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Studies on the absorption of beta-carotene and the distribution of total carotenoid in human serum lipoproteins after oral administration*

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SUMMARY

The carotenoid increment in human serum was measured after the oral administration of aqueous dispersions and lipid dispersions of β-carotene with different test meal regimens. β-carotene was readily absorbed in both forms. Absorption of the aqueous dispersion was similar when administered with either high lipid or protein-carbohydrate test meals. Aqueous dispersions were poorly absorbed by fasting subjects. The lipoprotein distribution of the serum carotenoid increment was determined in normal, hypercholesterolemic, and hyperlipemic subjects to investigate the absorption and transport pattern for β-carotene. Peak carotenoid increments occurred first in the chylomicron and S_f 10–400 lipoprotein fractions, and considerably later in the S_f 0–10 fraction. A major part of the serum carotenoid increment was found in the S_f 0–10 lipoprotein fraction. This is reflected in the delayed peak found in serum carotenoid tolerance curves. Very little newly absorbed carotenoid was found in the high density lipoprotein fraction. Carotenoid-to-cholesterol and carotenoid-to-fatty acid ester ratios were determined after carotenoid administration and after a lipid test meal containing no carotenoid. These ratios indicated that the peak carotenoid increments represented newly absorbed carotenoid associated with specific lipoprotein fractions rather than rapid carotenoid exchange between lipoprotein fractions that varied in their total concentration. The absorption and transport of carotenoid was compared with the absorption and transport of other lipids. Portal absorption of at least a part of the ingested carotenoid is proposed as a possible explanation for the observed carotenoid transport pattern.

Palmer and Eckles (1) suggested more than 45 years ago that carotenoids exist in serum as protein complexes. Since then considerable information has accumulated on the distribution of lipid-soluble vitamins and provitamins in the different lipoproteins of human serum. Krinsky *et al.* (2) found that the carotenoid hydrocarbons, β-carotene and lycopene, were concentrated primarily in the low density S_f 0–10 lipoprotein fraction of human serum, whereas the carotenoid alcohol, lutein, was more evenly distributed between low and high density lipoproteins. These investigators also observed that vitamin-A alcohol in fasting serum was not associated with any of the lipoproteins, whereas vitamin-A esters, which are found in serum after the oral administration of vitamin A, were associated primarily with S_f 10–400 lipoproteins. Garbers *et al.* (3) subsequently demonstrated that vitamin-A alcohol was associated with the α_1-globulins of rat serum, and Beaumont and Beaumont (4) found vitamin-A increments in both chylomicrons and low density lipoproteins after oral administration of the vitamin to humans. This rapid absorption of vitamin-A esters and their transport in chylomicron and S_f 10–400 lipoprotein fractions is very similar to the absorption and transport of triglycerides (5). The pattern is not the same for all lipid and lipid-soluble substances. For example, McCormick *et al.* (6) recently found that human low density lipoproteins contained a major part of the total serum tocopherol, while the high density lipoproteins contained the remainder. After a test meal containing tocopherol, the increment in serum tocopherol appeared first in the chylomicron and S_f 10-400 lipoprotein fractions and, subsequently, in the S_f 0–10 and high density lipoproteins.

Although the carotenoid distribution in serum lipoproteins isolated from fasting subjects has been investigated (2), the distribution of newly absorbed

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carotenoid has not been studied. Following oral administration, peak serum concentration increments for iodinated triglyceride and vitamin-A ester, tocopherol, and carotenoid occur after significantly different time intervals (2, 5, 6, 7, 8, 9). Since the carotenoid tolerance curve differs from that of both vitamin-A ester and tocopherol, the lipoprotein distribution of newly absorbed carotenoid might also differ. In the present investigation, the carotenoid distribution in serum lipoproteins was studied before and at various time intervals after the oral administration of β-carotene to human subjects.

METHODS

β-Carotene Administration. β-carotene[1] was administered as an aqueous dispersion or as crystalline β-carotene dispersed in margarine[2] to normal, hypercholesterolemic, and hyperlipemic subjects. Fasting subjects received 60 to 200 mg of β-carotene either alone (followed by an 8-hour fast), or together with high lipid (40 to 80 g fat) or protein-carbohydrate (8 g protein, 104 g carbohydrate, and no fat) test meals. β-carotene was also administered to one subject with ox bile[3] alone and to another with ox bile plus a protein-carbohydrate test meal. Lipomul® Oral[4] was administered without β-carotene in a control experiment.

Separation of Lipoproteins. Blood specimens (30 ml) were obtained before and at stated time intervals after the test meal. The blood was allowed to clot at 4° for 4 hours and the serum was withdrawn. Serum was fractionated by a density-gradient ultracentrifugal-flotation procedure (2, 5, 6, 10) and separated into chylomicrons, S_f 10–400, S_f 0-10, and high density lipoproteins (11). Lipoprotein fractions from duplicate aliquots of serum were then analyzed for their carotenoid, cholesterol, and fatty acid ester content.

Chemical Analyses. Two milliliters of serum or a lipoprotein fraction isolated from 4 ml of serum was denatured by the addition of an equal volume of 95% ethanol at room temperature. Carotenoids were then extracted by vigorous shaking with 4 ml hexane (Phillips 66). The emulsion was broken by centrifugation and the hexane layer removed for analysis. The absorbancy of the hexane solution was determined at 450 mμ against a hexane blank in a Beckman Model B spectrophotometer. The carotenoid levels[5] were estimated from the extinction value for crystalline β-carotene dissolved in hexane: β-carotene, $E^{1\%}_{1cm}$ = 2480 at 450 mμ.

Lipoprotein fractions from duplicate sera were transferred to volumetric flasks and extracted with ethanol-ether 3:1 (v/v) as previously described (10). These lipid extracts were then analyzed for total cholesterol and fatty acid ester content by the methods of Abell *et al.* (12) and Stern and Shapiro (13), respectively.

RESULTS

Concentration of Serum Carotenoids after Oral Administration of β-Carotene. The effect of several different test regimens on serum carotenoid levels obtained after oral administration of β-carotene is summarized in Figure 1. Aqueous dispersions of β-carotene were readily absorbed when administered with either lipid or protein-carbohydrate test meals and gave a somewhat larger increment than crystalline β-carotene dispersed in margarine. Only slight serum increments were found when aqueous dispersions of β-carotene were administered to subjects without a test meal. Serum carotenoid levels were not enhanced by the administration of ox bile together with an aqueous dispersion of β-carotene to subjects on fasting and protein-carbohydrate test regimens. However, ab-

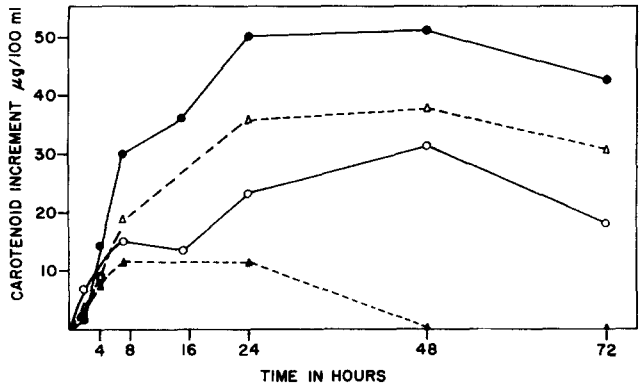

FIG. 1. Mean serum increments in β-carotene for subjects with different test regimens: 5 subjects with aqueous dispersion β-carotene and lipid test meal (●), 2 subjects with lipid dispersion β-carotene and lipid test meal (○), 2 subjects with aqueous dispersion β-carotene and protein-carbohydrate test meal (△), 2 subjects with aqueous dispersion β-carotene and no test meal (▲).

[1] The aqueous dispersion, β-carotene in a sugar and gelatin matrix, and crystalline β-carotene were kindly supplied by Hoffmann La Roche, Inc., Nutley, New Jersey.

[2] As crystalline β-carotene was mixed with a solid margarine, it was probably not completely dissolved in the margarine, and thus is described as a lipid dispersion.

[3] One gram of ox bile was administered as an elixir.

[4] Lipomul® Oral (Upjohn Co., Kalamazoo, Michigan) is a corn oil emulsion containing DL-α-tocopheryl acetate as an antioxidant.

[5] While human serum contains β-carotene, lycopene, and lutein, and these carotenoids have different absorbance maxima (2), the absorbance at 450 mμ estimates total carotenoid and measures the increment in β-carotene after its oral administration.

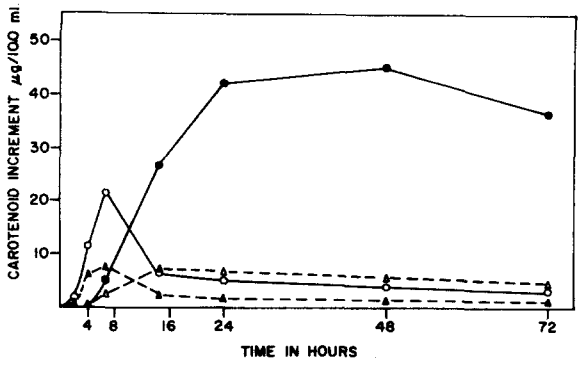

FIG. 2. Mean lipoprotein increments in β-carotene for 3 normal subjects: chylomicrons (▲), S_f 10–400 lipoproteins (○), S_f 0–10 lipoproteins (●), high-density lipoproteins (△).

sorption in both the fasting and bile studies was probably complicated by rapid fecal elimination. Thus, in one subject, the addition of bile to the test meal led to a mild diarrhea and concommitant diminution in β-carotene absorption.

The Lipoprotein Distribution of the Carotenoid Increment in Normal Subjects. The mean carotenoid increment in the serum lipoprotein fractions of three normal subjects after the oral ingestion of an aqueous dispersion of β-carotene with lipid and protein-carbohydrate test meals is recorded in Figure 2. Carotenoids in the chylomicron and S_f 10–400 lipoprotein fractions usually peaked in 7 hours. The maximum increment in the S_f 0–10 lipoprotein fraction occurred at 24 or 48 hours and was much higher than that in any other fraction. Although the carotenoid level in the high density lipoproteins appeared somewhat elevated beyond 15 hours, a consistent and significant increment in this fraction was not obtained. The mean recovery of serum carotenoids in lipoprotein fractions was 102%.

The Lipoprotein Distribution of the Carotenoid Increment in Hypercholesterolemic and Hyperlipemic Subjects. An aqueous dispersion of β-carotene was administered with a lipid test meal to a hypothyroid individual. This subject exhibited markedly elevated S_f 0–10 and moderately elevated S_f 10–400 lipoprotein fractions characteristic of the hypercholesterolemia seen in myxedema (10). The distribution of the carotenoid increment in the lipoproteins was similar to that seen in normal subjects (Fig. 3). However, the maximum carotenoid increment in the S_f 10–400 lipoprotein fraction, 30 μg per 100 ml at 7 hours, was increased, and the carotenoid content of this fraction was still elevated significantly at 15 hours. As in the normal, the maximum carotenoid increment in the S_f 0–10 fraction occurred at 48 hours. The total serum carotenoid reflected both the S_f 10-400 and S_f 0–10 carotenoid maxima by exhibiting two peaks.

To investigate further the effect of an elevated S_f 10-400 pool on carotenoid transport, an aqueous dispersion of β-carotene was administered with a lipid test meal to a subject with idiopathic hyperlipemia. The lipoprotein pattern in this subject was typical of idiopathic hyperlipemia, with markedly elevated

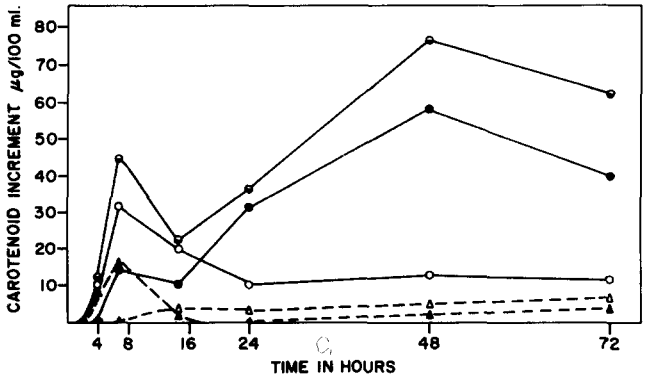

FIG. 3. Serum and lipoprotein increments in β-carotene for a hypothyroid subject who received 120 mg aqueous dispersion β-carotene and a lipid test meal: serum (◒), chylomicrons (▲), S_f 10–400 lipoproteins (○), S_f 0–10 lipoproteins (●), high-density lipoproteins (△).

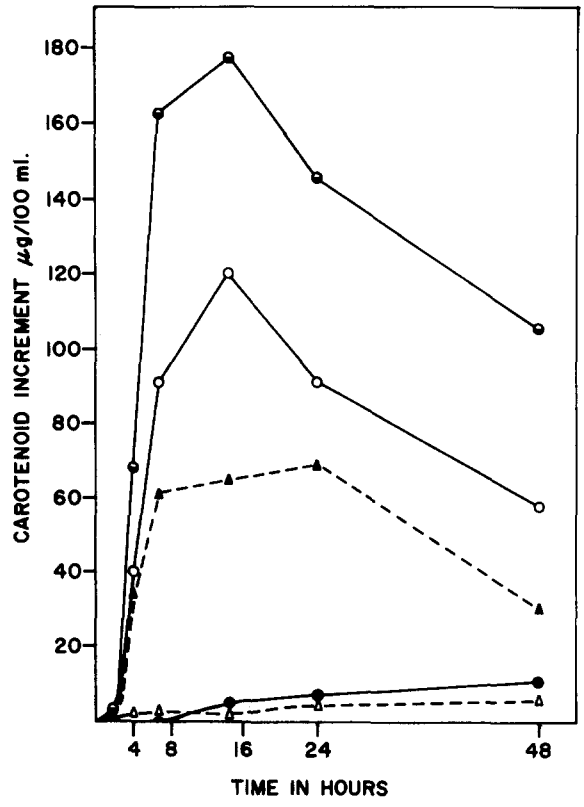

FIG. 4. Serum and lipoprotein increments in β-carotene for a hyperlipemic subject who received 120 mg aqueous dispersion β-carotene and a lipid test meal: serum (◒), chylomicrons (▲), S_f 10–400 lipoproteins (○), S_f 0–10 lipoproteins (●), high-density lipoproteins (△).

TABLE 1. CAROTENOID CONCENTRATION AND CAROTENOID-TO-CHOLESTEROL RATIOS IN SERUM LIPOPROTEIN FRACTIONS AFTER CAROTENOID-LIPID AND LIPID TEST MEALS

Test Meal and Fraction		Carotenoid in μg/100 ml*						
		Time in Hours						
		0	2	4	7	24	48	72
A.†	Carotenoid-lipid							
	chylomicron	2.4	3.2	8.1	5.2	4.0	3.6	3.6
		(0.53)	(0.71)	(1.56)	(1.16)	(0.91)	(1.16)	(1.19)
	S_f 10–400	8.5	12.5	23.8	31.1	18.1	19.1	13.3
		(0.56)	(0.64)	(1.06)	(1.24)	(1.05)	(0.77)	(0.85)
	S_f 0–10	156	151	137	152	194	185	178
		(1.38)	(1.28)	(1.32)	(1.37)	(1.88)	(1.80)	(1.73)
B.‡	Lipid							
	chylomicron	1.6	1.6	1.2	0.8			
		(0.52)	(0.32)	(0.40)	(0.21)			
	S_f 10–400	6.5	8.5	10.1	7.3			
		(0.33)	(0.31)	(0.34)	(0.31)			
	S_f 0–10	59	61	63	63			
		(0.41)	(0.41)	(0.39)	(0.40)			

* Carotenoid (μg)-to-cholesterol (mg) ratio in parentheses.
† Subject A received 200 mg aqueous dispersion β-carotene with test meal containing 60 g lipid.
‡ Subject B received test meal containing 120 g lipid as Lipomul® Oral.

chylomicron and S_f 10–400 lipoprotein fractions and markedly decreased S_f 0–10 and high density lipoprotein fractions (14). The lipoprotein distribution of the carotenoid increment in the serum of this subject is shown in Figure 4. Large increments were confined to the S_f 10–400 and chylomicron fractions. The initial increments in these fractions occurred at 4 hours as they did in normal and hypercholesterolemic subjects. The maximum increment, however, was delayed and extended between 7 and 24 hours for the chylomicron fraction and did not occur in the S_f 10-400 fraction until 15 hours. Total serum carotenoid also exhibited a maximum at 15 hours. Although carotenoid increments in the S_f 0–10 and high density lipoprotein fractions were very small, they increased somewhat throughout the 48-hour test period. Total serum carotenoid in the hyperlipemic subject increased more proportionally and also in absolute amount than did the corresponding values in normal and hypercholesterolemic subjects. Thus, the maximal increment of 178 μg/100 ml doubled the total serum carotenoid in the hyperlipemic subject, whereas maximal increments of between 20 and 70 μg/100 ml represented considerably smaller percentage increases in normal and hypercholesterolemic subjects given similar test meals.

Carotenoid Exchange Between Lipoprotein Fractions. Several observations suggest that rapid carotenoid equilibration and exchange between lipoprotein fractions do not occur. Peak carotenoid increments in the chylomicron and S_f 10–400 lipoprotein fractions occurred much earlier than those in the S_f 0-10 lipoprotein fractions (Figs. 2 and 3). Elevated carotenoid-to-cholesterol ratios (Table 1) show that these lipoprotein fractions contained relatively more carotenoid at the time of their peak increments. Thus, the carotenoid peak does not correspond merely to the increased chylomicron and S_f 10-400 lipoprotein concentrations found after a meal. Furthermore, the carotenoid content of a chylomicron fraction was unchanged 2 hours after a test meal containing lipid and no carotenoid (Table 1), even though fatty acid esters in this fraction increased from 0.01 to 0.28 mEq/100 ml. In the same experiment, small carotenoid increments proportional to increments in lipoprotein concentration were obtained in the S_f 10–400 fraction. However, these increments do not represent rapid exchange from S_f 0–10 and high-density lipoproteins since the carotenoid content of these fractions increased slightly (S_f 0–10) or remained unchanged (high density).

DISCUSSION

As carotenoid absorption is generally considered an aspect of lipid absorption (15, 16), it is significant that the peak increment following the ingestion of β-carotene (24 to 48 hours) appears considerably later than the peak increment following the ingestion of vitamin A (2), tocopherol (6), or iodinated triglycerides (5). Serum increments obtained in individual absorption studies with these lipids are calculated as the percentages of their maximum serum increments and compared in Figure 5. This delay is observed with aqueous and lipid dispersions and with lipid and protein-carbohydrate test meals. Carotenoid increments are similar with the different test meal regimens,

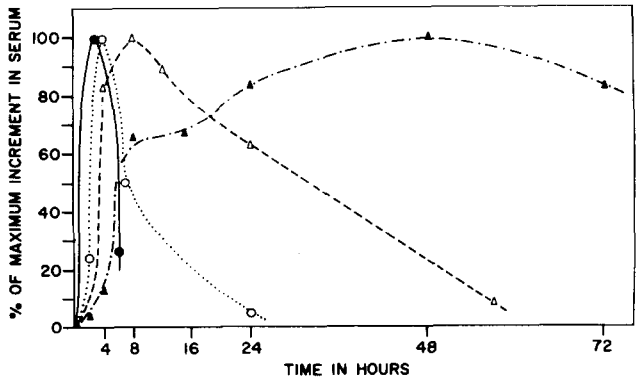

FIG. 5. The serum increment in vitamin-A ester (2), iodinated triolein (5), tocopherol (6), and carotenoid, calculated as percentages of their maximum serum increment after the oral administration of these lipids to individual subjects: vitamin-A ester (●), iodinated triolein (○), tocopherol (△), carotenoid (▲).

although very little absorption was found when carotenoid was administered without a test meal. These results are consistent with previous studies demonstrating that the addition of lipid did not enhance biological activity (17) or decrease fecal elimination of carotenoids (18), and they do not support experiments suggesting the importance of dietary fats alone in absorption (17). The data suggest that the absorption and transport of carotenoids differ from the absorption and transport of other lipids.

Differences in the transport pattern are also suggested by the lipoprotein distribution of newly absorbed carotenoid. The appearance and peak of the carotenoid increment in the S_f 0–10 lipoprotein fraction is considerably delayed. This is illustrated by the comparisons in Figure 6 where carotenoid and tocopherol increments in the S_f 10–400 and S_f 0–10 lipoprotein fractions are represented as the percentages of their maximum serum increment and plotted as a function of time. Comparable S_f 10–400 peaks for carotenoid and tocopherol disappear rapidly. The carotenoid increment in the S_f 0–10 fraction differs from the tocopherol increment in that the maximum is delayed and shows a broad plateau.

The study of lipid transport mechanisms is complicated by possible exchange equilibria of a given lipid between the several lipoprotein species in plasma as well as between vascular and extravascular pools of this lipid. For example, free cholesterol readily exchanges between lipoprotein fractions (19). Indeed, the equilibration of free cholesterol between several vascular and extravascular pools accounts for the relatively long half-life of labeled cholesterol in plasma (20). Tocopherol exchange between S_f 0–10 and high density lipoproteins has also been demonstrated (6). However, tocopherol does not appear to exchange between S_f 10–400 and S_f 0–10 or high density lipoprotein fractions. Since triglycerides and vitamin-A esters do not appear in appreciable amounts in S_f 0–10 and high density lipoproteins, the exchange of these lipids between S_f 10–400 lipoproteins and other fractions poses no problem. While carotenoids are found in all lipoprotein fractions (2), simultaneous peak increments in both carotenoid and carotenoid-to-cholesterol ratios for specific lipoprotein fractions after carotenoid ingestion and the absence of similar carotenoid increments when lipoprotein fractions are elevated by the ingestion of lipid alone indicate that rapid carotenoid equilibration and exchange between pools represented by lipoprotein fractions are unlikely.

Triglycerides are absorbed from the intestine, transported as chylomicrons through the lymphatics to the systemic circulation, and metabolized extravascularly

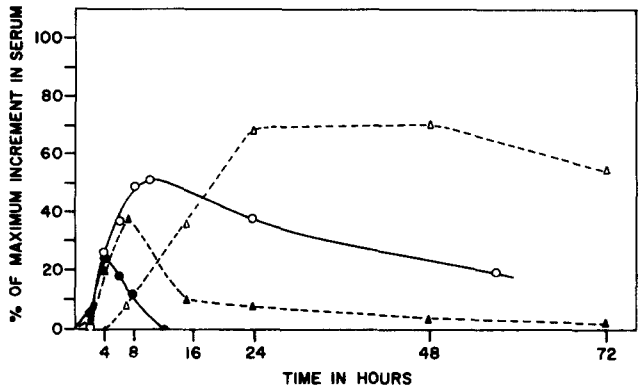

FIG. 6. The S_f 10–400 and S_f 0–10 lipoprotein increments in tocopherol (6) and carotenoid, calculated as mean percentages of their maximum total serum increments after the oral administration of tocopherol to 2 subjects and β-carotene to 3 subjects: tocopherol in S_f 10–400 lipoproteins (●), tocopherol in S_f 0–10 lipoproteins (○), carotenoid in S_f 10–400 lipoproteins (▲), carotenoid in S_f 0–10 lipoproteins (△).

liberating free fatty acids (5, 14, 21). During lipid absorption these free fatty acids are reincorporated into triglycerides in the liver and transported as S_f 10-400 lipoproteins (5, 14). These lipoproteins are converted in turn to the S_f 0–10 lipoproteins with the liberation of the triglyceride moiety as free fatty acid (5, 14, 22). Chylomicrons have a very short half-life (several minutes) and S_f 10–400 lipoproteins a somewhat longer half-life (several hours) (21, 22). The S_f 0–10 half-life is several days (22). This transport pattern explains both the iodinated triglyceride tolerance curve and lipoprotein distribution of labeled triglyceride. Since vitamin A is absorbed and transported similarly[6] (4, 23, 24), its tolerance curve (2, 4, 25) and lipoprotein distribution, as vitamin-A ester, is like that of triglyceride (2, 4). Tocopherol is absorbed and transported initially by chylomicrons and S_f 10–400 lipoproteins (6). The tolerance curve is prolonged, however, as tocopherol is subsequently transported in and is in rapid equilibrium between S_f 0–10 and high density lipoproteins (6).

Drummond *et al.* (26) first demonstrated that carotenoids were transported through the lymphatics. Lymphatic transport, subsequently confirmed by other investigators (16, 17, 24), explains the initial carotenoid increment in the chylomicron fraction. It is further supported by a marked carotenoid increment in the chylomicron fraction of a subject with hyperlipemia and a possible clearing defect in chylomicron metabolism. However, chylomicron-lymphatic transport does not

[6] The small vitamin-A ester increment in chylomicrons found by Krinsky *et al.* (2) undoubtedly reflects rapid chylomicron turnover (21) rather than the postulated S_f 10–400 transport mechanism.

explain the delayed appearance and peak increment in the S_f 0–10 lipoprotein fraction. The peak tocopherol increment in the S_f 0–10 lipoprotein fraction, for example, occurs at a much earlier time.

There are two possible explanations for the delay in the S_f 0–10 carotenoid peak. Carotenoids may be removed from the chylomicrons and S_f 10–400 lipoproteins in the liver and the carotenoids in this pool subsequently incorporated into other S_f 0–10 lipoproteins. Alternatively, a part of the total carotenoid may be absorbed by a different route and transported to the liver where it forms a distinct carotenoid pool and is available for direct incorporation into newly synthesized S_f 0–10 lipoproteins. The second hypothesis has some experimental support. Drummond *et al.* (26) were able to recover only 20% of administered carotenoid in human chyle. Forbes (27) found very little carotenoid in the chyle of an infant with a chylothorax, while vitamin A was readily absorbed by this route. Wagner *et al.* (28) were able to detect only traces of radioactive β-carotene in the lymph of rats with a thoracic duct fistula. Low recovery in these studies may only indicate poor absorption or conversion to vitamin A. Kowalski *et al.* (29), however, demonstrated that portal absorption is a major route for carotenoid administered to dogs either as an aqueous dispersion or in an oil soluble form. Even though hypocarotenemia and steatorrhea are both associated with the malabsorption syndrome (30, 31), it does not necessarily follow that β-carotene and other lipids are absorbed by the same route. Hypocarotenemia may be related to rapid fecal elimination rather than the specific lymphatic absorption of all lipids except low molecular weight fatty acids.

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