnic centrifugation are shown in **Fig. 4.** The brush border enzyme had a modal density of 1.19 g/ml whereas that of the endoplasmic enzyme was 1.16 g/ml. Esterase activities against *p*-nitrophenyl acetate and tocopheryl acetate showed very similar distribution patterns, both having modal densities of 1.16 g/ml that closely followed the distribution of the endoplasmic reticulum marker.

DISCUSSION

These experiments compare the in vivo absorption of α-tocopherol and its acetate ester from jejunal closed loops in the rat. Both forms of the vitamin

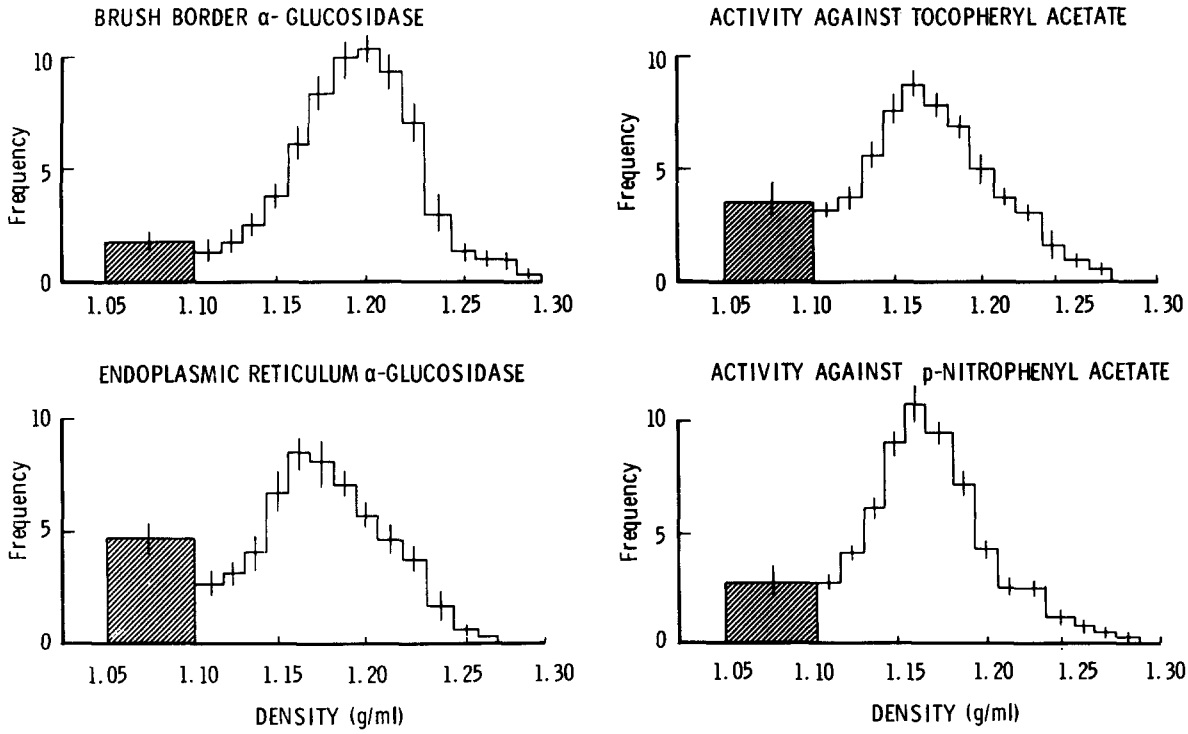

Fig. 4. Isopycnic centrifugation of post-nuclear supernatants from isolated jejunal enterocyte homogenates. Graphs show frequency-density histograms for brush border and endoplasmic reticulum marker enzymes and for activity against tocopheryl and *p*-nitrophenyl acetate. Frequency (mean ± 1 SD) is defined as the fraction of the total recovered activity present in the individual fraction divided by the density span covered by that fraction. The activity present over the density span 1.05–1.10 g/ml ▨ represents enzyme remaining in the sample layer and presumed to reflect soluble activity. Percentage recoveries; brush border, α-glucosidase, 86; endoplasmic reticulum, α-glucosidase 109; activity against tocopheryl acetate, 107; activity against *p*-nitrophenyl acetate, 81.

were solubilized in a physiological form and presented for absorption, with exclusion of both pancreatic and biliary secretions. The luminal disappearance of labeled free tocopherol was found to be maximal by 20 min and over this time period no difference was found in the luminal disappearance of α-tocopherol and α-tocopheryl acetate; approximately 40% of the administered vitamin was absorbed. This was initially surprising as Gallo Torres (6) had stressed that luminal hydrolysis of tocopheryl esters was a prerequisite for their efficient absorption. The finding of esterase activity in the postabsorptive fluids and of free tocopherol in the loops that had originally contained micellar tocopheryl acetate suggested that sufficient hydrolysis of the ester had occurred in the lumen to account for the observed absorption. A number of observations, however, indicate that this may not be the case. First, only 16.5% of the tocopheryl acetate remaining in the fluid from the closed-loop experiments was in the free form and second, only a minor proportion (approximately 7%) of the total available (i.e., mucosal) esterase activity against tocopheryl acetate was present in the postincubation fluid, suggesting that the intestinal lumen was probably not the primary site of action of this activity.

Gallo Torres (6) studied the absorption of tocopheryl esters by following the appearance of the vitamin in lymph after diversion of either or both biliary and pancreatic secretions and found that both secretions were necessary for the efficient absorption of tocopheryl acetate. A major difference in the experimental design between the two studies was that Gallo Torres instilled tocopheryl acetate dispersed in ethanol, whereas in our studies mixed micellar solutions of equal concentrations of free tocopherol or its acetate ester were used. Thus, in the studies of Gallo Torres, the degree of in vivo micellar solubilization and thus absorption would be highly dependent on the degree of luminal hydrolysis, as the free vitamin is more polar and thus more easily solubilized than the ester. In a previous study (36) we have shown that at pH 6.3 approximately six times as much free tocopherol can be solubilized than tocopheryl acetate and at pH 8.5 the difference in solubilization is at least twofold. The hypothesis that the degree of luminal solubilization is more important than hydrolysis per se for absorption would also explain the findings of Nakamura et al. (8) who found a correlation between the susceptibility of various tocopheryl esters to in vitro hydrolysis and their absorption in vivo. This could therefore reflect differing rates of production and solubilization of the more polar free tocopherol. Thus hydrolysis of vitamin E esters by pancreatic esterase may be an important and rate-limiting, but not necessarily obligatory, step for absorption. It would facilitate the solubilization of the vitamin within mixed micelles and present the vitamin to the brush border membrane of the enterocyte; tocopherol being absorbed by passive diffusion (37).

The origin of the esterase activity present in the closed jejunal loops after thorough washing was investigated. This activity could theoretically have been derived from pancreatico-biliary secretions, the mucosa, or bacteria. A number of observations suggest that the jejunal mucosa was the source of the esterase activity. Activity was detected in mucosal homogenates from both normally reared and germ-free rats which was subsequently localized to the endoplasmic reticulum of the jejunal enterocyte. This mucosal activity showed different characteristics from that obtained from pancreatic preparations. Although the mucosal enzyme appeared to be "released" into the intestinal lumen by bile salts, this was unlikely to have arisen simply from the adherence of pancreatic enzymes, as esterase activity was still "released" in a closed-loop experiment following a period of bile salt perfusion. A likely explanation, which has been proposed by others, is that bile salts cause increased desquamation of enterocytes from the tips of the villi (38, 39). Gallo et al. (40) have recently described a cholesterol esterase in the rat intestine that they located by immunocytochemistry and were "tempted to speculate was associated with the endoplasmic reticulum". They studied esterification rather than hydrolytic activity and provided evidence that the activity was pancreatic in origin. It is impossible, however, to state whether their enzyme activity and the one reported in this study are identical or otherwise. This will require purification and characterization of the enzymes.

Other workers have described intestinal esterases localized to the brush borders (41), cytosol (42) as well as the endoplasmic reticulum (43, 44). The characteristics of the intestinal esterase reported in this study show both similarities and marked differences to previous reports.

The lack of hydrolysis of cholesteryl esters was in contrast to the findings of others (41, 45). Murthy and Ganguly (45), for example, demonstrated brush border mucosal cholesterol esterase that had 5–10% of the activity of pancreatic esterase. Their assay, however, used a 3-hr incubation period and, in common with others, they used a dispersion of cholesteryl oleate in 10% ethanol. The stimulatory effects of bile salts on esterase activity against tocopheryl esters has been found by others using both cholesterol and vitamin A esters and intestinal mucosa from both the rat and chicken (41, 43, 45–47).

The localization of the enzymatic activity to the endoplasmic reticulum of the enterocyte and the finding of only a small proportion of the total amount of activity in the small intestinal lumen suggest a predominantly intracellular role for the enzyme. The kinetic studies showed that the mucosal enzyme had a greater affinity for tocopheryl esters and that its activity departed from linearity against time and enzyme concentration earlier than the pancreatic enzyme. These characteristics are consistent with an intracellular role for the mucosal enzyme. That the activity of the mucosal enzyme was increased in the presence of sodium taurocholate is not inconsistent with the intracellular role of the enyzme. For, although bile salts are principally absorbed by an active ileal transport process, some are absorbed by passive non-ionic diffusion in the jejunum (48, 49) where they are known to stimulate other intracellular reactions such as the re-esterification of fatty acids (50, 51).

Our studies, therefore, suggest that micellar solubilization of tocopheryl acetate without prior luminal hydrolysis may be sufficient for the uptake of the vitamin by the jejunal enterocyte. Under normal physiological conditions pancreatic esterase would account for the major hydrolytic activity against tocopheryl and related esters. It is probable, however, that small amounts of micellar solubilized esters would be hydrolyzed intraluminally by "released" mucosal esterase and some would also escape hydrolysis and be absorbed intact where they might be hydrolyzed intracellularly by the mucosal enzyme or, alternatively, pass intact into the lymph. If this activity is present in the human jejunum, it may play a role in pancreatic deficiency states where levels of pancreatic esterase are greatly reduced (52).

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