Specificity of the Retinol Transporter of the Rat Small Intestine Brush Border†

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ABSTRACT: The uptake of vitamin A (*all-trans*-retinol) by the absorptive cell of the small intestine is the necessary first step in its utilization by the organism and appears to involve a specific carrier that operates by facilitated diffusion. We investigated the specificity of that process by determining the absorption of *all-trans*-, 13-*cis*-, and 9-*cis*-retinol, 3-dehydroretinol, and retinal (vitamin A aldehyde) by gut sheets from the small intestine of suckling rats. We found that radiolabeled *all-trans*-retinol and 3-dehydroretinol were absorbed at similar rates and that approximately 60% of the total absorption could be competed for by unlabeled *all-trans*-retinol. A similar level of inhibition could be achieved for *all-trans*-retinol absorption by treating the intestinal sheets with *N*-ethylmaleimide. The noncompetable, noninhibitable component of *all-trans*-retinol absorption corresponded to the total absorption rate for 13-*cis*- and 9-*cis*-retinol and retinal. Additionally, we found that the relative rates of transport of these retinoids were unrelated to their relative affinities for the abundant absorptive cell retinoid carrier protein, cellular retinol-binding protein, type II, and were not driven by esterification. This confirms that the absorption of retinol is facilitated by a transporter and establishes that it is specific for the *all-trans* alcohol forms of vitamin A.

Vitamin A is present in the diet in two principal forms: as the provitamin carotenoids, particularly β-carotene, from vegetables, and as retinyl esters from animal products. Previous research has demonstrated that while β-carotene is directly internalized by enterocytes for subsequent metabolism (El-Gorab et al., 1975; Hollander & Ruble, 1978), retinyl esters must be hydrolyzed in the intestinal lumen before absorption can occur (Mahadevan et al., 1963). This suggested that a specific transporter that could recognize retinol was present.

Evidence for such a transporter was developed in studies utilizing either perfused intestines (Hollander & Muralidhara, 1977) or everted gut sacs (El-Gorab et al., 1975; Said et al., 1988), which revealed that uptake is a passive carrier-mediated phenomenon. The specificity of the putative carrier was not extensively examined, although it appeared not to transport retinal or retinoic acid (Said et al., 1988). These studies also demonstrated that the uptake is temperature dependent and is unaffected by metabolic inhibitors (Said et al., 1988). However, other studies using model membrane systems demonstrate that retinol can freely diffuse both across membranes (Fex & Johannesson, 1988) and between membranes and binding proteins (Noy & Blaner, 1991). The enterocyte contains CRBP(II).[1] This protein, a member of the superfamily that includes fatty acid-binding proteins, is extremely abundant, comprising approximately 1% of the soluble protein of the jejunal mucosa (Ong et al., 1994). Holo-CRBP(II) serves as the substrate for LRAT, which converts the bound retinol to retinyl esters using endogenous phosphatidylcholines (lecithins) as acyl donors. The retinyl esters are subsequently incorporated into chylomicrons for export into the lymph [for a review of intestinal metabolism of vitamin A, see Ong (1993, 1994)]. The latter observations raise the possibility that the uptake of retinol by enterocytes could be a simple diffusion phenomenon, driven by the large binding capacity for retinol of the CRBP(II) present in the intestine and by subsequent metabolism to retinyl esters, with specificity in uptake imparted by the binding specificity of CRBP(II).

We chose to test rigorously these two possible mechanisms of absorption by comparing the ability of rat intestinal sheets to absorb and subsequently metabolize various retinoids. The retinoids tested included changes in the isomeric state (9-*cis*- and 13-*cis*-retinol), in the oxidative state (retinal), and in the ring structure (3-dehydroretinol) of the vitamin. These retinoids have previously been examined for biological potency *in vivo* (Shantz & Brinkman, 1950; Ames et al., 1955a,b; Weiser & Somarjai, 1992), but no information is available on their efficiency of absorption. The retinoids were absorbed at different rates via a specific carrier-mediated mechanism that discriminated between retinoids and was inhibited by NEM, and at similar rates by passive diffusion. The observed differences in absorption could not be explained either by differing affinities for CRBP(II) or by differing metabolism by LRAT, indicating that the discrimination was by the carrier itself.

EXPERIMENTAL PROCEDURES

Materials. Sprague-Dawley suckling rats (15–17 days old) were purchased from Sasco. *all-trans*- and 13-*cis*-retinol, *all-trans*-, 13-*cis*- and 9-*cis*-retinal, and taurocholate were from Sigma. NaB^3H_4 was from American Radiolabeled Chemicals and [^{14}C]PEG-4000 was from either Dupont NEN or Amersham. 3-Dehydroretinol was a generous gift from Hoffman-LaRoche.

Preparation of Retinoids. All retinoids are the *all-trans* isomer unless otherwise noted. 3-Dehydroretinal and [^{3}H]-retinal were prepared from 3-dehydroretinol and [^{3}H]retinol, respectively, using the method of Bridges and Alvarez (1982). Radioactive *all-trans*-, 9-*cis*-, and 13-*cis*-retinols and *all-trans*-3-dehydroretinol were prepared from their corresponding aldehydes by reduction with NaB^3H_4 as described previously (Liau et al., 1981). 9-*cis*-Retinol was prepared by reduction of 9-*cis*-retinal with unlabeled $NaBH_4$. Retinyl laurate esters were prepared from lauroyl chloride and either *all-trans*-,

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[1] Abbreviations: CRBP(II), cellular retinol binding protein, type II; LRAT, lecithin–retinol acyltransferase; NEM, *N*-ethylmaleimide; PEG, poly(ethylene glycol); DMSO, dimethyl sulfoxide; RBP, retinol binding protein; PMSF, phenylmethanesulfonyl fluoride.

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13-*cis*-, or 9-*cis*-retinol as described (Huang & Goodman, 1965).

Preparation of Suckling Rat Gut Sheets. Previous studies have demonstrated that gut sacs and sheets are useful model systems for studying intestinal uptake of vitamin A (Mahadevan et al., 1963; Olson, 1964; El-Gorab et al., 1975; Said et al., 1988). Gut sheets from male suckling rats were prepared using a modification of the method of Said et al. (1988). The animals were killed by exposure to CO_2, and the small intestine was removed. Sections approximately 6 cm long were taken from the jejunum, starting 4 cm from the pylorus. The sections were washed with ice-cold Krebs–Ringer phosphate buffer (20 mM sodium phosphate, 125 mM NaCl, 4.93 mM KCl, 1.23 mM $MgSO_4$, 0.85 mM $CaCl_2$, and 10 mM glucose, pH 6.5) and then slit longitudinally and rinsed vigorously for an additional 30 s. They were then cut into 0.5 cm sections and randomized.

All experiments were performed under dim or yellow light. The incubation buffer was prepared by adding the appropriate retinoid (100 μM in DMSO) to Krebs–Ringer buffer with 0.5% taurocholate (w/v) to a final concentration of 0.06 μM. A trace amount of [^{14}C]PEG-4000 was added as a nonabsorbable marker. For competition experiments, the appropriate competitor was added to the incubation buffer in DMSO. The concentration of DMSO was kept below 1%. For all experiments, three gut sheets were incubated at 37 °C in 5 mL of incubation buffer in a 30 mL beaker. The samples were shaken at 80 oscillations/min, with 95% O_2 bubbled vigorously through the medium. After incubation, the sheets were quickly rinsed in cold buffer, weighed, and digested in 0.4 mL of 7.5 M NaOH at 70 °C overnight. After cooling, 15 mL of Cytoscint (ICN) was added and the radioactivity determined. Uptake of substrate was corrected for adherent fluid as determined by [^{14}C]PEG-4000. This correction accounted for less than 10% of the total tissue-associated radioactivity. At least six rats were used for each experiment.

Assay of Retinol Esterification. Intestinal microsomes were prepared and assayed as described previously (MacDonald & Ong, 1988; Dew et al., 1993). For determination of the isomeric specificity of the product esters, intestinal microsomal LRAT was solubilized as described (Dew et al., 1993). The solubilized protein was diluted 5-fold into the assay buffer to a concentration of approximately 0.6 mg/mL. The assay mixture consisted of 25 μL of the solubilized preparation (containing approximately 3 μg of protein) with the appropriate [^{3}H]retinol isomer (6 μM) in 500 μL of 0.2 M potassium phosphate buffer (pH 7.2) containing 1 mM dithiothreitol, 40 μM dilauroylphoshatidylcholine, and 10% DMSO. The assay was initiated by addition of the retinol and allowed to proceed in a 37 °C water bath for 10 min. The reaction was terminated by addition of 2 mL of cold ethanol, transferred to a 15-mL extraction tube, and extracted with 8 mL of hexane. To separate the phases, 2 mL of water was added. A measured amount of the hexane layer was transferred to a drying tube and dried under N_2. The product esters were dissolved in 100 μL of the mobile phase containing 13-cis-, 9-cis-, and *all-trans*-retinyl laurate standards. The elution times of the [^{3}H]-esters were determined by radioactivity and compared to the elution times of the standards, as monitored by absorption at 325 nm. Analysis was performed by HPLC on a Whatman Partisil 5, 4.6 mm × 25 cm, 5-μm particle silica column with a Supelco LC-Si 2-cm guard column using an ISCO Model 2350 pump, Model 2360 gradient programmer, and V^4 detector with a Spectra-Physics SP4270 integrator. The mobile phase was 0.3% ethyl acetate in hexane (v/v).

Extraction of Retinoids from Gut Sheets. The gut sheets from three rats were incubated for 4 min as described above, rinsed, and immediately frozen on dry ice and stored at −70 °C until further analysis. The absorbed retinoids were extracted essentially as described (Pappas et al., 1993). Briefly, the tissue was thawed, weighed, and homogenized with a Polytron PT-3000 at 22 000 rpm for 1 min in 6 mL of ethanol containing *tert*-butyl hydroxytoluene (100 μg/mL). One volume of 4.25 M NaCl in 0.025 M NaOH was added, followed by extraction (twice) with 1 volume of hexane. The hexane extracts were pooled, dried under N_2, and resuspended in 500 μL of hexane. The samples were applied to alumina columns (10% water deactivated, 1.2 g) and washed with hexane. The ester fraction was eluted with 2% ether in hexane (v/v), the retinal fraction (in samples for retinal metabolism) with 15% ether in hexane, and the retinol fraction with 50% ether in hexane as described previously (Pappas et al., 1993; Dew et al., 1993). The retinol and retinal fractions were dried under N_2 and resuspended in 100 μL of hexane, and an aliquot was taken for determination of radioactivity. The retinyl ester fractions were collected directly into scintillation vials and dried under N_2, and the radioactivity was determined.

Assay of Comparative Binding of Retinoids to CRBP(II). Apo-CRBP(II) was purified from an *Escherichia coli* expression system as described (Dew et al., 1993). The comparative binding of various retinoids was determined by their ability to compete for *all-trans*-[^{3}H]retinol binding to CRBP(II). Apo-CRBP(II) (500 μL of 1 μM in 0.01 M Tris–acetate, pH 8.3) was incubated with *all-trans*-[^{3}H]retinol (1.2 μM) and increasing concentrations of cold competitor. The retinoids were combined prior to their addition in 5 μL of DMSO. After incubation overnight at 4 °C in the dark, the bound retinoids were separated from free retinoids by chromatography on a 0.5 mL DEAE column (DE-52, Whatman). The column was equilibrated with 0.01 M Tris–acetate (pH 8.3) before addition of 400 μL of the incubation mixture. The column was then washed with 6 mL of the equilibrating buffer. The retinoid–protein complex was eluted with 2 mL of 0.33 M Tris–acetate (pH 8.3) directly into a test tube containing 100 μL of a 10 mg/mL bovine serum albumin solution. The sample was vortexed briefly and an aliquot transferred to a scintillation vial. Two volumes of ethanol were added and the sample radioactivity was determined. Background was determined by omission of the binding protein. The recovery was approximately 80% of the calculated protein-associated radioactivity.

Statistical Analysis. All statistical analyses were done using Instat, ver. 2.0, from GraphPad and as described by Glantz (1992).

RESULTS

Absorption of Retinoids by Gut Sheets and Competition by all-trans-Retinol. The ability of jejunal gut sheets to absorb *all-trans*-, 9-*cis*-, and 13-*cis*-retinol, 3-dehydroretinol, and retinal was examined as a function of time and was found to be linear for at least 5 min, as represented in Figure 1 by *all-trans*-retinol, 3-dehydroretinol, and retinal. The rate of absorption varied significantly among the retinoids examined. The *all-trans* alcohols (*all-trans*- and 3-dehydroretinol) had very similar rates of uptake [41 ± 2 and 36 ± 7 pmol/(g of tissue·min), respectively], whereas 9-*cis*-retinol [14 ± 1 pmol/(g of tissue·min), $P < 0.001$], retinal [17 ± 1 pmol/(g of tissue·min), $P < 0.001$], and 13-*cis*-retinol [24 ± 3 pmol/(g of tissue·min), $P < 0.01$] were absorbed at significantly lower rates than that for *all-trans*-retinol.

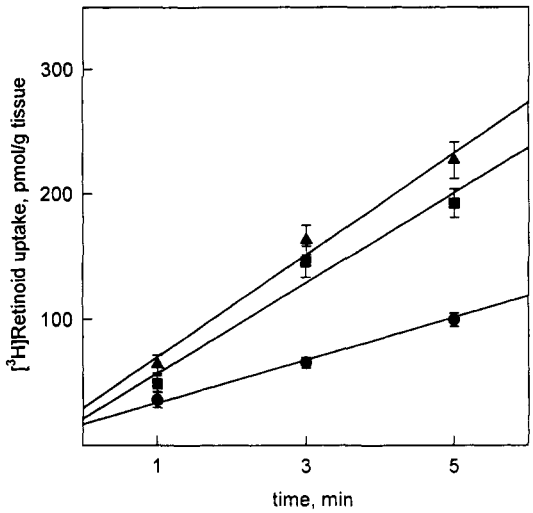

FIGURE 1: Rate of absorption of retinoids by gut sheets. Incubations were as described in the experimental procedures with 0.06 μM *all-trans*-[^{3}H]retinol (▲), 3-[^{3}H]dehydroretinol (■), or [^{3}H]retinal (●). The data shown are the mean ± SEM of at least six experiments.

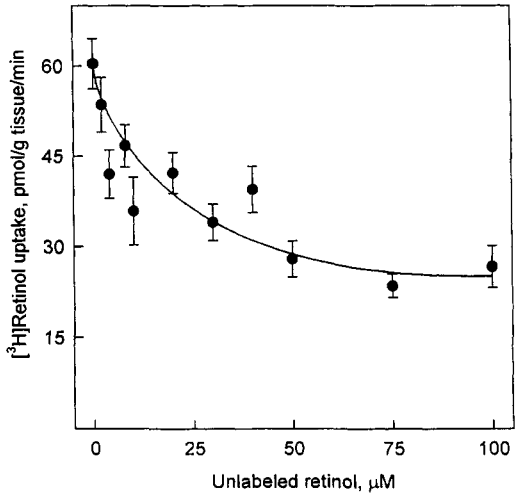

FIGURE 2: Competition of *all-trans*-[^{3}H]retinol absorption by unlabeled retinol. Incubations were for 4 min with 0.06 μM *all-trans*-[^{3}H]retinol and increasing concentrations of unlabeled *all-trans*-retinol. The data shown are the mean ± SEM of at least six experiments.

When gut sheets were incubated with 0.06 μM *all-trans*-[^{3}H]retinol and increasing concentrations of unlabeled retinol, the amount of absorbed [^{3}H]retinol was decreased in a concentration-dependent manner to approximately 40% [about 20 pmol/(g of tissue·min)] of that seen without added competitor (Figure 2). These data were transformed into total tissue-associated retinol as a function of retinol concentration, which generated a biphasic curve with saturable and nonsaturable components. The nonsaturable component was determined graphically (Shingleton et al., 1989) and was subtracted from total tissue-associated retinol to generate a curve for the saturable uptake component (data not shown). Analysis of this uptake yielded a K_T of 15 μM and a V_{max} of 10 nmol of retinol/(g of tissue·min), similar to previously reported values determined for gut sacs of 16 μM and 4 nmol of retinol/(g of tissue·min) (Said et al., 1988). The nonspecific component could be calculated to be approximately 21 pmol of retinol/(g of tissue·min) at a retinol concentration of 0.06 μM.

This calculated rate of nonspecific uptake of *all-trans*-retinol was not distinguishable from the rate of total uptake for retinal and 9-*cis*- and 13-*cis*-retinol. This indicated that the transporter for retinol was discriminating among the retinoids examined, with some retinoids entering only by passive diffusion. This was established by examining the ability of unlabeled *all-trans*-retinol to compete for the absorption of radiolabeled 13-*cis*- and 3-dehydroretinol and retinal. Unlabeled retinol effectively competed for the absorption of 3-dehydroretinol and *all-trans*-retinol (Figure 3). No competition was observed for 13-*cis*-retinol and retinal, consistent with uptake occurring only by passive diffusion, which would not be affected by the presence of unlabeled competitor.

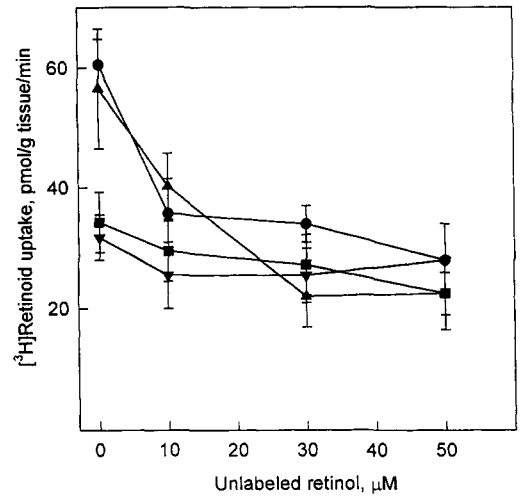

FIGURE 3: Competition of [^{3}H]retinoid absorption by unlabeled *all-trans*-retinol. Incubations were for 4 min with 0.06 μM radiolabeled *all-trans*-retinol (●), 3-dehydroretinol (▲), 13-*cis*-retinol (■), or retinal (▼) and increasing concentrations of unlabeled *all-trans*-retinol. The data shown are the mean ± SEM of at least six experiments.

Inhibition of all-trans-Retinol Uptake by NEM. A variety of protein modification reagents were examined for their ability to inhibit *all-trans*-retinol uptake by gut sheets. Diethyl pyrocarbonate, iodoacetamide, diethyl *p*-nitrophenyl phosphate, PMSF, and diisopropyl fluorophosphate had no significant effects on retinol absorption. However, NEM was found to be an effective inhibitor. At a concentration of 500 μM NEM, 60% of the total absorption was blocked (Figure 4). This inhibition encompassed all of the carrier-mediated absorption, as can be seen by comparison with the amount of [^{3}H]retinol absorbed in the presence of an excess of unlabeled retinol. In a similar study, NEM was found to have no effect on the rate of absorption of retinal (data not shown).

Determination of Relative Binding Affinities of Various Retinoids for CRBP(II). CRBP(II) binds all of the retinoids examined except 9-*cis*-retinol (MacDonald & Ong, 1987). To determine the relative binding affinities of different retinoids for CRBP(II) for comparison to relative uptake rates, apo-CRBP(II) was incubated with a constant, saturating amount of *all-trans*-[^{3}H]retinol and increasing amounts of unlabeled 13-*cis*-retinol, 3-dehydroretinol, or retinal. After protein was separated from the free retinoids, the amount of *all-trans*-[^{3}H]retinol bound was determined as a percentage of the retinol bound in the absence of any competitor (Figure 5). As expected, a one molar excess of unlabeled *all-trans*-retinol resulted in 50% displacement of the [^{3}H]retinol. In contrast, both retinal and 3-dehydroretinol required a 2–4-fold molar excess to achieve the same displacement, while 13-*cis*-retinol required an approximately 8-fold molar excess.

Esterification of Various Retinoids by LRAT. Intestinal microsomal LRAT effectively esterified *all-trans*-retinol and 3-dehydroretinol when the substrate was presented either free

Table 1: Kinetic Constants for Esterification of Retinols by Intestinal Microsomal LRAT

	free retinol		retinol–CRBP(II)	
	K_m (μM)	V_{max} [pmol/(min·mg of protein)]	K_m (μM)	V_{max} [pmol/(min·mg of protein)]
all-trans-retinol	0.6 ± 0.2[a]	188 ± 14	0.14 ± 0.02	149 ± 3
3-dehydroretinol	0.5 ± 0.1	145 ± 11	0.21 ± 0.03	148 ± 4
13-*cis*-retinol	5.0 ± 1.4	286 ± 27	1.9 ± 0.2	166 ± 3

[a] Kinetic constants were determined using the program k_{cat}, ver. 1.3 (Biometallics, Inc.), using data from at least two determinations.

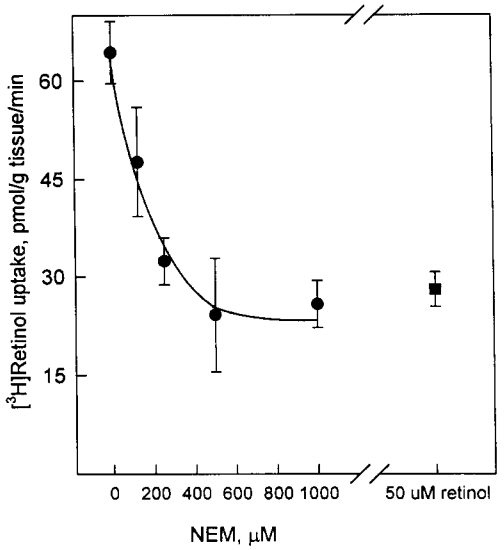

FIGURE 4: Inhibition of *all-trans*-retinol absorption by NEM. Incubations were for 4 min with 0.06 μM all-*trans*-[^{3}H]retinol and either increasing concentrations of NEM (●) or 50 μM *all-trans*-retinol (■). The data shown are the mean ± SEM of at least six experiments.

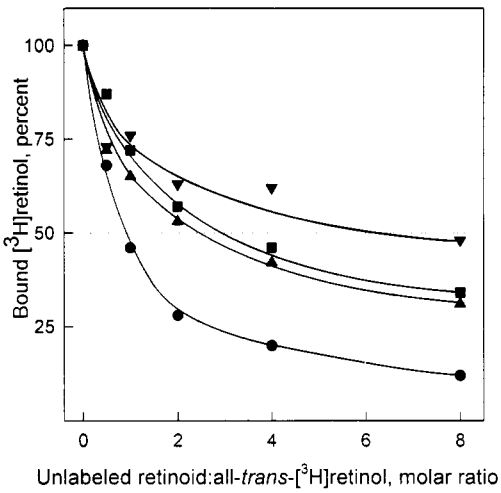

FIGURE 5: Competition for *all-trans*-[^{3}H]retinol binding to CRBP(II) by other retinoids. Apo-CRBP(II) was incubated with *all-trans*-[^{3}H]retinol and increasing concentrations of *all-trans*-retinol (●), retinal (■), 3-dehydroretinol (▲), or 13-*cis*-retinol (▼) overnight at 4 °C. Holo-CRBP(II) was separated from the free retinoids on a DEAE-cellulose column as described. Binding of *all-trans*-[^{3}H]retinol was determined as a percentage of the amount bound in the absence of any competitor. Each point is the mean of at least three determinations.

or bound to CRBP(II) (Table 1). As previously reported, *all-trans*-retinol–CRBP(II) was utilized more effectively than the free retinol as indicated by the measured K_m values (Herr & Ong, 1992). Here a similar decrease in K_m for 3-dehydroretinol–CRBP(II) over the unbound retinoid was also observed. 13-*cis*-Retinol was not readily utilized by LRAT,

Table 2: Metabolism of Retinoids by Intestinal Sheets

	% retinol	% retinyl esters	% retinal
all-trans-retinol	85.4[a]	14.6	n.a.[b]
3-dehydroretinol	80.1	17.9	n.a.
13-*cis*-retinol	84.7	15.3	n.a.
9-*cis*-retinol	87.9	12.1	n.a.
retinal	45.2	8.4	46.4

[a] Data are presented as the percentage in each fraction of the total cpms recovered after a 4 min incubation. Each point is the average of two determinations, each using intestinal sheets from three rats. [b] Not applicable.

with K_m values for both the bound and the free retinoid approximately 10-fold higher than those for the *all-trans* isomers. Attempts to determine the kinetic parameters for esterification of 9-*cis*-retinol were unsuccessful, since substrate concentrations adequate to saturate the enzyme could not be achieved. However, at a concentration of 0.5 μM, 9-*cis*-retinol was utilized 56% as effectively as *all-trans*-retinol. Examination of the isomeric state of the esters produced with 13-*cis*- and 9-*cis*-retinol confirmed that the product esters retained the isomeric configuration of the substrate retinol isomers.

Retinyl Ester Formation by Gut Sheets. If esterification is the rate-limiting step in absorption, it is possible that this differential esterification could account for the differences in rate of uptake. Also, since NEM effectively inhibits LRAT (Herr et al., 1991), the inhibition of uptake by NEM might be then due to inhibition of LRAT. However, when the amount of retinol esterification was examined in gut sheets incubated with radiolabeled retinoids, in the short time of the uptake experiment, the relative amounts of esterification were low (Table 2), accounting only for approximately 15% of the tissue-associated radioactivity. Not unexpectedly, absorbed retinal was effectively reduced to retinol, since this is a normal step in β-carotene metabolism, and the late suckling rat intestine contains a specific retinal reductase (Ong et al., 1991). Note that the gut sheets were continuing to support physiological function during the course of preparation and experimentation, since at least two of the enzymes involved in vitamin A metabolism inside the cell remained functional.

DISCUSSION

The evidence presented here indicates that the intestinal mucosa contains a transporter for retinoids that is quite specific. This was shown by its ability to transport *all-trans*-retinol and 3-dehydroretinol, as demonstrated by both competition studies and inhibition of uptake by a protein modification reagent, and its failure to recognize 9-*cis*- and 13-*cis*-retinol or retinal. The noncompetable component of *all-trans*-retinol absorption was essentially the same as the total absorption observed for both 9-*cis*- and 13-*cis*-retinol and retinal. Because the absorption of 9-*cis*- and 13-*cis*-retinol and retinal was not competed by unlabeled *all-trans*-retinol, and such absorption (tested with retinal) was not inhibited by NEM, all could be accounted for by passive diffusion. This relatively high non-carrier-mediated uptake may reflect in part the effects of

taurocholate on the integrity of the intestinal membranes and may be higher than would be expected *in vivo*.

A recent study (Levin, 1993) reported that amounts of retinol absorbed by the human intestinal cell line Caco-2 were determined by the levels of CRBP and CRBP(II) expressed in those cells. Since CRBP(II) is present at high levels in the normal intestine, rates of uptake of various retinoids by the gut sheet might then be mediated by their relative affinities for CRBP(II). To address this possibility, we determined the *relative* K_d's of CRBP(II) for these retinoids. Previous determinations of apparent K_d values have utilized the technique of fluorimetric titration, following either the increase in retinol fluorescence upon binding to the protein or the quenching of the protein's fluorescence which also occurs with retinoid binding. This method has been used by several groups (Li et al., 1987; MacDonald & Ong, 1987; Levin et al., 1988) and resulted in reported K_d's of 10–100 nM for retinol–CRBP-(II). Similar values were obtained for 13-*cis*-retinol and retinal (MacDonald & Ong, 1987). However, these similarities may have been due in part to an overestimation of the amount of free retinoid present in the assay system, since this method does not correct for retinoid that is unavailable for binding due to isomerization, oxidation, or adhesion to the glass of the cuvette. Such losses can create a "leveling" effect where all bound retinoids appear to have similar K_d values. Therefore, we utilized a column-based assay to determine the relative affinities of *all-trans*-, 13-*cis*-, and 3-dehydroretinol and retinal for CRBP(II) by competing for *all-trans*-[^{3}H]retinol binding to CRBP(II). The observed affinities of CRBP(II) for 3-dehydroretinol and retinal assessed by this method were about 3-fold less than the affinity for *all-trans*-retinol. This difference was previously observed by MacDonald and Ong (1987) using a fluorometric competition assay, but it was speculated that this apparent difference in K_d's might instead reflect differences in rates of association if equilibrium was not achieved during a relatively brief (30 min) incubation. Our results were obtained after incubation overnight and indicate that there is a true difference in these K_d's. The affinity of CRBP(II) for 13-*cis*-retinol was even lower, approximately 8-fold less than that of *all-trans*-retinol. No competition studies for *all-trans*- and 13-*cis*-retinol have previously been reported, and the earlier fluorimetric comparisons are subject to the caveats discussed above. Note that even though 13-*cis*-retinol was bound less tightly than *all-trans*-retinol, it still had a K_d in the nanomolar range (based upon previous determinations of the K_d for *all-trans*-retinol) and was readily bound by CRBP(II).

These differences in binding affinity did not reflect the relative rates at which these retinoids were absorbed by gut sheets. Note in particular the case of retinal and 3-dehydroretinol: both retinoids were bound by CRBP(II) with indistinguishable affinities, but 3-dehydroretinol was absorbed at a significantly higher rate. Also, under the conditions employed here, the passive uptake of retinal and 9-*cis*-retinol was similar, even though only retinal binds to CRBP(II) with measurable affinity (MacDonald & Ong, 1987). Carrier-mediated uptake was also not measurably modulated by affinity of the transported retinoid for CRBP(II), since the rates of uptake for *all-trans*-retinol and 3-dehydroretinol were the same even though their K_d values differed by 3-fold. Thus CRBP(II) cannot be an important determininant in rate of retinoid uptake under the conditions examined here. There are several possible explanations for the apparent discrepancy between this observation and that reported for Caco-2 cells. Caco-2 cells underexpress CRBP(II) (Quick & Ong, 1990) compared to normal human enterocytes (Inagami & Ong, 1992), so "overexpression" of the binding protein may, in fact, raise the levels to more nearly physiological concentrations in these cells. Since the amount of retinol absorbed was determined after either a 4 or 24 h incubation, an increase in the level of CRBP(II) might increase the total accumulation of retinol within the cells by providing a larger reservoir of binding sites without actually affecting the rate of absorption. This is particularly true if esterification of the retinol and regeneration of apo-CRBP(II) would become a rate-limiting factor in uptake after significant amounts of retinol were internalized. In studies with testicular Sertoli cells (Shingleton et al., 1989), examination of the internalization of retinol from RBP by these cells demonstrated that the rate of absorption declined when sufficient retinol had been internalized to fill all of the available CRBP in those cells. The subsequent rate of absorption might then have been dependent on the rate of esterification to generate apo-CRBP. As in Sertoli cells, the level of LRAT in Caco-2 cells is very low (Quick & Ong, 1990). Therefore, increasing the levels of CRBP(II) in these cells could significantly affect the total cellular associated retinol. It is also possible that Caco-2 cells do not express the transporter, since they do not always retain all of the functions of the normal cell, such as the ability to cleave β-carotene (Quick & Ong, 1990).

In studies of the uptake of retinol from either RBP or CRBP by membranes, it was postulated that the rate of uptake by target cells might be determined by the rate of its subsequent metabolism (e.g., esterification) (Noy & Blaner, 1991). This did not appear to be the case here, since only a small portion of the internalized retinols were esterified. Further, treatment of the gut sheets with PMSF, known to inhibit LRAT (Herr et al., 1991), did not decrease the rate of uptake. Also, each of the retinols examined were esterified to the same extent, despite having significantly different rates of absorption. However, it is possible that marked differences in the intestinal processing of these retinoids would be discernible over a longer time period than that examined here. *In vitro* examination of the kinetic parameters of LRAT with isomers of retinol revealed that the enzyme did not esterify *cis* isomers as rapidly as it did *all-trans*-retinol at low concentrations. This might affect the rate of exit from the enterocyte and consequently have some effect on biopotency. Earlier studies on substrate specificity in LRAT from the eye revealed a broad tolerance for variations in the ring and chain structures. Isomers, however, were not examined (Canada et al., 1990).

Previous studies have determined the efficacy of 3-dehydroretinol, various isomers of retinyl acetate, and retinal as substitutes for *all-trans*-retinyl acetate in bioassays based on rat growth and liver storage of vitamin A (Shantz & Brinkman, 1950; Ames et al., 1955a,b) or by using vaginal smear assays (Weiser & Somarjai, 1992). These groups found that 13-*cis*- and 9-*cis*-retinyl acetates were respectively 75% and 22% as effective as *all-trans*-retinyl acetate, while retinal and 3-dehydroretinol were at 91% and 40%, respectively. One reason for these different biopotencies may be due to the organism discriminating against certain retinoids at different levels of metabolism. In the intestine alone, there are several steps which discriminate among retinoids: differential uptake by the enterocyte, differential binding of nonphysiological retinoids by CRBP(II), and differential esterification by LRAT. This last observation is of particular interest, since LRAT is also involved in vitamin A storage and metabolism in the liver. Thus its inability to efficiently use *cis* isomers could have serious effects on their bioavailablity.

In summary, we have demonstrated that, in suckling rat jejunum, the absorption of vitamin A is mediated by an NEM-sensitive specific carrier. This carrier discriminates between the isomeric and oxidative states of the retinoid, but is unaffected by a minor change in ring structure. The specificity of this carrier cannot be dependent upon the affinity of CRBP-(II) for these retinoids, since utilization by the transporter does not reflect the relative affinity of CRBP(II) for these retinoids.

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