The effect of bile and bile salts on the uptake and cleavage of β-carotene into retinol ester (vitamin A ester) by intestinal slices

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SUMMARY Bile of the rat and of other species stimulates the uptake of β-carotene from a micellar solution of Tween 40 (polyoxyethylene anhydrosorbitol monopalmitate) and its cleavage into retinol ester by intestinal sections of the rat incubated in vitro. Sodium glycocholate and several other conjugated bile acids substitute completely for bile. Glycocholate also stimulates β-carotene cleavage by intestinal sections of the chicken, hamster, and lamb, but not of the turtle or opossum.

The stimulatory effect of several bile acids tested in a medium containing Tween 40 is roughly proportional to the number of hydroxyl groups present. With the exception of cholic acid, only conjugated bile acids have this activity. The binding of β-carotene to the intestine occurred rapidly, and could not be readily correlated with the formation of retinol ester.

Although the physicochemical state of carotene in solution is undoubtedly important, the conjugated bile acids probably stimulate the formation of retinol ester by enhancing the absorption of β-carotene by means of an interaction with the membrane of intestinal mucosal cells.

BILE HAS long been known to stimulate the absorption of β-carotene from the gastrointestinal tract (1, 2). Since bile emulsifies lipids, its action in stimulating carotenoid absorption has generally been attributed to this property (3). However, β-carotene dispersed in a micellar solution with a nonionic detergent is *not* absorbed and converted into retinol ester (vitamin A ester)[1] by the intestine, unless bile or sodium glycocholate is also present (5, 6). This latter observation suggests that bile not only disperses β-carotene in the intestinal lumen, but also stimulates its uptake in some undefined way.

The absorption of retinol, on the other hand, is not specifically dependent on bile. In vitamin A-deficient choledochocolonostomized dogs, retinol administered in oil was biologically active, whereas β-carotene in oil was inactive unless glycodeoxycholate or deoxycholate was administered concurrently (7). Retinol, upon dispersion in a nonionic detergent, is readily absorbed and esterified by the intestine (5), and is transferred across the intestinal wall, providing that the serosal solution also contains a nonionic detergent (8).

In the present investigation, the stimulatory effect of bile on β-carotene absorption and cleavage by intestinal sections in vitro is examined from several viewpoints: the generality of the effect of several species, the influence of bile acid structure and conjugation, and the effect of bile acids on other metabolic reactions in the intestine. Short reports of some aspects of this work have appeared (5, 9, 10).

METHODS

Preparation of β-Carotene-C^{14}

Uniformly labeled β-carotene-C^{14} was isolated from cultures of *Chorella pyrenoidosa* grown on $C^{14}O_2$ as previously described (6). β-Carotene-C^{14} of high specific activity was also prepared from cultures of *Phycomyces blakesleeanus* incubated with acetate-1-C^{14} by an extensive modification of the method of Lilly et al. (11). *Phycomyces blakesleeanus* sporulates well on a medium of 5 g of puffed wheat (Quaker Oats Company), 0.9 ml of glycerol, 30 mg of L-asparagine, and 30 ml of water. Spores were harvested in water, and 1 ml of the suspension (10^6 spores) was used to inoculate 100 ml of Lilly's medium

* With the technical assistance of Peggy Richardson and Jean Herron.

[1] The terms retinol ester and retinol are used instead of vitamin A ester and vitamin A, in accordance with recently approved rules of nomenclature (4).

(11) in a stationary Roux bottle under constant illumination. When pigmentation began (3–4 days), 1 ml of acetate-1-C^{14} solution containing 2.5 × 10^7 cpm in 1.4 mg was added each day for 4 successive days. On the 10th day, the mold was filtered with suction, ground in a mortar with anhydrous Na_2SO_4 and sand, and left overnight under distilled diethyl ether. The ether extract was filtered, evaporated to a small volume, and saponified under nitrogen with methanolic KOH. The unsaponifiable fraction was extracted with *n*-hexane, washed with water, dried over Na_2SO_4, concentrated to a small volume, and chromatographed on 10 g of deactivated Al_2O_3 containing 5–6% H_2O. β-Carotene was eluted with 30–70 ml of hexane. After removal of the solvent, β-carotene was recrystallized to constant specific activity from benzene–methanol 1:2 (v/v). Usually three crystallizations were required. About 0.5 mg of pure β-carotene with a specific activity of 1000–2000 cpm/μg was obtained from each Roux bottle. The over-all conversion of radioactive acetate to β-carotene was 0.5–1%, which compares well with that obtained by Lilly et al. (11). When preparations of β-carotene-C^{14} isolated from *Chlorella* and from *Phycomyces* were incubated with rat intestine *in vitro*, identical results were obtained. Hexane solutions of β-carotene-C^{14} were kept in low actinic, red glass vessels (Pyrex brand lifetime red laboratory glassware) at −20°.

Reagents and Solutions

Deactivated alumina and precipitated MnO_2 were prepared as previously described (6). Radioactive β-carotene was purified on alumina just before each experiment, and was solubilized in a solution containing 0.30% Tween 40 and Krebs-Ringer bicarbonate buffer, pH 7.4 (6). Hepatic bile of the rat was collected for 1 day from a cannula inserted into the bile duct close to the liver. Fresh gall bladder bile of the rabbit, pig, and turtle was obtained immediately after death, and human gall bladder bile was obtained at autopsy. All bile samples were frozen at −20° until used. Retinol acetate-C^{14} was a gift of Dr. O. Wiss of Hoffman-LaRoche and Co. (Basle, Switzerland). Glycolithocholic acid, taurolithocholic acid, and lithocholic acid were kindly given by Dr. Elwood Jensen of the University of Chicago, and chenodeoxycholic acid, taurodeoxycholic acid, and glycodeoxycholic acid by Dr. Henry Danielsson of the Karolinska Institutet (Stockholm, Sweden). Tween 40 (polyoxyethylene anhydrosorbitol monopalmitate, density at 25° = 1.022) was a gift of the Atlas Powder Co. (Wilmington, Del.). Glycocholic acid, taurocholic acid, glycocholanic acid, glycochenodeoxycholic acid, glycohyodeoxycholic acid, glycodehydrocholic acid, and *N*-cholyl-L-alanine were prepared by the method of Norman (12), separated by reversed phase partition chromatography (13), and checked for purity by paper chromatography (14). *N*-Palmitoyl glycine was synthesized by Abderhalden's procedure (15) and crystallized from 95% ethanol. Hyodeoxycholic acid and chenodeoxycholic acid were obtained from the California Foundation for Biochemical Research (Los Angeles, Cal.), cholanic acid from Steraloids, Inc. (Flushing, N. Y.), and cholic acid from Nutritional Biochemicals Corporation (Cleveland, Ohio). Cholic acid was recrystallized from 70% ethanol.

Incorporation of Acetate-1-C^{14} into Unsaponifiable Fraction

Intestinal sections were incubated with 1 mg of acetate-1-C^{14} (10^6 cpm/mg) in a medium containing 0.1% of glucose in Krebs-Ringer bicarbonate, pH 7.4, under 95% O_2–5% CO_2 for 1 hr at 37°. Intestinal sections were rinsed in saline and saponified in 25% KOH in 90% methanol for 2 hr at 60°. After the addition of water, the unsaponifiable fraction was extracted thrice into hexane, washed repeatedly with water, and dried over Na_2SO_4. An aliquot was plated and counted for radioactivity.

Preparation of Intestinal Sections and Their Incubation with β-Carotene-C^{14}

White male rats (Rolfsmeyer Farm, Madison, Wis.) weighing 150–300 g were fasted 2–6 hr prior to the experiment. Animals were anesthetized with ether, and opened along the linea alba. The duodenum and about half of the jejunum were quickly removed, briefly immersed in saline, and immediately placed in a Petri dish containing Krebs-Ringer bicarbonate solution in continuous equilibration with 95% O_2–5% CO_2 at 37°. Upon being cut open longitudinally in the bath, the slit intestine everted itself. Stretching of the gut and the drawing of one intestinal surface over the other, which are common aspects of most gut eversion procedures, were thereby avoided. The everted gut was gently shaken to remove the lumen contents and adhering pieces of food, and was quickly washed twice in isotonic saline. Successive segments of intestine about 2–3 cm in length were cut into 25-ml Erlenmeyer flasks containing 10 μg of β-carotene (20,000 cpm) in 4 ml of a solution containing 0.3% Tween 40, 0.1% glucose, Krebs-Ringer bicarbonate buffer, 7.4, and given amounts of bile or bile acids. Flasks containing no bile salt and 0.4% glycocholate were included in each experiment. Usually five to seven segments, weighing about 1 g in all, from different parts of the upper intestine were present in each flask. The solutions were shaken under 95% O_2–5% CO_2 at 32° in a Dubnoff shaker for 60 min.

Assay Procedure for Retinol-C^{14} Ester

The intestinal sections from each flask were washed three times in warm isotonic saline, and homogenized

TABLE 1 RELATIVE STIMULATION OF β-CAROTENE CLEAVAGE IN RAT INTESTINE BY BILE AND 0.4% GLYCOCHOLIC ACID

Bile	$R = \frac{\mu g \text{ Retinol Ester with Optimal Bile Concentration}}{\mu g \text{ Retinol Ester with 0.4\% Glycocholate}}$
Rat	0.58, 0.47
Human	0.95, 0.78
Rabbit	1.02
Pig	1.13
Turtle	1.18, 1.16

With each set of experiments on a given bile, control flasks with 0.4% glycocholate and with Tween 40 alone were run. In all cases, the retinol ester formed with 0.4% glycocholate was 0.3–1.0 μg, and with Tween 40 alone was 0–0.02 μg.

in 100 ml of hexane-ethanol 3 :1 containing 100 μg each of carrier β-carotene, retinol ester, retinal, and retinol (6). The hexane phase was concentrated and chromatographed on deactivated alumina as previously described (6). Generally retinol-C^{14} appeared mainly as the ester, and further characterization was not required. In some cases, however, the ester was converted into retinal semicarbazone via retinol and retinal (6). Fractions from alumina columns were evaporated under nitrogen, plated on aluminum planchets, and counted under Geiger operation in a windowless gas-flow counter equipped with an automatic sample changer and read-out timer (Nuclear-Chicago Corporation). Corrections were made for self-absorption when necessary.

RESULTS

The Requirement of Bile for β-Carotene Cleavage

When strips of rat intestine were incubated with β-carotene suspended in Tween 40, the formation of retinol ester depended on the presence of bile. As shown in Fig. 1, rat hepatic bile and the gall bladder bile of other species stimulated the cleavage of β-carotene into retinol ester by rat intestine. A maximal effect of gall bladder bile occurred at 0.1–0.2 ml. Since gall bladder

TABLE 2 THE CLEAVAGE OF β-CAROTENE INTO RETINOL ESTER BY INTESTINAL SECTIONS OF VARIOUS SPECIES IN VITRO*

Species	No. of Experiments	Weight of Intestine per Flask	μg Retinol Ester per g Wet Intestine	
			0.4% Glycocholate	No Glycocholate
Chicken	4	0.3	1.20	0.17†
Rat	12	1.0	0.46	0.03
Hamster	4	0.4	0.46	0.08†
Lamb	3	3.5	0.15	0.03
Turtle	6	2.5	0.01	0.004
Opossum	6	4.0	0.005	0.002

* Rats weighing 100–300 g, 100 g golden hamsters, 35 g chicks (1 day old), 4–7 kg turtles (*Pseudemys peninsularis*), 0.3–3 kg opossums, and a 20 kg lamb were used.

† Probably not significant. See text.

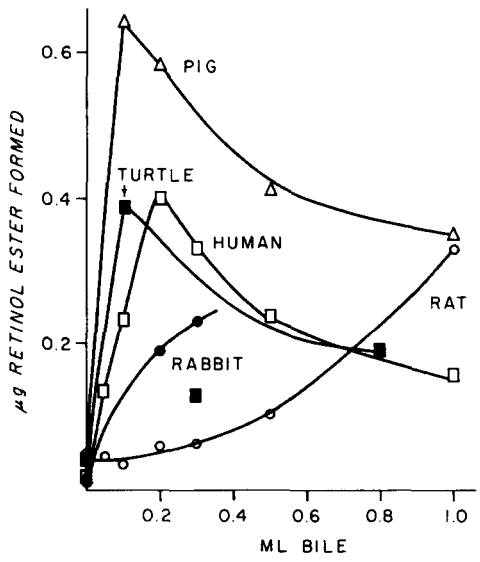

FIG. 1. The effect of bile of various species on the cleavage of β-carotene into retinol ester by rat intestine incubated in vitro in Krebs-Ringer bicarbonate buffer, pH 7.4, at 32° for 1 hr.

bile is roughly ten times as concentrated as hepatic bile, the absence of a maximum with rat bile is not surprising. Glycocholic acid at 0.4% concentration was as effective as gall bladder bile in stimulating retinol ester formation and was more effective than rat hepatic bile at the maximal concentration used (Table 1).

Glycocholate also stimulated carotene cleavage by intestinal tissue of the hamster, chicken, and lamb, but not that by intestinal tissue of the turtle and opossum (Table 2). The best conversion per gram wet weight of intestine was observed in the newborn chicken. In the absence of glycocholate little retinol ester, if any, was formed. The apparent formation of small amounts of retinol ester by chick and hamster intestines in the absence of glycocholate is not considered to be significant. Contamination of the retinol ester fraction with 0.06 μg or less of β-carotene artifacts is common, and conversion of the low experimental values for the chick and the hamster to a gram wet weight basis gave an apparent yield of retinol ester. The inability of intestinal slices of the turtle and opossum to cleave β-carotene into retinol ester is interesting. Since bile of several species stimulated carotene cleavage by rat gut (Fig. 1), a high specificity for endogenous bile acids in these other species is unlikely. Further investigation of this apparent defect is warranted.

The Influence of Conjugation and Structure of the Bile Salts

Conjugated and free bile acids containing from 0 to 3 hydroxyl groups were investigated for their ability to stimulate β-carotene cleavage. Varying concentrations of these compounds were employed, and in all cases a

TABLE 3 EFFECT OF VARIOUS FREE AND CONJUGATED BILE ACIDS ON β-CAROTENE CLEAVAGE*

Bile Acid	Substituents	Ratio of Retinol Ester Formed with Cited Bile Acid to That with Glycocholate		
		Glyco-	Tauro-	Free
Cholic	3α:7α:12α:-trihydroxy	1.00	1.58	0.66
Deoxycholic	3α:12α-dihydroxy	1.31	1.49	0
Chenodeoxycholic	3α:7α-dihydroxy	0.90	—	0.05
Hyodeoxycholic	3α:6α-dihydroxy	0.63	—	0.05
Lithocholic	3α-hydroxy	0.19	0.35	0.03
Cholanic	None	0.27	—	0.06
Dehydrocholic	3:7:12-triketo	0.01	—	0.04

* Maximal values of retinol ester formed in the presence of optimal concentrations of bile acid (0.2–0.4%) were used in calculating the ratios. Corrections were made for the small amount of radioactivity in the retinol ester fraction with Tween 40 alone. In all cases, the amount of retinol ester formed was 0.20–0.90 μg in the presence of 0.4% glycocholate, and 0–0.06 μg with 0.3% Tween 40.

maximal stimulatory effect was observed at 0.2–0.4% bile acid, followed by inhibition at higher concentrations. Since the formation of retinol ester varied in different experiments, and since higher concentrations of Tween 40 than 0.3% were required to solubilize the less polar bile acids, such as cholanic and lithocholic acids, the data are expressed as the ratio, *R*, of retinol ester formed with the optimal concentration of a given bile acid to that formed in the flask with 0.4% glycocholate and the same amount of Tween 40 (Table 3). The ratio for *N*-cholyl-L-alanine, which is not included in the table, was 0.76.

Other detergents such as sodium oleate, cephalin, and *N*-palmitoyl glycine were ineffective in stimulating β-carotene cleavage in vitro. Sodium dodecyl sulfate and dodecyl amine chloride were inactive in vivo (6).

Inhibition of Glycocholate Stimulation

The cleavage of β-carotene into retinol ester in the presence of glycocholic acid was inhibited by deoxycholic acid and by Tween 40, as seen in Table 4. Deoxycholate at 0.1% concentration almost completely blocked the stimulatory effect of 0.4% glycocholate. Tween 40 at 1% concentration reduced the amount of retinol ester formed to one-third of that at 0.3%. Since 0.3% Tween 40 was the minimal amount for effective solubilization of β-carotene, this concentration was generally employed. The addition of 0.4% egg lecithin depressed β-carotene cleavage from 0.62 μg to 0.23 μg. Interestingly, Tween 20 in concentrations up to 5% had little effect on the conversion of β-carotene into retinol ester in intestinal loops in vivo (6).

TABLE 4 INHIBITION OF β-CAROTENE CLEAVAGE BY DEOXYCHOLATE AND TWEEN 40 AT 32°

Detergents Present			Retinol Ester Formed
Glycocholate	Deoxycholate	Tween 40	
%	%	%	μg
0.4	0	0.3	1.09
0.4	0.1	0.3	0.11
0.4	0.2	0.3	0.05
0.4	0.3	0.3	0.03
0	0	0.3	0.04
0.4	0	1	0.38
0.4	0	2	0.14
0.4	0	6	0.03
0.4	0	11	0.03

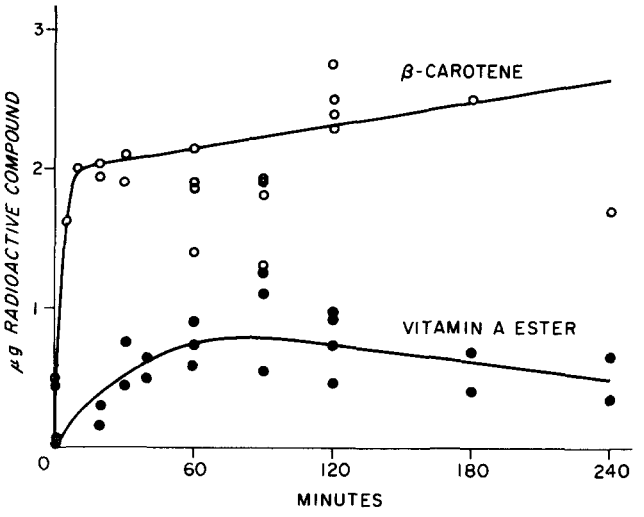

FIG. 2. The rate of β-carotene binding and retinol ester formation in rat intestinal segments incubated in vitro in Krebs-Ringer bicarbonate buffer, pH 7.4, at 32° for 1 hr in the presence of 0.4% sodium glycocholate.

The Binding of β-Carotene to the Intestinal Surface

The rate of β-carotene binding to the intestinal surface in vitro is rapid and is largely complete in a period of 10 min (Fig. 2). Retinol ester formation, on the other hand, continues more or less linearly for a period of 60 min. In the presence of all bile acids tested, more β-carotene was bound to the intestinal surface than was bound with Tween 40 alone. In a series of 30 experiments, the β-carotene bound in the presence of 0.4% glycocholate plus 0.3% Tween 40 was 1.62 ± 0.48 μg whereas that bound with 0.3% Tween 40 alone was 1.01 ± 0.52 μg. Statistically, this difference is highly significant ($P < 0.01$).

On the other hand, bile acids which stimulated retinol ester formation did not induce greater β-carotene binding than bile acids which did not. In the presence of metabolic inhibitors or in the absence of oxygen, carotene was also bound normally but retinol ester was not formed. In addition, compounds like trimethyldodecyl ammonium chloride, which injured the mucosal cells,

enhanced carotene binding but completely blocked retinol formation. Thus, the amount of β-carotene bound to the intestine was not indicative either of the retinol ester formed, or necessarily of the intracellular concentration of β-carotene.

The Effect of Bile Salts on Other Metabolic Processes in the Intestine

The formation of retinol ester from retinol-C^{14} or retinal-C^{14} by intestinal sections of the rat in vitro was not dependent on the presence of glycocholic acid (Table 5). Similarly, retinol ester formation from retinol or retinal in intestinal homogenates proceeded well in the absence of conjugated bile salts. The synthesis of nonsaponifiable lipid from acetate-1-C^{14} in longitudinally cut intestinal sections was unaffected by 0.2–1.5% Tween 40, but was somewhat inhibited by sodium glycocholate (Table 6). A more marked inhibition of sterol synthesis by glycocholate has been observed in perfused sections of jejunum (16). Deoxycholate, which is known to destroy the epithelial cells of the mucosa (17), profoundly inhibited sterol synthesis.

DISCUSSION

Retinol or retinal, when solubilized by 0.3% Tween 40, is lodged in a micelle with an anhydrous particle weight of about 100,000 g/mole (18, 19) and a single particle volume of about 160,000 A^3. If the hydrated micelle contains 2 g of H_2O per g of nonionic detergent (19, 20) and has a spherical shape, the micellar diameter would be approximately 0.01 μ. Such micelles are much smaller than the minimal size of 0.5 μ defined by Frazer (21) for the particulate absorption of lipids, and are tiny enough to enter the intermicrovillous spaces (ca. 0.04 μ) of the mucosal cells (22). Retinol and retinal solubilized in Tween 40 are readily absorbed and metabolized by the mucosal cells.

TABLE 5 EFFECT OF GLYCOCHOLATE ON RETINOL ESTER FORMATION FROM RETINOL AND RETINAL*

Substrate	Glycocholate	Retinol Ester Formed	
	%	μg	
		Exp. 69	*Exp.* 77
Retinol	0	3.88	2.32
	0.2	3.60	1.72
	0.4	3.72	2.22
	0.8	0.56	—
	1.0	—	0.74
		Exp. 71	*Exp.* 77
Retinal	0	4.4	1.32
	0.2	4.9	1.56
	0.4	4.2	1.73
	0.8	2.0	—
	1.0	—	0.68

* Retinol (15,000 cpm in 17 μg for Exp. 69, or 11,000 cpm in 12 μg for Exp. 77) or retinal (5,000 cpm in 130 μg for Exp. 71 and 10,000 cpm in 23 μg for Exp. 77) was suspended in 1.25% Tween 20 and incubated in a Dubnoff shaker with 1 g of intestinal sections in Krebs-Ringer bicarbonate buffer, pH 7.4, at 32° for 1 hr under 95% O_2–5% CO_2.

Unexpectedly β-carotene, although dispersed in the same fashion, is not metabolized by the gut unless bile or conjugated bile acids are present. Unconjugated acids, with the exception of cholate, are inactive. These differences in activity between free and conjugated acids do not correlate well with known changes in the physicochemical properties of bile acids upon conjugation. The pK_a of free bile acids is between five and six, whereas that of the glycine conjugates is three to four (23, 24). In micellar solution, the apparent pK_a may well be 2 pH units higher (25). However, at pH 7.4, the pH used in these experiments, the major form of both free and conjugated acids would be the salt. In addition the salts of both have similar solubilizing properties (26) which are not strongly affected by pH (23). Micelles of both conjugated and free bile acids have anhydrous particle weights[2] of less than 10,000 (27), and upon combining solutions of these bile acids with Tween 40, similar mixed micelles of intermediate size form[2] (28).

The optimal concentration of conjugated bile salts and cholate for β-carotene cleavage is 0.004–0.008 M, in the same range as the similar critical micelle concentrations for both free and conjugated acids (23, 26). In the presence of the nonionic detergent Tween 40, the bile acids probably enter a mixed micelle at somewhat lower concentrations. Interestingly, the actual concentration of conjugated bile salts in the upper intestinal lumen during digestion, 0.005–0.010 M, is the same as the optimal concentration for carotene cleavage (29). Although deoxycholate and detergents like sodium dodecyl sulfate and deodecyl amine chloride destroy cells (17), the inactivity of hyodeoxycholate, chenodeoxycholate, and other compounds tested could not be attributed to a loss of cellular integrity. Thus, although the physicochemical state of the solubilized carotene is undoubtedly important, the particular effectiveness of conjugated bile salts in stimulating carotene cleavage to retinol ester must be dependent on specific biological interactions with mucosal cells.

At this time the nature of these interactions is unknown. Some indirect evidence favors the possibility that the cell membrane and microvilli of the intestinal cell are the sites of action of conjugated bile salts. Unlike gut, the perfused liver does not require bile salts for the conversion of β-carotene into retinol ester (30). Since the absorption of compounds into liver cells from sinusoids is generally less restrictive than absorption from the

[2] N. R. Peterson, J. S. Herron, and J. A. Olson, unpublished observations.

gut, the existence of a bile acid requirement in mucosal cells might well be associated with its highly differentiated cell membrane. Although bile acids may be absorbed and resecreted into the lumen in the upper gut, only a small portion crosses the proximal mucosa into the blood (31, 32). As already postulated for monoglyceride absorption (24), they may serve a "transport function" for β-carotene as well on the surfaces of the epithelial cells. However, the possibility that the differences in dissociation constants of free and conjugated bile acids become of critical importance within the mucosal cell cannot be ruled out. Unfortunately the entry of β-carotene into the cell could not be differentiated by the methods employed from adsorption on intestinal surfaces. Perhaps as a result of this difficulty, the total carotene bound to the intestine could not be correlated with the amount of retinol ester formed. For example, the best intestinal preparations in vivo contained about 1 μg of retinol ester and 0.5 μg of β-carotene, intestinal segments incubated in vitro generally had 0.4 μg of retinol ester and 1.6 μg of carotene, and intestinal sections inactivated by cellular poisons or by strong detergents contained 2–5 μg of carotene and no retinol ester. The development of procedures whereby extracellular carotene and intracellular carotene might be distinguished would be welcome, and attempts to resolve this problem are under way in our laboratory.

In addition to their stimulation of carotene cleavage, conjugated bile acids similarly enhance the uptake and esterification of palmitate (17), the utilization of glucose by gut slices (17, 33), perhaps the absorption of cholesterol (34), the esterification of oleate (35), and the hydrolytic action of pancreatic lipase (25) and of other enzymes. On the other hand, the transport of glucose and amino acids across the jejunum, the synthesis of protein and nonsaponifiable lipid in the jejunum, and the accumulation of glucose in mucosal cells are inhibited by conjugated bile acids (16, 36, 37). Whether these multiple physiological actions of bile salts will be explicable in terms of a single molecular or micellar mechanism is as yet uncertain. These effects of conjugated bile salts are widespread in vertebrates (17), however, and these compounds may well have regulatory effects on the intestinal mucosa which are distinct from their intraluminar function of lipid emulsification.

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TABLE 6 EFFECT OF DETERGENTS ON THE INCORPORATION OF ACETATE-1-C^{14} INTO NONSAPONIFIABLE LIPID BY INTESTINAL SECTIONS IN VITRO

Detergent	Concentration	No. of Experiments	Nonsaponifiable Lipid
	%		*cpm*
Tween 40	0	18	1800
	0.2	12	2008
	0.5	12	2655
	1.5	12	1755
Glychocholate	0	18	2093
	0.2	12	2316
	0.4	12	1869
	1.0	12	1632
Deoxycholate	0	18	5750
	0.2	12	822
	0.4	12	709
	1.0	12	446

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