Pathways of absorption of retinal and retinoic acid in the rat

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ABSTRACT The chemical and anatomical pathways of absorption of dietary retinal, retinoic acid, and retinol were examined in rats containing lymph, bile, and duodenal cannulae. The experiments were designed to maintain physiological conditions to the greatest possible extent. In each rat an uninterrupted flow of bile into the duodenum was maintained by connecting the duodenal cannula to the bile duct of a second rat.

Labeled vitamin A compounds were introduced into the duodenum in very small amounts (7–14 μg) in the form of a bile–lipid mixture resembling normal intestinal contents. Under these conditions, most (70–80%) of the radioactivity recovered after the feeding of labeled retinol or retinal was found in the lymph, predominantly in saturated retinyl esters. In contrast, 92–95% of the radioactivity recovered after the feeding of labeled retinoic acid was found in the bile, and was contained in a mixture of polar metabolites, most of them more polar than free retinoic acid. Two-thirds of the small amount of radioactivity found in lymph after retinoic acid-^{14}C feeding was in the form of free retinoic acid.

The results indicate that under normal conditions the major pathway of retinal absorption involves its reduction to retinol, which is then esterified and transported via the lymphatics in a manner similar to that of dietary retinol. A small proportion of retinal is apparently normally oxidized, and is then transported via the portal vein and excreted in the bile in a manner similar to that of dietary retinoic acid. The relative importance, in quantitative terms, of these two pathways of retinal metabolism can vary, depending on the status of the animal.

KEY WORDS vitamin A · retinal · retinoic acid · retinol · retinyl esters · rat · absorption · metabolism · intestine · lymphatics · portal vein · bile · reduction · oxidation

RECENT STUDIES on the biosynthesis of vitamin A from β-carotene have demonstrated that the initial reaction in this biosynthetic sequence involves the central cleavage of β-carotene into two molecules of retinal (1–4). This reaction occurs during the intestinal absorption of β-carotene and is catalyzed by a soluble mucosal enzyme (1–4). Some uncertainty exists, however, as to the events that occur during the subsequent metabolism of the mucosal retinal. On the one hand, studies in vivo, both in the rat (5) and in man (6), have demonstrated that vitamin A, newly formed from dietary β-carotene, is mainly transported in the lymph in the form of retinyl esters. These studies suggest that newly-formed retinal is reduced to retinol, and subsequently esterified, in the intestinal mucosa. Reduction of retinal to retinol is catalyzed by a soluble mucosal aldehyde reductase which requires NADH or NADPH as co-factor (7). On the other hand, it is known that intestinal mucosa also contains an enzyme capable of oxidizing retinal to retinoic acid (8, 9), and it has recently been suggested that a significant portion of mucosal retinal is oxidized to retinoic acid and absorbed via the portal vein (9). There is some evidence suggesting that retinoic acid, if absorbed, would not accumulate in tissues, but would be largely excreted in the bile in the form of polar metabolites (10–12). Thus, large amounts of radioactivity were found in the bile of rats after the intraportal injection of labeled retinoic acid (10, 11). Two possible pathways of retinal metabolism hence exist: (*a*) reduction to retinol and transport as retinyl esters via the lymphatics; (*b*) oxidation to retinoic acid and transport via the portal vein. The relative importance of these alternative pathways under normal conditions is not clear.

In the experiments described below, this question was examined by comparing the intestinal absorption and metabolism of dietary retinal, retinoic acid, and retinol in rats containing both lymph and bile fistulae. The ex-

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periments were designed to maintain conditions as nearly physiological as possible. Thus, in each experiment, bile was continuously replaced via a duodenal cannula connected to the bile duct of a second, donor rat. Furthermore, all vitamin A compounds were administered in very small amounts in the form of a bile–lipid mixture resembling normal intestinal contents. The results demonstrate that under normal conditions retinal is mainly reduced to retinol and absorbed via the lymphatic route.

METHODS AND MATERIALS

Labeled Substrates

Retinyl-6,7-^{14}C acetate (specific radioactivity 6.83 μc/μmole) was a generous gift from Hoffmann-La Roche, Basel, Switzerland.

Retinol-6,7-^{14}C was prepared by saponification of the retinyl acetate-^{14}C (see below for details). The nonsaponifiable fraction was extracted with hexane, and the ^{14}C-labeled retinol was isolated by chromatography on a column of alumina (5).

Retinal-6,7-^{14}C was prepared by oxidation of retinol-6,7-^{14}C with MnO_2 (13), and the retinal was also purified by alumina chromatography (5).

Retinoic acid-6,7-^{14}C was prepared by the enzymic oxidation of retinal-6,7-^{14}C as described previously (1).

Unlabeled Substrates

Unlabeled retinal, retinol, and retinoic acid were purchased from Eastman Kodak (Rochester, N.Y.). Unlabeled retinyl palmitate, oleate, and linoleate were synthesized by reaction of retinol with the appropriate acyl chloride as described previously (5).

Feeding Mixtures

In each study, approximately 32 μg (0.7–0.8 μc) of the labeled vitamin A compound was dissolved in 25 μl of acetone. To this was added 0.1 ml of a mixture of oleic acid and monoolein (2:1, v/v); freshly collected rat bile was then added to a final volume of 2 ml. The mixture was agitated with a Vortex mixer to provide a relatively stable emulsion.

Surgical Procedures

Lymph and bile duct cannulations were performed on the same rat. Polyethylene tubing (Intramedic, Clay-Adams, Inc. New York Cat. No. PE 50) was implanted into the thoracic duct below the diaphragm by a modification of the method of Bollman, Cain, and Grindlay (14). The bile duct was severed 2–3 cm above its point of entrance into the duodenum. Cannulae of polyethylene tubing (PE 10) were inserted into both ends of the severed bile duct. One tube was inserted towards the liver for the collection of bile, and the second tube was inserted through the lower portion of the bile duct into the duodenum. The external ends of these two tubes were then connected, so as to provide an uninterrupted flow of bile from the liver into the duodenum.

The animals were kept in restraining cages and allowed access to a solution of 5% glucose in 0.45% saline. Studies were begun 12–24 hr after the completion of surgery. At the start of each experiment the duodenal and bile cannulae were separated from each other, and the animal was tested for its ability to absorb fat as follows. Through the duodenal cannula was fed a test meal of 0.2 ml of the oleic acid–monoolein mixture 2:1, emulsified with 0.3 ml of bile. When fat absorption and the formation of chylomicrons was evinced by the milky appearance of the chyle, 0.5 ml of the appropriate ^{14}C-labeled vitamin A feeding mixture was fed through the duodenal cannula. This (duodenal) cannula was then connected to the bile duct of a second (bile donor) rat, so that the uninterrupted flow of bile into the duodenum was continued for the remainder of the experiment. Lymph and bile were collected in amber graduated cylinders kept in ice. After 24 hr of collection the rats were killed; at this time the livers were excised, rinsed in saline, blotted, and weighed.

Extraction Procedure

Lymph, bile, and liver samples were extracted with 20 volumes of chloroform–methanol 2:1. A mixture of nonradioactive carrier vitamin A compounds, containing 100 μg each of retinal, retinol, a mixture of retinyl esters, and retinoic acid, was added to the chloroform–methanol extract. The total extract was filtered, and a portion was taken for radioassay. The remainder was split into two phases by addition of 0.01 N H_2SO_4 (5). One-tenth of each chloroform and of each methanol–water phase was taken for radioassay and the rest was saved for chromatographic and other analyses.

Column and Thin-Layer Chromatography

Chromatography was performed on alumina columns as described previously (5).

The composition of the labeled retinyl esters eluted in fraction 2 (see Table 3) from the alumina columns was determined by chromatography on thin-layer plates of Alumina Gel G impregnated with silver nitrate, as described previously (5). Approximately 100 μg each of retinyl palmitate, oleate, and linoleate were added to the samples before chromatography. After development, the separated bands were viewed under UV light and scraped onto filter funnels, and the esters were eluted with chloroform.

Other Procedures

Saponification was carried out in amber tubes under N_2 with 2 N KOH in 70% ethanol. The samples were heated at 60–70°C for 1.5 hr, and the nonsaponifiable fraction was extracted with light petroleum ether (bp 39–53°C) or hexane. The saponifiable fraction was similarly extracted after acidification.

In some samples, acidic and nonacidic lipids were separated by extraction of a hexane solution of the lipids with 0.1 N NaOH in 50% ethanol (15). The nonacidic lipids remained in the hexane phase, which was removed; the acidic lipids were subsequently recovered by acidification of the ethanolic NaOH and extraction with hexane.

Radioassay

Samples were dissolved in 12 ml of the scintillation mixture described by Bray (16) and assayed in a Packard Tri-Carb scintillation counter at an efficiency of 78%. Corrections were made for quenching, where necessary, by the addition of toluene-^{14}C as internal standard.

RESULTS

The percentage recovery and distribution of radioactivity in the lymph, bile, and liver, after the feeding of the various ^{14}C-labeled vitamin A compounds to rats, is shown in Table 1. Most of the radioactivity recovered after feeding ^{14}C-labeled retinol or retinal was found in the lymph, while 15–20% was present in the bile. In sharp contrast, after feeding of retinoic acid-^{14}C, 90–95% of the recovered radioactivity was found in the bile. The liver contained 5–10% of the recovered ^{14}C after labeled retinal had been fed, but only 0.1–0.6% after labeled retinol or retinoic acid.

The distribution of radioactivity between the chloroform (lipid) and the methanol–water (nonlipid) phases is shown in Table 2. Nearly all of the radioactivity from all the lymph extracts was found in the lipid (chloroform) fraction. In contrast, 28–53% of the radioactivity in the bile extracts was found in the nonlipid (methanol–water) phase. Most of the radioactivity of the liver extracts was found in the lipid fraction, although 14–38% was recovered in the methanol–water phase after the feeding of retinol-^{14}C or retinoic acid.

Table 3 shows the distribution of radioactivity observed after chromatography of the lipid extracts of lymph, bile, and liver on alumina columns. Most of the radioactivity present in the lymph and liver extracts of rats fed retinol-^{14}C or retinal was found in the retinyl ester fraction. Most of the radioactivity in the bile lipid extracts, however, remained on the column, presumably

TABLE 1 RECOVERY AND DISTRIBUTION OF RADIOACTIVITY AFTER THE FEEDING OF ^{14}C-LABELED RETINOL, RETINOIC ACID, AND RETINAL TO RATS

Rat No.	Labeled Compound Fed	Amount Fed		Total ^{14}C Recovered*	Distribution of Recovered ^{14}C		
					Lymph	Bile	Liver
		μg	μc	% of ^{14}C fed		%	
1	Retinol	14	0.29	47.5	79.8	20.1	0.1
2	Retinoic acid	7	0.19	50.7	5.3	94.3	0.4
3	Retinoic acid	7	0.19	37.5	6.7	92.7	0.6
4A†	Retinal	8	0.19	41.5	79.8	20.2	—†
4B†	Retinal	8	0.19	44.0	70.3	19.4	10.3
5	Retinal	8	0.19	64.2	78.3	15.6	6.1

* Recovered in lymph, bile, and liver. It is presumed that the recovery of ^{14}C in these samples also represents the percentage absorption of the labeled substrate.
† Rat No. 4 was fed twice. At the end of the second experiment (4B), the liver was removed and extracted.

TABLE 2 PARTITION OF RECOVERED ^{14}C BETWEEN LIPID AND NONLIPID PHASES

Rat No.	Labeled Compound Fed	Lymph		Bile		Liver	
		C*	M*	C	M	C	M
				%			
1	Retinol	98.4	1.6	47	53	86	14
2	Retinoic acid	85.9	14.1	72	28	62	38
3	Retinoic acid	97.8	2.2	65	35	81	19
4A	Retinal	99.9	0.1	55	45	—†	—†
4B	Retinal	99.9	0.1	54	46	99	1
5	Retinal	99.9	0.1	66	34	99	1

* C, chloroform (lipid) phase; M, methanol–water (nonlipid) phase.
† Rat No. 4 was fed twice. At the end of the second experiment (4B), the liver was removed and extracted.

TABLE 3 DISTRIBUTION AND RECOVERY OF ^{14}C AFTER CHROMATOGRAPHY ON ALUMINA COLUMNS

Labeled Compound Fed:	Retinol		Retinoic Acid		Retinal		
Lipid Extract of:	Lymph	Bile	Lymph†	Bile†	Lymph‡	Bile‡	Liver‡
Total ^{14}C Recovered from Column* (%):	101	36	70	27	92	20	90
Column Fraction	*% distribution of recovered ^{14}C*						
1 (β-carotene)	6	0.2	3	3	9	1	4
2 (retinyl esters)	77	2	22	35	82	6	79
3 (retinal)	6	2	5	3	4	10	5
4 (retinol)	8	23	3	17	3	17	3
5 (polar; nonacidic)	3	27	2	11	1	20	2
6 (polar; acidic§)	1	45	65	32	2	45	7

* Per cent of applied ^{14}C recovered in the sum of the six fractions collected.

† Mean of two experiments. Each sample was analyzed separately, with good agreement obtained between the individual samples. The results were, therefore, averaged for presentation here.

‡ Mean of three experiments, with good agreement between individual samples. The results were averaged for presentation here.

§ Including retinoic acid.

because of the retention of very polar labeled compounds by the column.

The composition of the labeled retinyl esters isolated from the lymph and liver extracts of rats fed ^{14}C-labeled retinal is shown in Table 4. Labeled saturated esters predominated in all samples. Portions of the retinyl ester fractions from the lymph of rat No. 5 and from the liver of rat No. 4 were saponified. After saponification the radioactivity was recovered in the nonsaponifiable fraction, and was subsequently recovered in the retinol fraction (fraction 4) after alumina column chromatography. These procedures established that the ^{14}C found in the retinyl ester fraction (fraction 2) after column chromatography actually resided in esters of labeled retinol.

Portions of the lipid extracts of the lymph and bile samples from the rats fed retinoic acid-^{14}C (rats No. 2 and 3) were separated into acidic and nonacidic lipids by solvent partition. 65% and 76%, respectively, of the lymph-^{14}C was found in acidic lipids. These values were almost identical with the percentage of lymph ^{14}C recovered in the retinoic acid fraction (fraction 6) after alumina column chromatography (Table 3). This suggests that approximately two-thirds of the small amount of radioactivity in the lymph of these rats resided in free retinoic acid. The recovery of lymph ^{14}C after solvent partition was 87% and 90% in these two rats. In contrast, only 57% and 60% of the ^{14}C in the lipid extracts of the bile of these rats was recovered after solvent partition. The acidic fraction contained 35% and 13%, respectively, of the recovered ^{14}C. These data indicate that only a very small portion (maximum 5–15%) of the ^{14}C in the bile of these rats could possibly have been present as free retinoic acid.

Of the small amount of ^{14}C found in the lymph of the rats fed retinoic acid-^{14}C, 22% was recovered in the retinyl ester fraction (fraction 2, Table 3). After saponification of this fraction, 75% of the ^{14}C was found as labeled saponifiable lipid. This suggests the presence of an ester of labeled retinoic acid in the lymph of these rats. This ester would have to be a monoester, such as a cholesteryl ester, since triglycerides (tri-esters) are eluted mainly in column fraction 3, rather than in fraction 2.

In all the experiments so far described, the lymph became chylous after the test meal, and the vitamin A compounds were fed in small amounts. In two experiments, however, (rats No. 6 and 7, Table 5) the lymph remained clear before and after test meals containing retinal-^{14}C. In these rats, more of the ^{14}C recovered was found in the bile than in the lymph. In addition, in two experiments (rats No. 6 and 8, Table 5) retinal was fed in large amounts (0.9 mg). In these experiments, despite the large amount fed, both rats absorbed a substantial percentage of the retinal-^{14}C. As already discussed, the lymph of one of these rats (No. 6) did not become chylous, and most of the absorbed ^{14}C was found in the bile. In contrast, the lymph of rat No. 8 did become chylous after the test meal, and much more ^{14}C was recovered in the lymph than in the bile. In addition, 30% of the ^{14}C re-

TABLE 4 LABELED RETINYL ESTER COMPOSITIONS IN LYMPH AND LIVER OF RATS AFTER THE FEEDING OF ^{14}C-LABELED RETINAL

Rat No.	Sample	% Distribution of Labeled Retinyl Ester		
		Saturated	Mono-unsaturated	Di-unsaturated
4A	Lymph	58	31	11
4B	Lymph	59	33	8
4B	Liver	82	11	8
5	Lymph	67	23	11
5	Liver	81	12	7

TABLE 5 EFFECT OF ABNORMAL PHYSIOLOGICAL CONDITIONS ON THE ROUTE OF ABSORPTION OF RETINAL-^{14}C

Rat No.	Amount Fed		Total ^{14}C Recovered	Distribution of Recovered ^{14}C		
				Lymph	Bile	Liver
	μg	μc	% of ^{14}C fed		%	
6*	900	0.07	41	3	78	19
7†	6	0.14	8	21	73	6
8‡	900	0.12	15	49	21	30

* Rat No. 6 was fed a large amount of retinal; after the labeled compound had been fed in a mixture containing fat (see text), the lymph remained clear.

† Rat No. 7. As with rat No. 6, the lymph remained clear and gave no evidence of chylomicron formation. This animal did not receive bile continuous'y from a donor rat.

‡ Rat No. 8 was fed, as indicated in the table, a large amount of retinal. The lymph did become chylous.

covered from this rat was found in the liver. Most (78%) of this liver ^{14}C resided in retinyl esters, which suggests that the hepatic radioactivity was derived from labeled retinyl esters absorbed via aberrant lymphatic pathways (the normal pathways lead to the cannula).

In addition to the above experiments with triply cannulated rats, a study was conducted to examine the absorption of retinal-^{14}C in a rat containing only one cannula, in its thoracic duct. 22 hr after cannulation, this rat was fed via gastric intubation 0.3 ml of triolein containing 350 μg of retinal-^{14}C. Lymph was collected for 24 hr and the rat was then fed 0.3 ml of trilinolein containing 350 μg of retinal-^{14}C. The rat was killed 24 hr later and the liver was extracted. The recovery of fed ^{14}C in the lymph was 19% after the first feeding and 23% after the second. The liver contained 3–4% of the total amount of fed ^{14}C. Centrifugal flotation of the chylomicrons in the first lymph sample demonstrated that 77% of the lymph ^{14}C was present in lymph chylomicrons. Analysis by alumina column chromatography demonstrated that most of the label in the lymph and liver samples resided in labeled retinyl esters. The composition of the labeled retinyl esters in the lymph and liver samples was determined by TLC on $AgNO_3$-impregnated alumina gel, and is shown in Table 6. Saturated esters predominated in all three samples. A small effect of the fatty acid composition of the diet was seen, in that relatively more mono- than diunsaturated esters were found after the feeding of triolein, whereas the reverse was found after trilinolein had been fed.

DISCUSSION

The studies reported here were designed to elucidate the anatomical and chemical pathways of absorption of several vitamin A compounds. In order to obtain physiologically valid information, we attempted to maintain nearly physiological conditions. To this end, an uninterrupted flow of bile into the duodenum was maintained, and the labeled substrates were fed in very small amounts in the form of emulsions resembling normal intraluminal contents during fat absorption. The feeding mixtures contained monoolein and oleic acid in a ratio of 1:2 (v/v), rather than normal fat (triglyceride), because of the possibility that bile duct cannulation might have interfered with the flow of pancreatic juice into the intestine. It is well known that intraluminal lipolysis is dependent on the action of pancreatic lipase, and that the major products of lipolysis are monoglyceride and free fatty acid (17). The physiological status of each rat was assessed by examining its ability to absorb fat and to form lymph chylomicrons. Animals were used only if it was certain that they could still absorb fat in this form. In some preparations, lymph and bile flows were obviously impaired, or the lymph did not become chylous after fat administration; these rats were not used, except as indicated in the results to demonstrate a particular effect.

When nearly physiological conditions were achieved, as described above, most of the radioactivity recovered after the feeding of labeled retinol or retinal was found in the lymph. When labeled retinoic acid was fed, however, nearly all the radioactivity recovered was found in the bile. In the study with retinol-^{14}C, 80% of the absorbed radioactivity was recovered in the lymph. This finding was expected, since numerous published studies, carried

TABLE 6 EFFECT OF DIETARY FAT COMPOSITION ON THE COMPOSITION OF RETINYL ESTERS AFTER THE FEEDING OF ^{14}C-LABELED RETINAL TO RATS

Sample	% Distribution of Labeled Retinyl Est er		
	Saturated	Mono-unsaturated	Di-unsaturated
Lymph*	56	37	7
Lymph†	59	19	22
Liver	68	23	8

Feeding mixture contained:
* 350 μg of retinal-^{14}C in 0.3 ml of triolein.
† 350 μg of retinal-^{14}C in 0.3 ml of trilinolein.

out with a number of species, have demonstrated that retinol absorption mainly occurs via the lymphatic route (18–21). The fact that 20% of the radioactivity recovered was found in the bile suggests that under normal conditions some dietary retinol may be oxidized to retinoic acid in the intestinal mucosa and absorbed via the portal vein. Portal vein transport of dietary vitamin A may be even more significant under abnormal conditions, since it has been reported that ligation of the thoracic duct does not prevent the absorption of vitamin A in rats (22). In the present studies, after labeled retinal had been fed the results observed were almost identical with those obtained after the feeding of labeled retinol. Thus, in the three physiological experiments reported in Table 1, 70–80% of the radioactivity recovered after feeding retinal-^{14}C was found in the lymph, and 15–20% in the bile.

In striking contrast to the results observed with retinol and retinal, feeding labeled retinoic acid gave only 5–7% of the absorbed radioactivity in the lymph, whereas 92–95% was found in the bile. These findings establish that absorption of retinoic acid occurs via the portal venous route, and are consistent with the finding of Zachman, Dunagin, and Olson (10, 11) that retinoic acid, injected into the portal vein, was rapidly metabolized and excreted in the bile. The labeled compounds found in the bile consisted of a mixture of polar metabolites, most of which were more polar than free retinoic acid. Only very small portions (5–15%) of the ^{14}C in the bile of these rats could possibly have been present as free retinoic acid. These findings are consistent with the observations of Zachman et al. (11), that retinoic acid injected into the portal vein of rats was largely excreted in the bile in the form of more polar metabolites of retinoic acid. The major metabolite in rat bile has been tentatively identified as retinoyl β-glucuronide (12).

Since samples of portal blood were not analyzed in the present studies, no direct information is available about the chemical form in which retinoic acid was absorbed via the portal vein. It is possible, for example, that retinoic acid was absorbed as the free acid, perhaps in the form of its carboxylate anion bound to serum albumin. The similarities between the properties of the biliary metabolites observed here and those observed after intraportal injection of free retinoic acid (11) are consistent with this suggestion. It is also possible that retinoic acid was converted to its glucuronide conjugate in the intestinal mucosa, and then transported in portal blood in this form.

It is interesting to speculate why retinoic acid, a 20-carbon carboxylic acid, is not absorbed via the lymphatics as are the usual long-chain fatty acids. Perhaps, because of substrate specificity on the part of the enzymes involved in fatty acid activation and in glyceride ester bond formation, retinoic acid is unable to be converted into glyceride esters. In this regard it should be noted that two-thirds of the small amount of radioactivity found in lymph after administration of retinoic acid-^{14}C was found as free retinoic acid. Most of the remaining radioactivity in lymph was tentatively identified as a monoester of labeled retinoic acid. We can, therefore, hypothesize that retinoic acid might be unable to be incorporated into glyceride esters, but instead can be converted into water-soluble derivatives or can be bound to (and hence "solubilized" by) serum albumin. Since portal blood flow greatly exceeds that of intestinal lymph, the absorption of retinoic acid via the portal route can thus be explained. Only 0.5% of the radioactivity recovered after labeled retinoic acid had been fed was found in the livers of the rats. Previous workers (23–25) have found no accumulation of retinoic acid in the tissues, including the intestinal mucosa itself, after the feeding of this compound to animals, despite the fact that many symptoms of vitamin A deficiency can be relieved by administration of retinoic acid (26).

Almost all of the radioactivity recovered in the lymph after the feeding of labeled retinal (and retinol) was found in lipid-soluble compounds, mainly in retinyl esters. These studies hence demonstrate that, under physiological conditions (i.e., when the animal was able to absorb fat), absorption of retinal occurred mainly via the lymph, in the form of retinyl esters. Under these conditions, therefore, the major pathway of retinal absorption involved its reduction to retinol, which was then esterified and transported in a manner similar to that of dietary preformed retinol. Since this seems to be the main "physiological" pathway, it is likely that retinal newly-formed from β-carotene in the intestinal mucosa would also be metabolized predominantly via this pathway. Saturated retinyl esters predominated in the lymph after administration of retinal, regardless of the fatty acid composition of the diet. This finding is similar to that observed after the feeding of labeled retinol or β-carotene, both in the rat (5) and in man (6). The capacity of the rat intestine to reduce retinal and then to transport the retinol via the lymph greatly exceeded the small physiological daily requirement for vitamin A. Thus, when large amounts (350 or 900 μg) of retinal were fed, a considerable amount of the total radioactivity was absorbed via the lymphatic route.

Under physiological conditions as defined above, in addition to the ^{14}C found in the lymph, 15–20% of the radioactivity recovered after retinal-^{14}C feeding was found in the bile. Furthermore, when fat absorption was impaired, as indicated by the failure of the lymph to become chylous, most of the absorbed ^{14}C (from retinal-^{14}C) was found in the bile, rather than in the lymph. These results may explain the findings of Crain, Lotspeich, and

Krause (9), who injected large doses (1 mg) of labeled retinal, emulsified with Tween-80, into ligated intestinal loops of rats, and found 30–40% of the absorbed radioactivity in portal blood collected via an exchange transfusion technique. In our experiments, the labeled compounds in bile consisted of a mixture of polar metabolites, grossly similar to those observed after the feeding of retinoic acid-^{14}C. These data suggest that, under normal conditions, a small portion of dietary (or newly biosynthesized) retinal is oxidized to retinoic acid, which is then transported and metabolized in a manner similar to that of dietary preformed retinoic acid. The results also suggest that the relative importance, in quantitative terms, of the two pathways of retinal metabolism can vary, depending on the status of the animal.

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