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The Intestinal Absorption and Metabolism of Vitamin A and β-Carotene in Man *

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The fact that vitamin A is absorbed via the lymphatic route was first demonstrated by Drummond, Bell, and Palmer in 1935, in studies carried out in a patient with chylothorax (2). Since then this finding has been confirmed and amplified by studies with other animal species. It has been well established that retinol [1] is largely esterified during its intestinal absorption, and partial information about the processes of absorption and esterification has been available from the studies of Ganguly and his associates (3–5) and Pollard and Bieri (6). In contrast to retinol, however, much less detailed information has been available concerning the intestinal absorption and metabolism of the provitamin A, β-carotene.

Detailed studies of the events occurring during the intestinal absorption of β-carotene and retinol in the rat were recently reported by Huang and Goodman (7). Radioactive β-carotene or retinol was dissolved in oil and fed to rats containing cannulae implanted in the thoracic duct. Absorption of radioactivity occurred entirely via the lymphatic route, mainly in the form of lymph chylomicrons. Retinyl esters were the predominant labeled compounds in all samples of lymph and contained an average of 89% of the radioactivity absorbed after the administration of either labeled retinol or labeled purified β-carotene. There was virtually no absorption of unchanged labeled β-carotene into the lymph. The composition of the lymph retinyl esters was remarkably constant, regardless of the fatty acid composition of the diet and regardless of whether the retinyl esters were derived from dietary retinol or from β-carotene. Retinyl palmitate was the predominant ester in lymph, and saturated esters (retinyl palmitate plus stearate, in a ratio of approximately 2 to 1) consistently comprised two-thirds to three-fourths of the retinyl esters.

The studies reported here were designed to define the events occurring during the intestinal absorption of β-carotene and vitamin A in man. These studies represent both an extension, to man, of the studies carried out in the rat, and a continuation of earlier investigations on the transport of lipids via the thoracic duct in man (8).

Methods

Labeled compounds.[2] Retinol-15-^{14}C (specific radioactivity, 29.6 μc per mg) and retinyl-15-^{3}H acetate (specific radioactivity, 100 μc per mg) were stored under argon in sealed vials until used and were used without further purification. Previous assay of a different vial

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[1] The terms retinol, retinal, retinoic acid, and retinyl ester refer, respectively, to vitamin A alcohol, aldehyde, acid, and ester.

[2] The labeled compounds used in these studies were the generous gift of Dr. U. Gloor of Hoffmann-La Roche, Basel, Switzerland.

from the same preparation of retinol-15-^{14}C had indicated that at least 98% of the radioactivity chromatographed with unlabeled retinol on alumina column chromatography (7). β-Carotene-15,15'-^{3}H (specific activity, 1.09 mc per mg) was chromatographed on a column of deactivated alumina (7) just before use. Further information about this preparation of labeled β-carotene has been provided elsewhere (9).

Subjects and collection of samples. Four studies were carried out in three subjects. In all subjects, polyethylene cannulae were implanted in the thoracic duct in the neck by the procedure described by Werner (10). Lymph was drained directly into containers kept in a small closed refrigerator, thus ensuring that the samples remained cold and in the dark during the time of collection.

Subject AW (study I) was a 61-year-old man who presented with a 2-month history of bilateral supraclavicular swellings. Past history included the presence of an esophageal diverticulum and of gallstones. Biopsy revealed the swellings in the neck to consist of lipomas. Lymph duct cannulation was performed at the time of surgical exploration of the swelling in the left supraclavicular region. At the time of cannulation the serum cholesterol concentration was 266 mg per 100 ml, and the lymph cholesterol in the fasting subject was 182 mg per 100 ml. On the day after cannulation the patient was fed a formula meal containing 52 μc β-carotene-^{3}H (47 μg) dissolved in 2 ml olive oil and emulsified with 50 ml skim milk. Lymph samples were collected at 1- to 2-hour intervals for 22 hours. The lymph flow was in the range of 15 to 25 ml per hour for most of the collection period. The patient was not fed during the collection period, but was allowed to drink water ad libitum.

Subject IC (studies II and III) was a 58-year-old housewife who presented with a lump in the left breast of 1 year's duration. Past history revealed a 1-year history of hypertension and mild congestive heart failure, treated with digitalis and chlorothiazide. Needle aspiration biopsy of the left breast showed the presence of malignant cells. Chest and skeletal X rays revealed no visible metastases. There was no history of gastrointestinal symptoms or abnormalities. Cannulation of the thoracic duct in the neck was performed at the time of scalene node biopsy, which was done to exclude the possibility of cervical node metastases. The serum cholesterol concentration was 310 mg per 100 ml, and the fasting lymph cholesterol was 158. One hour after cannulation the patient was given a formula meal containing 50 μc (46 μg) β-carotene-^{3}H, 91 μg unlabeled β-carotene, and 2 mg α-tocopherol, all dissolved in 2 ml of a polyunsaturated commercial vegetable oil [3] and emulsified with 50 ml skim milk. Lymph was collected at 1- to 2-hour intervals for the next 44 hours. A second formula feeding, consisting of 20 μc (0.68 mg) retinol-15-^{14}C plus 2 mg α-tocopherol dissolved in 2 ml of Kronolja and emulsified with 50 ml skim milk, was administered 23 hours after the first formula. The lymph flow was in the range of 15 to 30 ml per hour during most of the first collection period (study II) and in the range of 25 to 35 ml per hour during the second collection period (study III). The patient remained fasting for at least 10 hours after each of the two formula feedings, but was then allowed to drink milk (study II) or to eat a small meal (study III).

Subject HD (study IV) was a 52-year-old male engineer admitted for resection of a gastric carcinoma. A weight loss of 7 kg had occurred during the preceding year. The serum cholesterol concentration was 268 mg per 100 ml. At the time of total gastrectomy the liver appeared free of metastases, but regional lymph nodes contained metastatic tumor. The pathological diagnosis of the resected specimen was anaplastic carcinoma. Two hours before the start of gastrectomy, lymph duct cannulation was performed in the neck, in connection with a study of the appearance of malignant cells in lymph during and after gastric surgery. Four hours before lymph duct cannulation the patient was fed 50 ml of olive oil containing 194 μc (1.94 mg) of retinyl-^{3}H acetate. Lymph samples were collected at short time intervals (15 to 30 minutes) during the first 2 hours after cannulation, followed by infrequent collections thereafter.

Extraction and chromatography. In each study, the time course of the absorption of radioactivity was determined by extracting 0.5 ml of each lymph sample with 10 ml $CHCl_3$: MeOH (2:1, vol:vol), followed by radioassay of the total lipid extract so obtained. The lymph samples containing the main peak of absorbed radioactivity were then pooled for subsequent analyses. Portions of the pooled lymph were layered under an equal volume of isotonic saline and centrifuged for 45 to 60 minutes at 15,000 × *g* to 20,000 × *g*. The top, creamy zone of packed chylomicrons and the clear, chylomicron-free bottom fraction were separately collected and extracted with 20 vol of $CHCl_3$: MeOH (2:1, vol:vol), followed by the addition of 5 vol of 0.01 N H_2SO_4. A mixture of unlabeled carriers, consisting of 250 μg of retinol plus 100 μg each of β-carotene, retinal, retinoic acid, and of each of four retinyl esters (palmitate, stearate, oleate, and linoleate) was added to each sample at the time of extraction. The lower, chloroform phase was collected and evaporated to dryness. The total lipid extract was weighed, dissolved in benzene, and stored under argon at −15° C.

In study IV the lymph samples obtained during the first 3 hours of collection were all found to contain approximately 25,000 dpm of ^{3}H per ml. The lymph samples were pooled, and the total lipid of whole lymph was extracted as described.

Portions of each lipid extract were chromatographed on columns of deactivated alumina [4] as described previously (7). The order of elution and volume of eluent per 5 g of alumina were as follows: fraction 1, β-carotene (20 ml of *n*-hexane); fraction 2, retinyl esters (20 ml of benzene-hexane, 3:17); fraction 3, retinal (20 ml of

[3] The Swedish trade name of the vegetable oil is Kronolja. It consists mainly of soybean oil and contains a high proportion of linoleic acid.

[4] Woelm, grade III.

benzene-hexane, 1:1); fraction 4, retinol (50 ml of benzene); fraction 5, more polar but nonacidic compounds (20 ml methanol); and fraction 6, acids including retinoic acid (20 ml methanol:25% acetic acid, 3:1). Portions of each column fraction, and of each total lipid extract, were assayed for radioactivity.

Composition of retinyl esters. The composition of the labeled retinyl esters in each column fraction 2 was determined by a combination of two different chromatographic separations, as described in detail previously (7). A carrier mixture of unlabeled retinyl esters, comprising 50 μg each of retinyl palmitate, stearate, oleate, and linoleate, was added to each sample just before chromatography. Separation of the retinyl esters on the basis of the degree of unsaturation of the fatty acid component was first accomplished by thin-layer chromatography on alumina gel G impregnated with silver nitrate. The saturated, and other, esters were then separated according to fatty acid chain length by reversed phase chromatography on silicone-impregnated paper. The retinyl ester composition of each sample was calculated after expressing the results of each chromatographic separation as the percentage distribution of recovered radioactivity in the different esters.

Identification procedures. Radioactivity in column fraction 2 from the chylomicron samples of studies I and II (after feeding β-carotene-^{3}H) was established to reside in retinyl esters by the procedures described previously (7). These procedures included saponification to form labeled retinol, followed by acetylation to form labeled retinyl acetate (which was identified by column and thin layer chromatography). The material present in each column fraction 2 was also subjected to thin layer chromatography on alumina gel G, together with appropriate carriers, so as to separate long chain fatty acid esters of retinol from short chain esters (such as retinyl acetate) and from cholesterol esters (7). Elution and radioassay of the separated compounds indicated that in every sample, in all four studies, virtually all of the radioactivity in column fraction 2 resided in long chain retinyl esters.

Other procedures. The fatty acid composition of the total lipid extract of each sample was determined by gas-liquid chromatography of the fatty acid methyl esters, as described previously (7).

Pure lecithin was isolated from two of the samples and was subjected to hydrolysis with phospholipase A, as described elsewhere (11). The compositions of the fatty acids attached to the β and to the α′ positions of lecithin were then separately determined by gas-liquid chromatography.

Radioassay (for ^{3}H or ^{14}C or both) was carried out by dissolving samples in 15 ml of 0.5% diphenyloxazole in toluene, followed by assay with a Packard Tri-Carb liquid scintillation counter. Significant quenching was not observed.

The sources of all materials and compounds used in this study were described previously (7).

Results

The time course of the absorption of radioactivity into the lymph, in the two subjects fed β-carotene-^{3}H, is shown in Figure 1. In both subjects

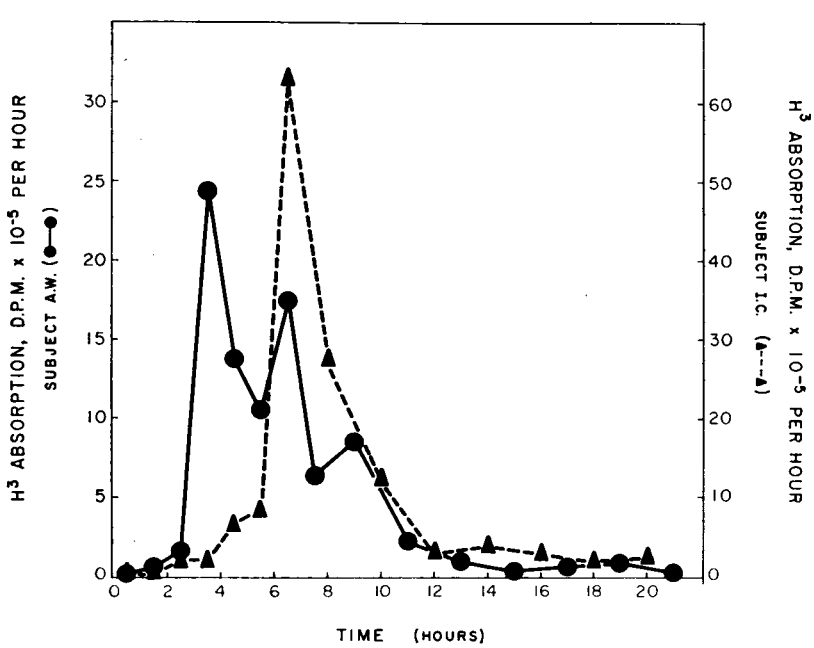

FIG. 1. THE TIME COURSE OF THE ABSORPTION OF RADIOACTIVITY INTO THORACIC DUCT LYMPH IN TWO HUMAN SUBJECTS FED β-CAROTENE-15,15′-^{3}H.

TABLE I

Distribution of radioactivity in human lymph after β-carotene-15,15'-^3H

Alumina column fraction	Percentage distribution of absorbed ^3H			
	Study I		Study II	
	Chylomicrons	Bottom fraction*	Chylomicrons	Bottom fraction*
1. β-Carotene	23	22	30	27
2. Retinyl esters	67	71	61	65
3. Retinal	3	2	3	2
4. Retinol	3	3	3	3
5+6. Polar; acids	4	2	3	3

* Lymph from which chylomicrons had been removed by centrifugation.

radioactivity was absorbed in a fairly sharp peak, which coincided with the peak of fat absorption into the lymph (as determined qualitatively by the degree of visible lactescence of the lymph samples). In study I (subject AW) the main peak of absorption occurred between 3 and 4 hours, and 88% of the absorbed ^3H appeared in the lymph between 3 and 10 hours. The apparent second peak of absorption seen in this study immediately after the main peak probably reflects irregular gastric emptying of the formula meal containing the labeled β-carotene. In study II (subject IC) the main peak of absorption occurred between 6 and 7 hours, and 81% of the recovered ^3H was absorbed between 5 and 11 hours. In both studies the absorption of radioactivity was virtually complete by 12 hours. The total amount of radioactivity recovered in thoracic duct lymph during the entire period shown in Figure 1 comprised 8.74% of the fed radioactivity in study I and 16.76% of the fed radioactivity in study II.

A similar time course of absorption of radioactivity was seen after feeding retinol-^{14}C. Thus, in study III the main peak of absorption of ^{14}C occurred between 3 and 5 hours, and 78% of the radioactivity absorbed into the lymph was absorbed between 2 and 8 hours. A total of 21.5% of the fed radioactivity was recovered in the lymph during a period of 20 hours.

Chylomicrons, isolated as described from the pooled samples representing the main peak of absorption of radioactivity, contained 70% of the absorbed radioactivity in study I and 80% of the absorbed radioactivity in study III. The relative amount of lymph radioactivity contained within lymph chylomicrons was not precisely determined in studies II or IV.

Table I summarizes the distribution of radioactivity in the chylomicron and the nonchylomicron ("bottom") portions of lymph in the two subjects given β-carotene-^3H (studies I and II). In all samples, most of the radioactivity was found in the retinyl ester fraction (fraction 2), which contained 61 to 71% of the radioactivity absorbed into the lymph. Analysis of a portion of the retinyl ester fraction from each chylomicron sample demonstrated that the radioactivity in this column fraction definitely resided in long chain fatty acid esters of retinol (see above, under *Identification procedures*). Significant amounts of radioactivity were also recovered in the β-carotene fractions (fraction 1's), which contained 22 to 30% of the absorbed radioactivity. To determine whether this radioactivity resided in unchanged β-carotene, we added unlabeled pure β-carotene (250 μg) to a portion of each fraction 1 and serially crystallized the β-carotene two or three times, to constant specific radioactivity. Most (81 to 85%) of the ^3H in each fraction 1 was established to reside in β-carotene by this procedure.

Comparable data for the distribution of radioactivity in lymph, in the two subjects fed labeled preformed vitamin A, are presented in Table II. In both subjects, most of the radioactivity absorbed into the lymph was found in the retinyl ester fraction, which contained 86 to 88% of the absorbed radioactivity in study III and 80% of the absorbed radioactivity in study IV. Further analysis showed that all of the radioactivity in these retinyl ester fractions resided in long chain fatty acid esters of retinol. This indicated that, in study

TABLE II

Distribution of radioactivity in human lymph after radioactive vitamin A

Alumina column fraction	Percentage distribution of absorbed label		
	Study III (retinol-^{14}C)		Study IV (retinyl-^3H acetate)
	Chylomicrons	Bottom fraction	Whole lymph
1. β-Carotene	1	2	5
2. Retinyl esters	88	86	80
3. Retinal	6	5	6
4. Retinol	3	3	6
5+6. Polar; acids	2	4	3

TABLE III

Fatty acid compositions of the total lipid of human lymph

Study; oil in test meal	Sample analyzed	Percentage distribution of fatty acid mass					
		Palmitate (16:0)*	Palmitoleate (16:1)	Stearate (18:0)	Oleate (18:1)	Linoleate (18:2)	Arachidonate (20:4)
I. Olive oil	Chylomicrons	27	3	10	43	11	Trace
	Bottom	27	3	11	35	15	Trace
II. Kronolja	Chylomicrons	14	1	6	24	38	8
	Bottom	19	2	9	26	33	6
III. Kronolja	Chylomicrons	13	1	4	24	40	8
	Bottom	18	2	8	25	33	6
IV. Olive oil	Whole lymph	19	2	3	53	22	Trace

* Number of carbon atoms: number of double bonds. Only the major component fatty acids have been listed in this Table. A number of minor components, comprising together less than 10% of the total fatty acid mass, were observed in each sample.

IV, the dietary retinyl acetate had been hydrolyzed completely before or during absorption, followed by a re-esterification of the released retinol with long chain fatty acids. Small amounts of radioactivity were also recovered in all of the other column fractions. Most of this latter radioactivity probably represented artifactual chromatographic "smearing" secondary to oxidation or other alterations occurring during the processing of the samples. Similar slight smearing of radioactivity is commonly observed during the handling of pure labeled retinyl esters or retinol. Attempts to identify the radioactive material recovered in column fraction 4 [see (7)] demonstrated that only about one-third to one-half the radioactivity in the several fraction 4's definitely resided in retinol.

The fatty acid composition of the total lipid extract of each lymph sample studied is summarized in Table III. Since triglycerides constitute the major lipid in both the chylomicron and nonchylomicron portions of lymph, the compositions listed in Table III largely represent the triglyceride compositions of the several samples. As expected, the fatty acid composition reflected the composition of the dietary fat in all samples. Thus, linoleic acid predominated in lymph after test meals containing the polyunsaturated vegetable oil (Kronolja) (studies II and III), and oleic acid predominated after test meals containing olive oil. Furthermore, the degree of predominance of oleic acid was greater in study IV, in which 50 ml of olive oil had been fed, than in study I, in which only 2 ml of olive oil had been fed.

The composition of the labeled retinyl esters present in the retinyl ester fraction of each sample studied, for all four studies, is presented in Table IV. All samples contained a mixture of four retinyl esters: retinyl palmitate, stearate, oleate,

TABLE IV

Labeled retinyl ester compositions in human lymph

Study; labeled compound fed	Oil in test meal	Sample analyzed	Percentage distribution of labeled retinyl esters			
			Palmitate	Stearate	Oleate	Linoleate
I. β-Carotene-^{3}H	Olive oil	Chylomicrons	57	25	12	6
		Bottom	54	25	14	7
II. β-Carotene-^{3}H	Kronolja	Chylomicrons	53	27	13	7
		Bottom	57	26	12	5
III. Retinol-^{14}C	Kronolja	Chylomicrons	60	22	10	8
		Bottom	60	24	10	6
IV. Retinyl-^{3}H acetate	Olive oil	Whole lymph	56	19	16	9

TABLE V

Fatty acid compositions of chylomicron lecithin

Study; oil in test meal	Fatty acid position on lecithin	Percentage distribution of fatty acid mass					
		Palmitate	Palmitoleate	Stearate	Oleate	Linoleate	Arachidonate
I. Olive oil	α'	45.5	0.8	34.8	11.8	5.0	Trace
III. Kronolja	α'	42.8	1.2	31.2	9.9	9.4	3.8
I. Olive oil	β	3.7	1.4	1.2	18.3	58.5	11.2
III. Kronolja	β	3.2	1.6	2.0	14.4	64.5	13.6

and linoleate; there was no evidence for the presence of polyunsaturated retinyl esters containing more than two double bonds in the fatty acid moiety.[5] Retinyl palmitate was the predominant retinyl ester in all samples, and the two saturated esters, retinyl palmitate and stearate, together comprised 75 to 84% (average 79%) of the total retinyl esters in all samples. The ratio of retinyl palmitate to stearate was relatively constant ranging from 2.0 to 2.9 (average 2.4) in the seven samples listed in Table IV. The composition of the labeled retinyl esters bore no resemblance to the composition of the dietary fat or of the lymph total lipid (Table III). Furthermore, the retinyl ester composition was relatively constant regardless of whether the retinyl esters were derived from preformed vitamin A or from β-carotene and regardless of whether the chylomicron or the nonchylomicron portion of lymph was analyzed.

Analyses were also carried out to determine the fatty acid composition of the lecithin present in the chylomicron samples of studies I and III. It is known that lecithin comprises the major phospholipid in rat (12) and human (13) lymph and that the composition of lymph lecithin is relatively fixed, regardless of the composition of the diet (11–13). Furthermore, it has been pointed out (7) that, in the rat, the composition of the fatty acids attached to the α' position of lymph lecithin strongly resembles the fatty acid composition of newly absorbed lymph retinyl esters.

Table V summarizes the composition of the fatty acids attached to both the α' and the β positions of chylomicron lecithin for the two samples studied. Similar compositions were found in both lecithin samples. Saturated fatty acids predominated at the α' position, comprising 74 to 80% of the α' fatty acids in the two samples. Linoleic acid predominated at the β position. The ratio of palmitate to stearate, at the α' position, was 1.31 and 1.37 in the two samples. These results are in accord with previously reported observations on the fatty acid distribution in human lymph lecithin (13).

Discussion

The studies reported here demonstrate that during the intestinal absorption of dietary preformed vitamin A, in man, the vitamin A is almost completely esterified with long chain fatty acids, and the retinyl esters are then transported via the lymphatic route mainly in association with lymph chylomicrons. During the absorption of dietary β-carotene, the carotene is largely converted to vitamin A, and the newly synthesized vitamin A is then esterified and transported in the lymph in the same fashion as dietary retinol. The composition of the lymph retinyl esters was remarkably constant in all the present studies, regardless of the fatty acid composition of the diet, regardless of whether the retinyl esters were derived from preformed vitamin A or from β-carotene, and regardless of whether the chylomicron or the nonchylomicron portion of lymph was analyzed. Retinyl palmitate predominated in all samples, and saturated esters (retinyl palmitate plus stearate, in an average ratio of 2.4 to 1) consistently comprised approximately 80% of the labeled esters.

These results indicate that the events occurring during the intestinal absorption of β-carotene and vitamin A in man are very similar to those occur-

[5] Although small amounts of radioactivity (5 to 11% of the total retinyl ester radioactivity) were consistently found at or just above the origin (below the linoleate zone) after thin layer chromatography, this radioactivity apparently resided in oxidation products formed during chromatography. Thus, after elution from the thin layer plate and rechromatography on an alumina column, less than 10% of this radioactivity was recovered in the retinyl ester fraction (column fraction 2).

ring in the rat. Thus, labeled retinyl esters predominated in rat lymph after the feeding of either labeled retinol or labeled β-carotene (7). Furthermore, the composition of the labeled retinyl esters found in rat lymph after a wide range of test meals was remarkably constant and was similar to the retinyl ester composition found here in human lymph. One difference was, however, observed between man and the rat: Man is capable of absorbing small but significant amounts of unchanged dietary β-carotene into the lymph, whereas virtually no dietary β-carotene can be absorbed intact into rat lymph. The quantitative difference in the ability of these two species to absorb intact β-carotene is, however, not very great, since most of the radioactivity absorbed into human lymph after feeding β-carotene-^{3}H was found in retinyl esters, and not in unchanged β-carotene. The human intestine therefore possesses only an extremely limited capacity for the absorption of unchanged dietary β-carotene.

Since the labeled β-carotene employed in these studies was labeled with tritium at the central two carbon atoms (C-15 and 15′), the question should be raised as to whether some ^{3}H label might not be lost during the metabolism of this β-carotene, particularly during its conversion to vitamin A. Recent studies with rat intestinal mucosa have demonstrated that the reaction mechanism of vitamin A biosynthesis consists of the central cleavage of β-carotene into two molecules of retinal, and that the hydrogen atoms attached to the central two carbon atoms of β-carotene are completely retained during this reaction (9, 14). β-Carotene-15,15′-^{3}H hence should be a suitable compound to use in studies of the metabolism of β-carotene. Because of its location the ^{3}H label would, of course, be lost during the conversion of newly formed retinal into retinoic acid, so that the present studies would not have detected the formation of retinoic acid had it occurred during absorption and metabolism of the β-carotene-^{3}H. Significant amounts of retinoic acid were not detected in rat lymph, however, in previous studies with ^{14}C-labeled β-carotene (7), and retinoic acid was not detected in human lymph, in the present study, after feeding retinol-^{14}C. The conversion of β-carotene into two molecules of retinal appears to be a dioxygenase reaction involving a direct reaction between molecular oxygen and the central carbon atoms of β-carotene (9). The newly formed retinal is then reduced to retinol (14), which in turn is esterified and transported via the lymph in the manner described herein.

The composition of the retinyl esters found in human and in rat lymph may be compared with the composition of the retinyl esters recently reported for a number of rat tissues (15). The composition of the labeled retinyl esters in each of six rat tissues was determined at several time intervals after the intravenous injection of chylomicrons containing newly absorbed labeled vitamin A (15). The retinyl ester compositions were remarkable in showing a consistent predominance of saturated esters in all tissues. Large differences were, however, seen in the relative amounts of labeled retinyl palmitate and stearate in different tissues. Thus, retinyl palmitate markedly predominated in liver, whereas similar amounts of retinyl palmitate and stearate were found in kidneys, and retinyl stearate predominated in adrenal glands. A marked predominance of retinyl palmitate in rat liver has also been reported by Futterman and Andrews (16). In addition, these workers determined the composition of the retinyl esters formed during incubation *in vitro* of homogenized retinas in the light (17); in four mammalian species the composition of the newly formed retinyl esters was similar to the retinyl ester composition observed here in lymph.

In a previous publication (7) it was pointed out that the fatty acid composition of retinyl esters in rat lymph is similar to the composition of the fatty acids present at the α′ position of lymph lecithin. It was also pointed out (15) that, since lecithin from practically all sources shows a striking predominance of saturated fatty acids at the α′ position, the predominance of saturated retinyl esters in all tissues extends the comparison between the fatty acid composition of retinyl esters and that of the α′ position of lecithin. In the present study, direct analysis of the fatty acid composition of lecithin from two of the chylomicron samples showed that, as with the retinyl esters, the composition of lecithin was relatively fixed and was hardly influenced by the composition of the test meal. The composition of the fatty acids attached to the α′ position of lecithin resembled the composition of the retinyl esters in being largely saturated; the ratio of palmitate to stearate at the

α′ position of lecithin was, however, distinctly lower than that seen in the retinyl esters. Despite considerable similarities, therefore, the fatty acid compositions of retinyl esters and of the α′ position of lecithin are certainly not identical.

The reason for the remarkably constant composition of the retinyl esters found in human and rat lymph is not known. It has been suggested (7) that retinol esterification during absorption may involve the direct reaction of retinol with a fatty acyl donor of composition similar to that seen in lymph retinyl esters. It has also been pointed out (7) that the similarity between the fatty acid composition of the α′ position of lymph lecithin and that of retinyl esters suggests that retinyl ester fatty acids may derive either from the α′ position of lecithin or from the precursor pool of the fatty acids of this position of lecithin. Some selectivity would, of course, have to exist to account for the different ratio of palmitate to stearate in retinyl esters and in lecithin. Further definition of the mechanism of retinol esterification during absorption will be required to decide between these and other, alternative possibilities.

Summary

β-Carotene-15,15′-^{3}H, vitamin A alcohol-15-^{14}C, and vitamin A-15-^{3}H acetate were fed to patients in whom polyethylene cannulae had been inserted in the thoracic duct in the neck. Serial samples of lymph were collected, and the lipid was extracted and chromatographed on columns and on thin layer plates of alumina. Absorption of radioactivity into the lymph mainly occurred between 3 and 10 hours. During this time washed chylomicrons contained 70 to 80% of the absorbed radioactivity. Labeled vitamin A esters predominated in all lymph samples, representing 80 to 90% of the absorbed radioactivity after the feeding of labeled preformed vitamin A, and 60 to 70% after β-carotene. The fatty acid composition of the vitamin A esters in lymph bore no resemblance to the composition of the diet and was remarkably constant, regardless of the fatty acid composition of the diet, regardless of whether the vitamin A esters were derived from dietary vitamin A or from β-carotene, and regardless of whether chylomicron or nonchylomicron lipid was analyzed. Vitamin A palmitate predominated in all samples, and saturated esters (vitamin A palmitate plus stearate, in an average ratio of 2.4 to 1) consistently comprised 75 to 85% of the labeled esters. Small amounts of vitamin A oleate and linoleate were also found in all samples. Unchanged labeled β-carotene comprised only 20 to 30% of the absorbed radioactivity, after ingestion of β-carotene-^{3}H. The human intestine possesses only an extremely limited ability to absorb unchanged dietary β-carotene into the lymph. The fatty acid composition of the lymph vitamin A esters was similar to but not identical with that of the α′ position of lymph lecithin.

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