Biochimica et Biophysica Acta, 401 (1975) 265–277

BBA 77037

THE ROLES OF BILE SALTS IN THE UPTAKE OF β-CAROTENE AND RETINOL BY RAT EVERTED GUT SACS*

MOHAMED I. EL-GORAB**, BARBARA A. UNDERWOOD and JOHN D. LOERCH

Nutrition Program, Division of Biological Health, College of Human Development, The Pennsylvania State University, University Park, Pa. 16802 (U.S.A.)

(Received January 22nd, 1975)

SUMMARY

1. The effects of bile salts, Tween 20 and hexadecyltrimethylammonium-bromide on the uptake of β-[^{3}H]carotene and [^{3}H]retinol by rat-everted gut sacs were studied in vitro under conditions simulating those present in the intestinal lumen during lipid absorption.

2. Micellar solutions significantly enhanced uptake over emulsions. Maximum uptake occurred at the critical micellar concentration of the bile salts mixture. At higher detergent concentrations β-carotene uptake declined sharply; retinol absorption remained high.

3. In β-carotene absorption bile salts functioned not only as micellar solubilizers but also may have been required for interaction with the cell membrane or as a transport carrier. In retinol uptake their primary function appeared only to be micellar solubilization. Both uptake and efflux of substrates were enhanced in bile salt micellar solutions compared to the other detergents.

4. β-carotene cleavage and conversion to retinyl esters occurred only in bile salts solutions. Retinol esterification was seen with all detergents. These effects increased as the tri-/dihydroxy bile salts ratio was increased.

5. β-carotene uptake appeared to be reversible and passive at low concentrations. Retinol uptake was reversible, 7–30 times more rapid, and partially inhibited by 2,4-dinitrophenol at higher concentrations. An energy-requiring step may have been rate limiting.

INTRODUCTION

Although bile salts play an important and well defined role in the intestinal absorption of fat, their specific functions in the absorption of fat-soluble vitamins is still unclear. We have reported that they are required in vitro for β-carotene and

* Presented in part at the meeting of the Federation of American Societies for Experimental Biology and Medicine, April 1974, Atlantic City, New Jersey, U.S.A.

** Present address: Nutrition Institute, 16 Kasr El-Aini St., Cairo, Egypt.

retinol solubilization [1], and the micellar phase has been proposed as the preferred or obligatory phase in vivo for absorption of certain poorly water-soluble drugs [2] as well as non-polar lipids such as sterols [3].

Yet bile salts may have a more extended function than simple micellar solubilization, as for example in their reported protection of cholesterol esterase from hydrolysis by trypsin and chymotrypsin [4]. Earlier reports [5, 6] have suggested that bile salts may also play an obligatory role in β-carotene absorption in which not only uptake, but cleavage, reduction and esterification of the resulting retinol are involved. The replaceability of bile salts by substitute detergents should provide a test of this specific requirement.

We report in the present study the possible roles of bile salts in the uptake of β-carotene and retinol under conditions simulating those present in the intestinal lumen during lipid absorption. Rat everted gut sacs were used to compare uptakes of β-carotene and retinol from micellar solutions and emulsions both of bile salts and of the non-physiological detergents Tween 20 and hexadecyltrimethylammonium-bromide. Rates of substrate efflux from preloaded sacs using the three detergents were also studied, as were the effects of bile salt concentrations and compositions, incubation times, substrate concentrations and the metabolic inhibitor 2,4-dinitrophenol.

MATERIALS AND METHODS

β-carotene, oleic acid, monoolein, triolein, L-α-lecithin (β,γ-dipalmitoyl) and Tween 20 (polyoxyethylene sorbitan monolaurate) were obtained from Sigma Chemical Co., St. Louis, Mo. All-*trans*-retinol, retinyl palmitate and hexadecyltrimethylammoniumbromide were obtained from Eastman Kodak Co., Rochester, N.Y. Bile salts (96–98 % pure) were obtained from Calbiochem, Los Angeles, Calif. β-[15, 15′-$^3H_2$]carotene was a generous gift from Hoffman-La Roche, Inc., Nutley, N.J. [^{3}H]Retinol and Aquasol were obtained from New England Nuclear, Boston, Mass.

Preparation of stock sodium phosphate buffer. A 0.15 M Na^+, 0.116 M PO_4^{-3} buffer (pH 6.3), made by mixing 550 ml 0.3 M NaH_2PO_4 with 450 ml 0.15 M Na_2HPO_4 then diluting with an equal volume of water, was used in all studies [7].

Preparation of stock detergent solutions. A mixed solution containing six conjugated bile salts was prepared by dissolving 30 mmol sodium glycocholate, 30 mmol sodium glycochenodeoxycholate, 15 mmol sodium glycodeoxycholate, 10 mmol sodium taurocholate, 10 mmol sodium taurochenodeoxycholate, 5 mmol sodium taurodeoxycholate and 50 mmol NaCl in 1 l of water. The resulting solution contained 0.1 M mixed bile salts and 0.15 M Na^+, had a glycine/taurine bile salt ratio of 3 : 1, and a tri-/dihydroxy bile salt ratio of 0.66 : 1. Solutions with other bile salt ratios were prepared similarly. Solutions of 0.1 M Tween 20, and 0.1 M hexadecyltrimethylammoniumbromide each containing 0.15 M Na^+ were also prepared.

Preparation of micellar incubation media. β-[^{3}H]carotene, or [^{3}H]retinol, in benzene was added to a mixture of oleic acid, monoolein and lecithin in *n*-hexane. The solvent was evaporated under N_2 and appropriate volumes of stock detergent solutions, buffer, NaCl, glucose and water were added. In addition to either β-[^{3}H]carotene or [^{3}H]retinol and the desired type and concentration of detergent (see tables and figures), the media contained 7.5 mM oleic acid, 2.5 mM monoolein,

0.68 mM lecithin, 0.15 M Na^+ and 0.10 mM glucose at a pH of 6.4±0.1. Micelles were prepared by gentle shaking under N_2 for 22–24 h at 37 °C followed by centrifugation for 18 h at 100 000 × *g*. The resulting clear micellar phase was used.

Emulsions rather than micellar solutions were used in some experiments. When specified, these were prepared using the same lipid concentrations as above except triolein was added (final concentration 1.13 mM) and the detergent concentrations were reduced to 2 mM, a level below the critical micellar concentration. Emulsions were obtained by sonication for 5 s in an Ultrasonics Sonifier Model W185D and were stable for the entire incubation period.

Preparation of everted gut sacs. Male rats of Wistar strain (locally interbred) weighing 280–300 g were stunned by a blow on the head after being fasted overnight. Their small intestines were quickly removed and rinsed with chilled oxygenated buffer. After discarding the first 3–4 cm below the pylorus, the upper third of the intestine was everted and used to prepare gut sacs of 2 cm length according to Wilson and Wiseman [8]. The serosal space was filled with fresh, chilled, oxygenated buffer.

Uptake. Sacs prepared from segments of the upper small intestine were randomized, paired, and incubated with the desired incubation medium. They then were rinsed four times in the stock detergent solution under study, the serosal fluid was removed and the sacs were blotted and homogenized in 10 ml of buffer. The total radioactivity of the incubation mixture, tissue homogenate and serosal fluid was determined by mixing 1 ml of the solutions with 10 ml Aquasol in a polyethylene vial and counting in a liquid scintillation spectrometer, Intertechnique Model SL 30. Quenching, if any, was determined and corrected for by means of an internal-external standard ratio. Protein in the tissue homogenate was determined by the Biuret method [9]. At least six sacs were used in determining each experimental value (see tables and figures).

RESULTS

Effect of bile salt concentrations on uptake of substrates

Results of varying bile salt concentration from 2 to 16 mM on the mucosal uptake of β-carotene and retinol are shown in Figs la and 1b, respectively. The uptake of both substrates rose sharply at the critical micellar concentration (8–10 mM) of the bile salts mixture. However, the uptake of β-carotene fell sharply at higher bile salt concentrations while retinol uptake did not decline significantly from its maximum value.

Effect of changing detergents and physical state of the medium

A comparison of mucosal uptake of β-carotene and retinol from emulsions and micellar solutions of the three detergents is shown in Table I. Uptake of both substrates occurred from all mixtures studied. The magnitude of uptake differed markedly according to the physical state of the incubation medium and detergent type. β-carotene uptake from micellar solutions in all cases was significantly higher than from the corresponding emulsion and was highest when the incubation medium contained bile salts regardless of the physical state of the incubation mixture. Uptake decreased in the following order: bile salts > Tween 20 > hexadecyltrimethylammoniumbromide. The uptake of β-carotene from micelles was 1.2–3.5 times greater than

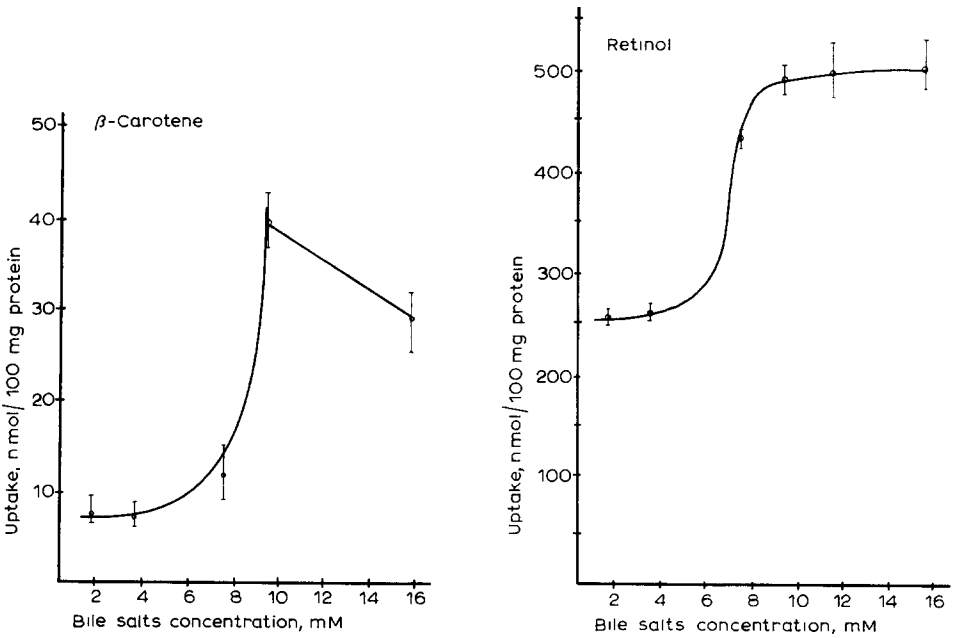

Fig. 1. (a and b) Effect of bile salts concentration on uptake of β-carotene and retinol from everted gut sacs. The incubation medium (3 ml volume) contained either 90 nmol β-[^{3}H]carotene (spec. act- 400 000 cpm/μmol) or 500 nmol [^{3}H]retinol (spec. act. 66 000 cpm/μmol), lipids (oleic acid, monoolein and lecithin), phosphate buffer (pH 6.3), Na^+, glucose and varying amounts of a stock detergent solution of six conjugated bile salts. Sacs were incubated for 60 min with shaking at 37 °C under an atmosphere of O_2/CO_2 (95 : 5, v/v), rinsed, blotted and homogenized in buffer for determination of protein (about 20 mg/sac) and radioactivity. Uptake was calculated as nmol substrate per 100 mg protein and displayed as means ±S.D. Six or more sacs were used for each determination.

TABLE I

EFFECT OF DETERGENT TYPE AND PHYSICAL STATE ON MUCOSAL UPTAKE OF β-CAROTENE AND RETINOL

Conditions as for Fig. 1 except that detergent type and physical condition was varied. For micellar uptake all detergent concentrations were 12 mM, above the critical micellar concentration of the bile salts mixture. For uptake from emulsions, formed by sonication, the detergent concentrations were reduced to 2 mM and triolein was added. Uptake is calculated as nmol/100 mg protein and expressed as means ±S.D. Six or more sacs were used for each experimental and control condition.

Detergent type	β-carotene uptake*		Retinol uptake*	
	Emulsion	Micelles	Emulsion	Micelles
Bile salts**	8.1±0.8	28.9±3.5	228.8±13.8	584.5±22.8
Tween 20	4.1±1.2	7.2±0.3	308.0± 3.9	518.0±20.0
Hexadecyltrimethylammoniumbromide	3.1±0.2	3.7±0.2	226.0± 1.0	480.0± 8.5

* Values for uptake from micellar solutions are significantly higher than from emulsions for each detergent ($P < 0.001$).

** Values for uptake from bile salts are significantly higher than for the other two detergents in the same physical state ($P < 0.001$). Tri-/dihydroxy ratio = 0.66.

from emulsion. For retinol, there was a 1.7–2.5-fold increase. Uptake of retinol tended to be higher from bile salt micelles than from other detergent micelles but this difference was not statistically significant.

Binding of substrate

β-carotene was firmly bound to the mucosal cells after incubation in micellar media of the various detergents. Extensive washing with bile salts or Tween 20 after incubation did not release a significant amount of radioactivity. In an attempt to determine whether this β-carotene was surface bound or intracellular, the sacs were incubated with labeled β-carotene in the usual way, then washed with a mixture of *n*-hexane/ethanol (3 : 1, v/v). It was anticipated that this procedure would fix the cells by denaturation of surface proteins, deter the leakage of intracellular β-carotene from the everted sacs, and simultaneously solubilize any surface-bound substrate. Substrate remaining after treatment was considered to be "intracellular".

Only 34–61 % of the β-carotene absorbed after 60 min incubation was removed by the solvent washing procedure (Table II). In contrast, more than 95 % of substrate taken up in short incubation periods was released by the washing. Of the detergents, bile salts functioned most effectively in promoting intracellular incorporation of β-carotene. In contrast, retinol crossed the mucosal membrane readily regardless of detergent type with 5–10 % being surface bound. In absolute terms, approx. 12 nmol of retinol were surface bound per sac (using an approximate figure of 20 mg protein per sac with the final retinol concentration 380 nmol in the incubation medium) versus 3.8 nmol of β-carotene (final concentration approx. 84 nmol). The proportions of bound to available substrate at the 1-h sampling time are approx. 3 and 4.5 %, respectively.

Effect of preincubation with bile salts on β-carotene uptake

In an attempt to examine further the effects of bile salts on β-carotene uptake,

TABLE II

EFFECT OF DETERGENT TYPE ON SOLVENT-RESISTANT BINDING OF β-CAROTENE IN MUCOSAL CELLS

Uptake conditions as for Fig. 1 except the detergent type was varied as indicated and detergent concentrations were 12 mM, above the critical micellar concentration of the bile salts mixture throughout. After incubation, sacs were washed with a mixture of *n*-hexane/ethanol (3 : 1, v/v) before proceeding with homogenization and subsequent analyses. Values for total uptake and intracellular (solvent resistant) uptake are expressed as nmol substrate per 100 mg protein, six sacs used per determination.

Detergent type	β-carotene uptake		
	Intracellular	Total	Percent of total
Bile salts*	19.0	28.9	66
Tween 20	2.8	7.2	39
Hexadecyltrimethylammoniumbromide	1.6	3.7	44

* Tri-/dihydroxy ratio = 0.66.

everted gut sacs were preincubated without substrate in a bile salts micellar solution or sodium phosphate buffer. They were then incubated in media containing labeled β-carotene in a micellar solution of either bile salts or Tween 20. Washing with a mixture of *n*-hexane/ethanol was used to distinguish between surface-bound and intracellularly bound substrate as in the previous section. If bile salts function only as micellar solubilizers within the lumen, preincubation of the sacs with the detergent should have no advantage over preincubation with the buffer. However, if they exert a specific effect either on membrane permeability or intracellular binding, preincubation with bile salts should enhance substrate uptake. Table III shows that bile salts preincubation caused a significant increase in levels of solvent-resistant binding of β-carotene. The increase occurred regardless of whether the final incubation was carried out with substrate solubilized in micelles of bile salts or of Tween 20. There was no significant difference in substrate bound to the mucosal surface (removable by solvent) attributable to the preincubation conditions. In contrast to these findings for sacs from the proximal jejunum, preincubation of sacs from the distal half of the small intestine with bile salts did not increase the surface-bound or intracellular uptake of β-carotene compared to buffer.

Efflux of β-carotene and retinol

Table IV shows the release of β-carotene and retinol from everted gut sacs after preloading with labeled substrate in micelles of various detergents. The efflux of substrates from sacs incubated with Tween 20 or hexadecyltrimethylammonium-bromide was significantly less than from sacs incubated with bile salts. No significant differences in efflux were attributable to different tri-/dihydroxy or glycine/taurine bile

TABLE III

EFFECT OF DETERGENT PREINCUBATION ON SOLVENT-RESISTANT BINDING OF β-CAROTENE IN MUCOSAL CELLS

Sacs were preincubated for 30 min without substrate or other components in either a 12 mM bile salts micellar solution obtained by dilution from a stock 0.1 M solution, or 0.116 M sodium phosphate buffer (pH 6.3). Physical conditions were as for Fig. 1. The sacs were then incubated for another 30 min in standard incubation media containing β-[^{3}H]carotene solubilized in 12 mM micellar solutions of either bile salts or Tween 20. After incubation, sacs were washed with a mixture of *n*-hexane/ethanol (3 : 1, v/v) before proceeding with homogenization and analysis. Values for surface-bound intake (removable by solvent) and intracellular (solvent resistant) uptake were calculated as nmol substrate per 100 mg protein and expressed as means ±S.D., six or more sacs per determination.

Medium used*		β-carotene uptake		
Preincubation	Incubation**	Surface-bound	Intra-cellular	Total
Bile salts	Bile salts	13.2±3.4	27.1±6.4	40.3±9.1
Buffer	Bile salts	11.0±1.5	11.7±3.9	22.7±2.8
Bile salts	Tween 20	5.9±1.5	9.5±0.8	15.4±2.5
Buffer	Tween 20	4.4±1.3	5.2±0.6	9.7±1.7

* For all bile salts media, tri-/dihydroxy ratio = 0.66.

** Increases in intracellular β-carotene uptake caused by preincubation with bile salts over buffer are significant at $P < 0.005$ for each incubation medium.

TABLE IV

EFFECT OF DETERGENT TYPE ON EFFLUX OF β-CAROTENE AND RETINOL FROM EVERTED GUT SACS

Sacs were preloaded with labeled substrate as for Fig. 1 except a 12 mM bile salts mixture was used throughout, the detergent composition and type were varied as indicated, and the incubation time was reduced to 30 min. Preloaded sacs were washed twice in the appropriate detergent solutions (no additives), again incubated for 30 min in the original medium (but without substrate), and homogenized. Efflux from the mucosal cells to the second incubation medium was determined both by assaying the radioactivity released to the medium and the amount remaining in the tissue homogenate. Results were calculated as the percent of substrate initially taken up and released as efflux. Data are expressed as means ±S.D., six or more sacs per determination.

Detergent type and composition	Efflux %	
	β-carotene	Retinol
Bile salts*		
Tri-/dihydroxy ratio**:		
0.66	77.7±2.7	35.9±2.7
1.32	79.9±1.4	38.2±4.6
2.64	77.9±3.2	39.7±8.6
Glycine/taurine ratio***:		
1 : 1	77.7±2.5	35.9±0.6
6 : 1	79.0±0.2	35.3±1.9
Tween 20	68.6±1.9	29.2±1.0
Hexadecyltrimethyl-ammoniumbromide	50.8±0.9	25.8±1.2

* Values for all bile salts detergents significantly higher than for the other two ($P < 0.005$).
** Glycine/taurine ratio held constant at 3.0.
*** Tri-/dihydroxy ratio held constant at 0.66.

salts ratios. In a related experiment the same ratios of bile salts as in Table IV were used in the standard uptake procedure for β-carotene and retinol. No significant changes were observed in uptake of either substrate as a result of varying the bile salts ratios of the incubation medium.

Effect of detergent type on β-carotene substrate cleavage and retinol substrate esterification

Although retinyl ester was formed from β-carotene when bile salts were present in the incubation medium, none was found when the other detergents were used for solubilization; however, retinol substrate was esterified regardless of the type of detergent present (Table V). Bile salts solutions with higher tri-/dihydroxy ratios tended to be increasingly effective in facilitating the cleavage of β-carotene substrate (based on the percentage of ^{3}H-labeled retinyl ester formed) and they were also more effective in aiding the formation of ester from the retinol substrate.

Rate of β-carotene and retinol uptake

The progressive mucosal uptake with time of β-[15, 15′-$^3H_2$]carotene and [^{3}H]retinol from mixed bile salts micelles is shown in Figs 2a and 2b, respectively.

TABLE V

EFFECT OF DETERGENT TYPE ON PRODUCTION OF BOUND RETINYL ESTER FROM β-CAROTENE AND RETINOL SUBSTRATES

Sacs were incubated as for Fig. 1 but with the labeled substrates solubilized either in bile salts solutions having varying tri-/dihydroxy ratios, in Tween 20 or in hexadecyltrimethylammoniumbromide, all at 12 mM micellar concentration. Sacs were homogenized after incubation and extracted with *n*-hexane/ethanol (3 : 1, v/v) containing 10 μg each of carrier β-carotene, retinyl palmitate and retinol. The hexane phase was removed, evaporated, redissolved in light petroleum and chromatographed on a column of deactivated alumina [10]. Results are expressed as the percent of substrate initially taken up and recovered from the homogenate as β-carotene and retinyl ester (β-carotene substrate) or retinyl ester (retinol). Six sacs per determination.

Detergent type	Percent recovered from β-carotene substrate as		Percent recovered from retinol substrate as retinyl ester
	β-carotene	Retinyl ester	
Bile salts*			
Tri-/dihydroxy ratio:			
0.66	17.2	68.6	75.0
1.32	20.0	72.2	81.0
2.64	19.6	75.8	84.0
Tween 20	100.0	0.0	65.2
Hexadecyltrimethyl ammoniumbromide	100.0	0.0	64.3

* Glycine/taurine ratio held constant at 3.0.

For β-carotene the rate was virtually linear throughout the 60-min period studied, at which time about 5 % of the substrate initially present in the incubation medium had been taken up by the gut sac, based on an approximate average figure of 20 mg protein per sac. Retinol uptake was also somewhat linear for the first 5–10 % of the available substrate (0–5 min) and reached almost 24 % of the total in 60 min. Initial uptake velocities, v_i, were calculated to be 22.5 nmol β-carotene/h per 100 mg protein (assuming first order, 90 nmol β-carotene initially present) and 3600 nmol retinol/h per 100 mg protein (from the slope of the tangent, 500 nmol retinol initially present). No significant radioactivity was found in the serosal fluid during either study.

Effect of micellar substrate concentrations on uptake rates

The effect of increasing substrate concentrations in micellar bile salts solutions on uptake by everted sacs is shown in Figs 3a and 3b, respectively. For β-carotene, uptake was typically first order, with approx. 10 % of the total available substrate taken up in a 60-min incubation period throughout most of the concentration range studied. Retinol uptake on the other hand was much more rapid and kinetically complex. The data on retinol absorption were also consistent with but not necessarily limited to a first-order mechanism in which approx. 50 % or more of the available substrate was taken up in the 60-min incubation period. At higher concentrations the total uptake fell to 10–15 %, indicating the possible effects of competition for binding

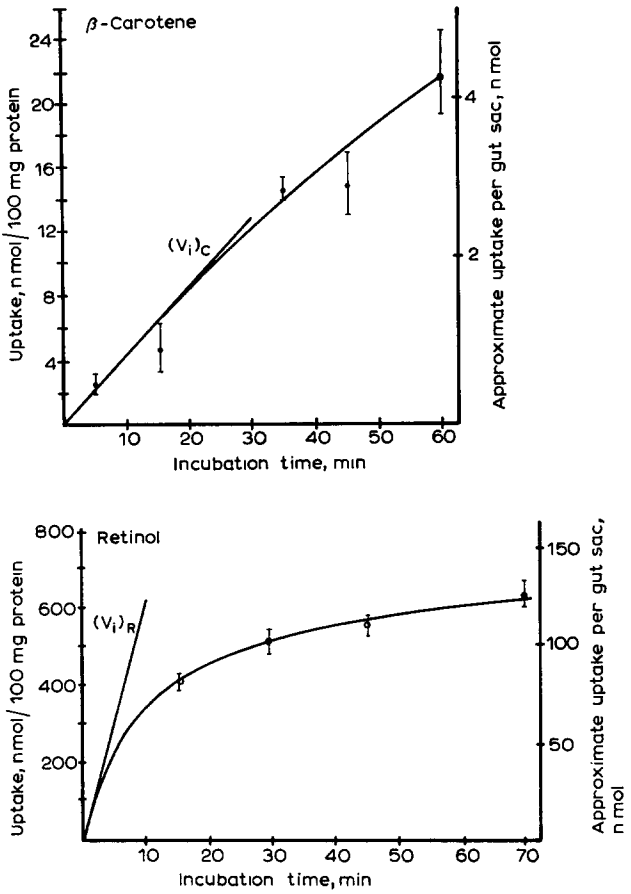

Fig. 2. (a and b) Progressive mucosal uptake of β-carotene and retinol from a micellar solution of mixed bile salts. Conditions as for Fig. 1 except incubation times were varied and the bile salts mixture was held at a micellar concentration of 12 mM. Approximate initial uptake velocities for β-carotene and retinol, $(v_i)_C$ and $(v_i)_R$, respectively, are represented by the slopes of the tangents to the experimental curves at time zero. Uptake expressed both as nmol/100 mg protein and, approximately, as nmol/gut sac. Data displayed as means ±S.D., six or more sacs per determination.

sites, the presence of some later rate-limiting step in the absorption process, or of product accumulation in the sacs. A plot of the retinol data of Fig. 3b in the Lineweaver-Burk form as $(\text{uptake})^{-1}$ versus $(\text{retinol concentration})^{-1}$ is inconclusive in sorting among these mechanisms. The data could be interpreted as being consistent with diffusion kinetics at retinol concentrations as low as those used in the β-carotene study, and possibly would have been at higher concentrations if initial uptake velocities could have been obtained. However, the possibility remains that the "best" fit to the retinol data may not approach a first-order diffusion model in which case some intermediate step may be partially rate determining even at the lowest retinol concentrations studied. β-carotene showed a slight tendency toward non-linearity of uptake at higher concentrations.

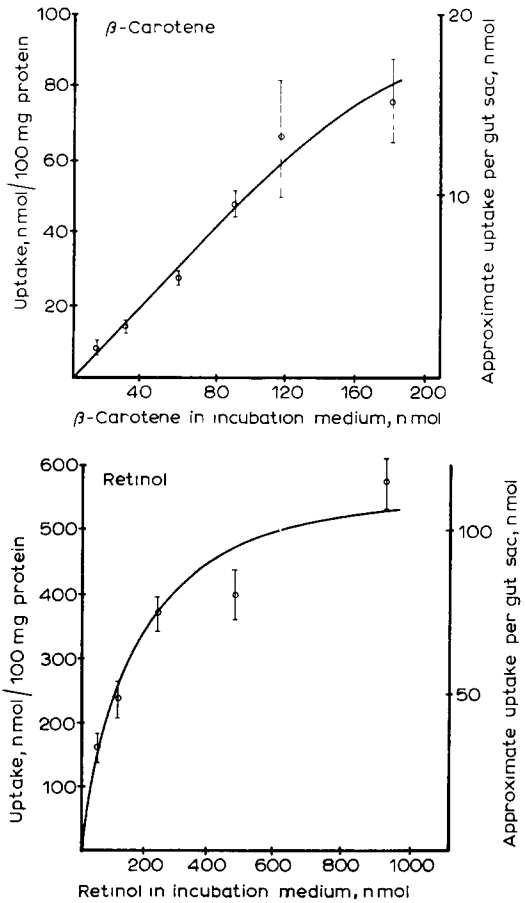

Fig. 3. (a and b) Effect of substrate concentration on uptake of β-carotene and retinol from micellar solutions. Conditions as for Fig. 1 except substrate concentrations were varied and the bile salts mixture was held at a micellar concentration of 12 mM. Uptake expressed both as nmol/100 mg protein and, approximately, as nmol/gut sac. Data displayed as means ±S.D., six or more sacs per determination.

Effect of 2,4-dinitrophenol on uptake of substrates

To obtain evidence concerning the possibility of a rate-limiting energy-requiring step in the uptake of β-carotene or retinol, incubation experiments were repeated using the standard 12 mM micellar bile salts incubation media of Fig. 1 in the presence and absence of $1 \cdot 10^{-4}$ M 2,4-dinitrophenol. Six sacs were used for each determination. Although there was no significant effect seen on β-carotene uptake (40.9±8.0 versus 44.2±6.7 nmol/100 mg protein for media with and without the inhibitor, respectively, expressed as means±S.D.), retinol uptake was markedly decreased by the inhibitor (383±52 versus 636±70 nmol/100 mg protein). This finding, significant at $P < 0.005$, suggests that mucosal uptake of retinol may take place in part and at higher substrate concentrations through an active, energy-requiring mechanism.

DISCUSSION

Most studies favor the concept that a micellar phase is the preferred physiological state for most efficient absorption of lipids from the intestinal lumen [3, 11]. It is not clear, however, whether micellar solubilization is obligatory for uptake of all lipids under normal conditions [11–15]. In our study, using rat-everted gut sacs, micellar solubilization markedly increased the rate of uptake of both β-carotene and retinol over emulsions. Care was taken to prepare all incubation media using the same lipid components, manipulating only the detergent concentrations below or above their critical micellar concentration. In contrast, most previous studies used albumin or a high pH to stabilize the emulsions [15–17].

Our results also showed a sharp inflection in uptake of both substrates at a bile salts concentration range corresponding to the critical micellar concentration of the mixture used. This provides evidence that the uptake of both substrates is highly sensitive to their physiochemical state in the incubation medium. The data are consistent with absorption by molecular diffusion from a micellar reservoir as suggested by Salee and Dietschy [18], in which micelles provide more intimate contact than emulsions with the mucosal surface.

However, bile salts may affect the absorption process other than simply through micellar solubilization, although their membrane-related or intracellular functions during lipid absorption are controversial [4, 16, 19]. Results from our study showed that preincubation of sacs with bile salts caused a significant increase in the "intracellular" radioactivity following incubation with labeled β-carotene. In contrast, binding to the mucosal surface occurred in the presence of micellar solutions of all three detergents suggesting that surface binding does not specifically require the presence of bile salts. The finding that no retinyl ester was formed when sacs were incubated with β-carotene solubilized in Tween 20 or hexadecyltrimethylammonium-bromide clearly indicates that bile salts are specifically required for β-carotene cleavage and promote the accessibility of the highly water-insoluble substrate to the water-soluble enzyme. It does not indicate whether the effect is on membrane permeability, on the dioxygenase enzyme directly or in facilitating intracellular transport to the cleavage site [5, 20, 21].

Retinol, on the other hand, crossed the mucosal membrane and was esterified regardless of the type of detergent used. This indicates that bile salts do not play a specific role in retinol absorption and can be replaced by other non-toxic detergents. The primary and perhaps only function of bile salts in retinol absorption is as micellar solubilizers. The higher percentage of retinyl ester formed from bile salt micelles compared to the other detergents could be an effect of the smaller size of the bile salt micelles [6, 22]. By their increased interaction with the cell membrane, thereby enhancing uptake, they could indirectly influence the activity of retinyl esterase by regulating the rate at which substrate was made available for the enzymatic reaction.

Additional support for the suggestion of an increased mobility of the smaller micelles is afforded from our efflux experiments. Both uptake and release of β-carotene solubilized in bile salt micelles exceeded that found with the other detergents. The absorption process appears to be a dynamic one involving a reversible binding of substrate. The binding sites are probably located on the microvillous surface although these efflux data do not distinguish definitively between membrane-bound and

intracellularly-bound material. Retinol uptake appeared to behave similarly. Cleavage of β-carotene, or esterification of retinol, would serve as a trap and maintain a concentration gradient across the cell membrane. No binding sites specific for different lipids have been characterized, but if such are present, concentration-independent differences in the rate of absorption of different lipids might be related to the relative availability of such sites. A bidirectional flux of fatty acids, cholesterol and bile salts has been described previously [17, 23]. However, it is quite possible that the observed reversible binding might be a property of the in vitro system used.

Under the conditions used in our studies, a linear relationship was seen between β-carotene uptake and its concentration in the incubation medium. These results indicate that uptake at low concentrations may be a first-order process consistent with a diffusion-limited mechanism. In the general first-order rate equation:

$$kt = \log \frac{(\text{initial substrate concentration})}{(\text{substrate concentration at time } t)},$$

the approximate value of k for β-carotene, k_C, varied from $0.84 \cdot 10^{-3}$ to $1.76 \cdot 10^{-3}$ min^{-1}, using uptake values of 5 and 10 %/h from Figs 2a and 3a, respectively. The corresponding calculated initial uptake velocities. $(v_i)_C$, were 4.5–9.5 nmol/h per gut sac with 90 nmol β-carotene as substrate where $(v_i) = k \cdot$ (initial substrate concentration). If retinol uptake is also first order for the entire incubation period at a substrate level of 90 nmol, the corresponding value for k_R is 0.118 min^{-1} using an uptake value of 51 %/h from Fig. 3b. The corresponding initial velocity, $(v_i)_R$, is approx. 63.5 nmol/h per gut sac, a rate 7–14 times that of β-carotene. Similar considerations, using the data of Fig. 2b and correcting for differences in initial substrate levels, yield the conclusion that retinol uptake is at least 15–30 times faster than that of β-carotene at initial substrate levels of 90 nmol.

Loran and Althausen [24] and Skála and Hrubá [25] suggested that vitamin A absorption is an active process. The results presented in our study are in accord with such an active, energy-requiring process, at least for retinol at the higher substrate concentrations. At these levels, during a 60-min uptake period, the kinetic data for retinol became complex and its uptake was inhibited by 2,4-dinitrophenol. In β-carotene uptake, only a hint of deviation from first-order kinetics developed and no significant inhibition by 2,4-dinitrophenol was seen. This may have been due to the lower substrate levels studied since retinol also exhibited near-first-order uptake kinetics at similarly low levels.

ACKNOWLEDGEMENT

This work was supported in part by National Institutes of Health Grant AM-13676.

REFERENCES

1 El-Gorab, M. and Underwood, B. A. (1973) Biochim. Biophys. Acta 306, 58–66
2 Bates, R. R. (1966) Ph. D. Thesis, Columbia University, New York
3 Bergström, B. and Borgström, B. (1955) Prog. Chem. Fats Lipids 3, 351
4 Vahouny, G. V. and Weersing, S. (1964) Arch. Biochem. Biophys. 107, 7–15

5 Olson, J. A. (1961) J. Biol. Chem. 236, 349–356
6 Olson, J. A. (1964) J. Lipid Res. 5, 402–408
7 El-Gorab, M. I. (1973) Ph.D. Thesis, Columbia University, New York, p. 40
8 Wilson, T. H. and Wiseman, G. (1953) J. Physiol. London 123, 116–125
9 Cornall, A. G., Bardawill, G. S. and David, M. M. (1949) J. Biol. Chem. 177, 751–766
10 Thompson, J. N., Erdody, P., Brien, R. and Murray, T. K. (1971) Biochem. Med. 5, 67–89
11 Dawson, A. M. and Webb, J. P. W. (1972) Proc. Soc. Expl. Biol. Med. 142, 906–908
12 Simmonds, W. J., Hofmann, A. F. and Theodor, E. (1967) J. Clin. Invest. 46, 874–890
13 Hamilton, J. D. (1971) Biochim. Biophys. Acta 239, 1–8
14 Hoffman, N. E. (1970) Biochim. Biophys. Acta 196, 193–203
15 Johnston, J. M. and Börgström, B. (1964) Biochim. Biophys. Acta 84, 412–423
16 Porte, Jr, D. and Entenman, C. (1965) Am. J. Physiol. 208, 607–614
17 Feldman, E. B. and Borgström, B. (1966) Biochim. Biophys. Acta 125, 148–156
18 Salee, V. L. and Dietschy, J. M. (1971) J. Clin. Invest. 50, 80a
19 Dawson, A. M. and Isselbacher, K. J. (1960) J. Clin. Invest. 39, 730–740
20 Devel, Jr, H. J. (1955) The Lipids (Devel, Jr, H. J., ed.), Vol. 2, p. 286, Interscience, New York
21 Goodman, D. S., Huang, H. S., Kanai, M. and Shiratori, T. (1967) J. Biol. Chem. 242, 3543–3554
22 Small, D. M. (1971) The Bile Acids (Nair, P. P. and Kritchevsky, D., eds), Vol. 1, pp. 249–356, Plenum Press, New York
23 Mishkin, S., Yalovsky, M. and Kessler, J. (1971) Biochim. Biophys. Acta 225, 335–346
24 Loran, M. R. and Althausen, T. L. (1959) Am. J. Physiol. 197 (6), 1333–1336
25 Skála, I. and Hrubá (1964) Am. J. Physiol. 206 (2), 458–460