The transport of α-tocopherol and β-carotene in human blood

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Abstract The concentrations and distributions of major lipids (cholesterol, phospholipid, and triglyceride), tocopherol and carotenoids were determined in the plasma lipoprotein fractions (VLDL, LDL, and HDL) of (*1*) normal human subjects, (*2*) patients with hyperlipoproteinemia, and (*3*) patients with erythropoietic protoporphyria treated with oral β-carotene and/or α-tocopherol. The distribution of tocopherol (in percent) was most closely correlated with the distribution of total lipids in the individual lipoproteins, while the major portion of β-carotene was present in the low density lipoproteins, irrespective of the lipid distribution in the lipoproteins (except for one subject with hyperchylomicronemia). The α-tocopherol and β-carotene concentrations of plasma and RBC in patients treated with tocopherol and carotene were determined periodically for a one-year period. Plasma and RBC tocopherol concentrations showed a rapid, parallel increase in response to tocopherol supplementation. In contrast, the plasma and RBC carotene concentrations showed a much slower and nonparallel increase in response to carotene administration. When carotene supplementation was stopped, the elevated carotene levels in both plasma and RBC persisted for several months; the elevated plasma carotene level persisted longer than the raised RBC carotene levels. These results suggest that α-tocopherol and β-carotene are transported differently in the circulation and that the tissue storage and mobilization of these compounds are different.

Supplementary key words erythropoietic protoporphyria · antioxidant · hyperlipoproteinemia · singlet oxygen quenching

A recent development in the management of erythropoietic protoporphyria (EPP) has been the administration of β-carotene to decrease sensitivity to sunlight (1, 2). Erythropoietic protoporphyria is a disorder characterized by elevated protoporphyrin levels in RBC and feces and by cutaneous photosensitivity to wavelengths in the 400 nm region (3). The mechanism of protection by β-carotene could involve direct sunscreen action, singlet oxygen quenching (4–6), or antioxidant activity (7). An experimental model that has been developed for studying the mechanism of skin photosensitivity in EPP is porphyrin-catalyzed photohemolysis of RBC (8–11) Since both β-carotene (10) and α-tocopherol (12) have been shown to provide a degree of protection in this model system, a study was undertaken of the action of these compounds in patients with EPP and of a comparison of the transport of α-tocopherol and β-carotene in human blood.

Orally administered radioactive tocopherol appears rapidly in the plasma and tissues of rats and chickens (13) where transport of the vitamin is carried out by lymph and plasma lipoproteins (14). When high levels of tocopherol are in the diet of man, increased concentrations appear in plasma, RBC, and tissues (15, 16). Tocopherol exchanges rapidly between RBC and plasma lipoproteins in vitro (17, 18), and this exchange is thought to be the basis for the parallel response of RBC and plasma concentrations after a large, oral dose of the vitamin. While carotene levels in the plasma and tissues of humans rise after ingestion of increased amounts of carotene (19, 20), the details of this response are not known. Data on whether or not carotene exchanges rapidly between plasma lipoproteins, RBC, and plasma membranes are not available, although such information has been obtained for tocopherol and free cholesterol. In addition, carotene levels of human RBC and the response of RBC to dietary carotene supplementation had not been previously measured when this study was initiated. This report presents the carotene levels of the individual plasma lipoproteins and of the RBC of normal subjects and of subjects with hyperlipoproteinemia, as well as of patients with EPP receiving supplemental β-carotene and α-tocopherol. Distinct differences were observed between the transport of tocopherol and carotene in blood.

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; TLC, thin-layer chromatography; EPP, erythropoietic protoporphyria.

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MATERIALS AND METHODS

D-α-(5 Methyl-^{3}H)tocopherol, obtained from Amersham-Searle, Arlington Heights, Illinois, was purified prior to its use by thin-layer chromatography (TLC) on silica gel G plates, using the solvent system benzene–absolute ethanol 99:1, and eluted with benzene. Unlabeled *d*-α-tocopherol (Eastman Kodak, Rochester, N.Y.) was added to the radioactive tocopherol to adjust the specific activity to $5-10 \times 10^5$ dpm/μg. This stock solution of [^{3}H]tocopherol was stored in benzene under nitrogen at −20°C and rechecked periodically by TLC to maintain purity >95%.

β-[15,15-^{3}H]carotene and β-carotene were supplied by Hoffmann-LaRoche, Inc., Nutley, N.J. β-[^{3}H]-carotene was mixed with unlabeled β-carotene to yield a final specific activity of 2.3×10^5 dpm/μg. This product was purified prior to use by column chromatography on Alumina (Woehm, grade IV, 10% H_2O) and elution with petroleum ether as described below for the carotene assay. The β-[^{3}H]carotene was stored under nitrogen at −20°C and checked periodically by column chromatography to maintain purity >95%. Capsules of β-carotene (30 mg) and tablets of DL,α-tocopheryl acetate (200 mg) were provided by Hoffmann-LaRoche, Nutley, N.J.

Human plasma lipoprotein fractions were prepared by the standard method of flotation ultracentrifugation (21) in which KBr was used to adjust the plasma densities. The plasma was centrifuged sequentially at densities of 1.006 (16 hr), 1.063 (30 hr) and 1.21 (40 hr) at 4°C in a Spinco model L ultracentrifuge at 100,000 *g* with a Type 40 rotor (Spinco Div., Beckman Instruments, Palo Alto, Cal.). The isolated lipoproteins were dialyzed against isotonic phosphate buffer, pH 7.4 containing 0.01 M Na_2HPO_4, 0.124 M NaCl and 0.3 mM Na_2EDTA, and stored at 4°C. Tocopherol and carotene assays were carried out on RBC the same day as blood collection and on the plasma and lipoprotein fractions within one week of blood collection, since both lipids are sensitive to light and air. Plasma and lipoprotein samples have been stored at 4°C for up to three weeks with no loss of tocopherol or carotene activity.

Analyses

Plasma and plasma lipoprotein fractions were extracted in chloroform–methanol 2:1 and the lipids were purified by the procedure of Folch, Lees, and Sloane Stanley (22). The final chloroform phase was analyzed for total cholesterol by the procedure of Franey and Amador (23), for phospholipids by the procedure of Chen, Toribana, and Warner (24), and for triglycerides by the procedure of Van Handel and Zilversmit (25). To determine the free to esterified cholesterol ratio, the lipid extract was separated by thin-layer chromatography on silica gel G in petroleum ether–diethyl ether–acetic acid 90:10:1. Free and esterified cholesterol were eluted with chloroform, and analyzed as above (23).

β-Carotene was assayed in plasma, plasma lipoprotein fractions, and RBC by the method of Krinsky, Cornwell, and Oncley (26) for plasma and plasma lipoprotein fractions, except for the extraction of carotenoids. The extraction procedure used was a method originally developed for the isolation of tocopherol from plasma and RBC and consists of a saponification step in the presence of a high concentration of pyrogallol to serve as an antioxidant, prior to the actual extraction (27). For assaying carotene in RBC, whole blood was collected in 0.2 mM Na_2EDTA, and the RBC were separated by centrifugation in a Sorvall, GLC-1 centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.) at about 500 *g*. The RBC were washed five times with 5 volumes of isotonic phosphate buffer, then adjusted to a hematocrit of approximately 50%; the hematocrit was accurately measured to permit the calculation of carotene in μg per ml of packed RBC. Two ml of the washed RBC were pipetted into 50-ml glass stoppered centrifuge tubes. For the estimation of carotene in normal human RBC, it was necessary to use 16 ml of washed RBC (8 tubes) due to the low concentration of carotene. Into each tube an aliquot of α-tocopherol (0.3 mg) was added for additional protection against oxidation. At this point β-[^{3}H]-carotene (11,000 dpm and 0.48 μg) was added to one of the eight tubes in order to determine the percentage recovery of β-carotene. Ten ml of a pyrogallol solution (20 mg/ml absolute ethanol) was added to the RBC suspension while mixing, and the sample was then incubated at 70°C in a water bath for 2 min. 0.5 ml of saturated KOH was then added, mixed well, and the contents incubated for 30 min at 70°C. The tubes were cooled in ice, and 7.5 ml of distilled water added, followed by 22 ml of hexane. The mixture was shaken vigorously by hand for 2 min and centrifuged lightly to separate the phases. Twenty ml of the hexane phase was removed for column chromatography and 1 ml was dried and counted in Liquifluor (New England Nuclear Corp. Boston, Mass.) in a liquid scintillation spectrometer (Packard Instruments, Downers Grove, Ill. model 3375). The hexane extract was evaporated to near dryness, (0.2–0.4 ml) and applied to a column containing 3 g of alumina (aluminum

TABLE 1. Distribution of major lipids, α-tocopherol and β-carotene in plasma lipoprotein fractions of normal and hyperlipoproteinemic human subjects

Subject, Age, Sex	Fraction	Phospholipid		Triglyceride		Total Cholesterol		α-Tocopherol		β-Carotene Fraction 1	
		mg/dl	%	mg/dl	%	mg/dl	%	μg/ml	%	μg/ml	%
A. Normal subjects[a]											
(1) H.K. 54, M	Plasma	286		107		269		16.6		0.49	
	VLDL		9		68		7		18		7
	LDL		36		21		55		39		63
	HDL		54		11		37		43		28
(2) L.B. 31, M	Plasma	179		88		165		11.5		0.29	
	VLDL		13		69		8		19		5
	LDL		42		21		63		48		78
	HDL		45		10		26		33		17
(3) D.D. 50, M	Plasma	219		37		268		13		0.34	
	VLDL		4		*b*		3		19		*b*
	LDL		46		80		64		58		78
	HDL		48		20		31		*b*		22
B. Hyperlipoproteinemic subjects[a,c]											
(4) P.A. 69, F	Plasma	368		350		447		37.8		0.79	
	VLDL		34		67		41		50		30
	LDL		44		29		51		38		55
	HDL		20		4		8		11		14
(5) J.S. 38, M	Plasma	332		1160		208		22.5		0.25	
	VLDL + chyl.		67		96		58		79		88
	LDL		17		2		31		12		10
	HDL		16		2		11		8		3
(6) G.L. 49, M	Plasma	327		580		323		45.9		0.13	
	VLDL		51		89		49		70		5
	LDL		27		6		36		19		78
	HDL		22		4		14		10		17
(7) F.M. 20, M	Plasma	245		220		245		15.2		0.18	
	VLDL		33		82		45		56		60
	LDL		24		11		31		19		27
	HDL		43		7		23		22		13

[a] Blood was drawn from subjects in the morning after an overnight fast. The percent distribution of the lipids in the lipoprotein was calculated by dividing the amount of lipid in each lipoprotein fraction by the sum of the amounts in all three fractions. Recovery of the lipids in the lipoprotein fractions was greater than 90% based on plasma lipid values.

[b] Insufficient material for analysis.

[c] Subject P.A., hyperlipidemia (elevated VLDL); J.S., hypertriglyceridemia with chylomicronemia; G.L., hyperlipidemia (elevated VLDL); F.M., dysbetalipoproteinemia.

oxide, Woehm, neutral, grade IV, 10% water). The column was successively eluted with 12 ml of three different solvents: petroleum ether, 4% acetone in petroleum ether, and 40% acetone in petroleum ether. β-Carotene was eluted in the first solvent and its concentration was measured in a Gilford Spectrophotometer (Gilford Instrument Labs, Oberlin, Ohio) ($E^{1\%}_{1\,cm}$ = 2570 at 450 nm in petroleum ether). Lycopene was eluted in fraction 2 and lutein in fraction 3 as reported by Krinsky et al. (26); these compounds are reported to be the major carotenoids found in human plasma. One ml from each of the three fractions was counted for radioactivity and the remainder evaporated to near dryness under nitrogen. Fractions 1 and 2 were made up of 1 ml with petroleum ether and fraction 3 with chloroform. Lycopene concentration was estimated at 472 nm (26) in petroleum ether ($E^{1\%}_{1\,cm}$ = 3420) and lutein concentration at 453 nm (28) in chloroform ($E^{1\%}_{1\,cm}$ = 1800). Spectra of the carotenoids in the visible range were recorded on a Cary model 14 spectrophotometer (Cary Instruments, Monrovia, Cal.).

The procedure for estimation of plasma carotene was essentially the same as that for the RBC except that 3 ml of normal human plasma was used along with 6 ml of the pyrogallol solution, 0.9 ml of saturated KOH, 3 ml of water, and 12 ml of hexane for the final extraction.

The details of the tocopherol assay have been reported previously (27). The plasma and RBC tocoph-

erol were extracted and separated from other compounds in the hexane extract by thin-layer chromatography on silica gel G in benzene–absolute ethanol 99:1.

RESULTS

Table 1 shows the tocopherol, carotene, phospholipid and triglyceride, and total cholesterol levels in plasma and their distributions among the plasma lipoproteins for three normal subjects and four subjects with hyperlipoproteinemia. These subjects were not receiving supplemental tocopherol or carotene. It is apparent that the plasma tocopherol levels of the hyperlipidemic subjects are higher than those of the normal subjects, as reported previously (29, 30). Plasma tocopherol levels have been reported to be more closely correlated with plasma total lipid levels ($r = 0.85$) than with the individual lipids ($r = 0.64–0.72$) in a study of normal and hyperlipoproteinemic subjects (29). In comparing tocopherol distribution in the individual lipoproteins, it is evident that tocopherol is transported primarily by the lipoprotein class that contains the largest amount of total lipid (obtained by adding triglyceride, phospholipid and total cholesterol values), which in normal human subjects are the low density lipoproteins (LDL). As shown in Table 1, section A, the LDL fraction (d 1.006–1.063) of subjects 2 and 3 contained the largest percentage of both total plasma lipid and of tocopherol. Subject 1 had slightly more tocopherol in the HDL than in the LDL fraction, although the values were nearly the same for the two fractions. Section B of Table 1 presents data for subjects with various hyperlipoproteinemias, but all having elevated triglyceride levels. In these patients the greatest percentages of total plasma lipid and tocopherol were found in the VLDL. This indicates that the distribution of tocopherol depends on the relative amounts of the lipoproteins.

The percent distribution of β-carotene is not closely correlated with the lipid distribution of the lipoproteins. Carotene is transported predominantly in the LDL fraction in normal human subjects (Table 1A). Subjects P.A. and G.L., with elevated VLDL levels (Table 1B), had the largest amount of β-carotene associated with the LDL, even though the VLDL contained the largest percentage of total lipid. Subject F.M. (Table 1B) had "floating beta" or Type III dysbetalipoproteinemia, where an abnormal LDL or "remnant" is isolated with normal VLDL at $d < 1.006$, and subject J.S. had hyperchylomicronemia. Both of these subjects were exceptions in that the major amount of carotene was found in the $d < 1.006$ fraction. It is known that β-carotene is absorbed into the lymph system in man (31) so, depending on the diet, chylomicrons could contain significant amounts of β-carotene. Only subject J.S. had chylomicrons in his plasma as evidenced by visual inspection of the plasma after it had remained at 4°C overnight (cream layer) and by agarose gel electrophoresis. No attempt was made to separate the chylomicrons from the VLDL; therefore, in this case the VLDL fraction contains both chylomicrons and VLDL. Hyperlipoproteinemic subjects are broadly defined as follows: hypertriglyceridemia, with triglyceride levels above 130 mg/dl and hypercholesterolemia, with cholesterol levels above 275 mg/dl. Normal plasma α-tocopherol and β-carotene levels in this laboratory range from 5 to 18 μg/ml and 0.14 to 0.79 μg/ml, respectively.

Table 2 presents the distribution of major lipid, tocopherol, and carotene in the plasma lipoproteins of several patients with EPP receiving supplemental oral β-carotene (section A), α-tocopheryl acetate (section B) and both drugs (section C). In section A it is apparent that, even with an increase in β-carotene levels by a factor of almost 10, the distribution in the lipoproteins is unchanged and is similar to that observed in Table 1, i.e., the major portion of the carotene is located in the LDL. The amount of tocopherol in the plasma and its distribution among the lipoproteins is not affected by the increased levels of β-carotene. In section B of Table 2, one subject had received 2 gm of oral tocopheryl acetate daily for 3 weeks; the plasma tocopherol level was elevated by a factor of 2–3, but the distribution of tocopherol was unchanged in the lipoproteins and did not affect the distribution of β-carotene. It is interesting to note that the plasma β-carotene level of this patient was elevated, although he had stopped taking supplemental β-carotene 3 weeks earlier. Section C of Table 2 shows data from two subjects with EPP receiving supplemental tocopherol and carotene simultaneously. While both tocopherol and carotene levels are strikingly elevated, their distribution among the lipoproteins is the same as observed previously.

In general the distribution of fraction 2 and fraction 3 carotenoids in the lipoproteins was similar to that of β-carotene except that a somewhat larger proportion of fraction 3 was transported by the HDL. The plasma concentrations and lipoprotein distributions of the fraction 2 and fraction 3 carotenoids were not affected by either tocopherol or carotene supplementation. Plasma concentrations of fraction 2 (lycopene) averaged 0.321 ± 0.13

TABLE 2. Distribution of major lipids, α-tocopherol and carotenoids in plasma lipoprotein fractions of patients with erythropoietic protoporphyria receiving dietary supplementation

Subject, Age, Sex	Fraction	Phospholipid mg/dl	Phospholipid %	Triglyceride mg/dl	Triglyceride %	Total Cholesterol mg/dl	Total Cholesterol %	α-Tocopherol μg/ml	α-Tocopherol %	β-Carotene Fraction 1 μg/ml	β-Carotene Fraction 1 %
A. β-Carotene supplementation.[a]											
(1) R.S. 26, M	Plasma	198		76		164		12.5		3.0	
	VLDL		9		55		10		24		2
	LDL		35		27		54		42		76
	HDL		56		17		35		34		21
(2) L.L.	Plasma	199		144		168		11.5		6.2	
	VLDL		23		75		25		41		14
	LDL		28		13		44		24		61
	HDL		48		12		30		35		24
(3) M.R. 35, F	Plasma	213		40		165		9.8		6.9	
	VLDL		5		*b*		4		11		1
	LDL		29		*b*		46		33		58
	HDL		66		*b*		49		56		39
B. α-Tocopheryl acetate supplementation.											
(4) J.L.	Plasma	226		179		208		42.0		2.94	
	VLDL		20		75		22		52		6
	LDL		31		12		47		28		75
	HDL		48		13		28		20		16
C. Both β-carotene and α-tocopheryl acetate supplementation.											
(5) M.M. 13, F	Plasma	226		111		226		25.5		6.11	
	VLDL		13		74		7		31		2
	LDL		49		19		72		43		87
	HDL		37		8		21		26		11
(6) P.M. 17, M	Plasma	235		110		219		26.1		2.15	
	VLDL		15		70		7		26		3
	LDL		45		21		70		44		84
	HDL		40		9		22		30		11

[a] Patients with erythropoietic protoporphyria were not fasting. Treatment schedule at the time of this study: Patient R.S., 120 mg/day of β-carotene for 5 weeks; L.L., 120 mg/day of β-carotene for 3 weeks; M.R., 120 mg/day of β-carotene for 8 weeks; J.L., 2 g of *dl*, α-tocopheryl acetate for 6 weeks; M.M. and P.M., 60 mg/day of β-carotene and 2 g/day of *dl*, α-tocopheryl acetate for 6 weeks.
[b] Not measured.

μg/ml and the range was 0.11–0.67 μg/ml. For fraction 3 the average plasma concentration was 0.67 ± 0.25 μg/ml with a range of 0.34–1.12 μg/ml.

In a correlation analysis of the percent distribution of α-tocopherol and β-carotene with the percent distribution of the major lipids in the three lipoprotein fractions, it was observed that tocopherol uniformly is more closely correlated than carotene with the major lipids (**Table 3**). In the VLDL and the LDL fractions, tocopherol distribution correlates more closely with free cholesterol distribution than with the distribution of the other individual lipids. In HDL the tocopherol distribution appears to correlate about the same with phospholipid distribution ($r = 0.93$) and free cholesterol distribution ($r = 0.92$). Tocopherol distribution also correlated very well with total lipoprotein (0.93–0.97) and total lipid distribution (0.94–0.96), and correlated least well for the triglycerides (0.71–0.77) in all the lipoprotein fractions. β-Carotene distribution appears to be more closely correlated with major lipid distributions in HDL than in LDL or VLDL, but in all cases the correlation is less than with tocopherol.

When cross-correlations, e.g., correlating VLDL cholesterol distribution with LDL cholesterol distribution, were tested it was found that a high negative correlation (−0.67 to −0.94) existed for the lipids in VLDL compared with the same lipids in LDL, and in VLDL compared with HDL, but that no significant correlation was found between LDL and HDL lipids. This evidence is consistent with the theory that there is a relationship between VLDL and LDL, and VLDL and HDL, but no direct connection between LDL and HDL (32).

Fraction 1 from normal human RBC (**Fig. 1A**) gave an absorption spectrum in petroleum ether in

TABLE 3. Correlations of the percent distribution of α-tocopherol and β-carotene with the percent distribution of the major lipids in individual lipoprotein fractions[a]

	Phospholipid	Triglyceride	Total Cholesterol	Free Cholesterol	Cholesteryl Ester	Total[b] Lipid	α-Tocopherol	β-Carotene	Total[b] Lipoprotein
VLDL									
α-Tocopherol	0.65	0.71	0.95	0.96	0.91	0.96	1.00	0.69	0.93
β-Carotene	0.49	0.62	0.77	0.76	0.71	0.71	0.69	1.00	0.79
LDL									
α-Tocopherol	0.86	0.74	0.89	0.95	0.83	0.96	1.00	0.68	0.94
β-Carotene	0.62	0.35	0.71	0.74	0.60	0.70	0.68	1.00	0.70
HDL									
α-Tocopherol	0.93	0.77	0.93	0.92	0.90	0.94	1.00	0.87	0.97
β-Carotene	0.83	0.68	0.86	0.79	0.78	0.87	0.84	1.00	0.84

[a] Number of samples is 14; for a correlation coefficient of 0.90, $P < 0.001$; of 0.70, $P < 0.005$; and of 0.60, $P < 0.02$. The correlation coefficients represent the comparison of the percent distribution of the various lipids with tocopherol and with carotene in each lipoprotein fraction. These correlation coefficients were calculated on a Univac 1108 computer utilizing the correlation analysis program BMD-03D from BMD-Biomedical Computer Programs, third ed. 1973, Univ. of Calif. Press, Berkeley.

[b] Total lipid values were calculated by adding the weight values for phospholipid, triglyceride, free cholesterol and cholesterol ester (multiplied by 1.7 to include the fatty acid moiety). Total lipoprotein values were calculated by adding the total lipid and the protein weight in each lipoprotein fraction.

the visible range that was the same as that of authentic β-carotene, which has maxima at approximately 480, 450, and 425 nm. Patients with EPP receiving supplemental β-carotene had much higher RBC levels so the absorption spectrum from fraction 1 was sharper (Fig. 1B) and identical to that of authentic β-carotene (Fig. 1C). Thin-layer chromatography of fraction 1 from RBC in benzene–absolute ethanol 99:1 demonstrated that only one compound was present that had the same R_f as authentic β-carotene.

The values for plasma and RBC β-carotene levels of normal subjects are listed in **Table 4**. The plasma levels (Table 4) average 0.399 μg/ml and range from 0.146 to 0.793 μg/ml. In this study

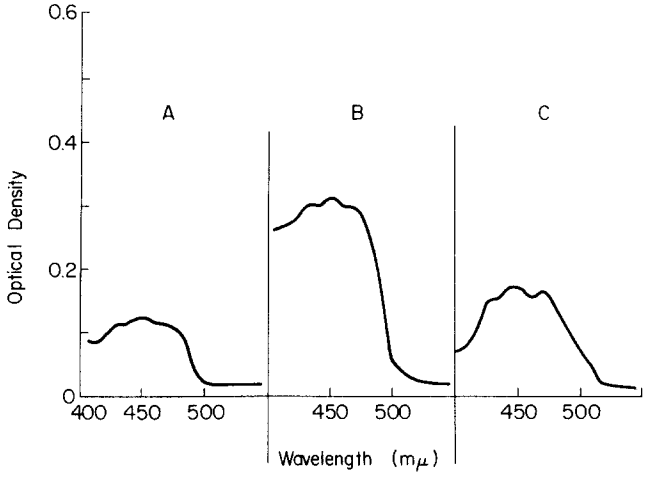

Fig. 1. Absorption spectra in petroleum ether recorded in the visible range of A) fraction 1 from normal human RBC, B) fraction 1 from RBC of patients with EPP on β-carotene therapy and C) authentic β-carotene.

the total plasma concentration of β-carotene plus lycopene averaged 0.72 μg/ml, which is in agreement with the value of 0.73 μg/ml reported by Krinsky et al. (26). The RBC β-carotene levels averaged 0.019 μg/ml and ranged from 0.008 to 0.036 μg/ml packed RBC (Table 4). In these assays the recovery of [^{3}H]carotene was 88 ± 6% in plasma and 69 ± 8% in RBC (average ± SEM). The concentrations of fraction 2 and fraction 3 in RBC were 0.007–0.072 and 0.042–0.113 μg/ml packed cells, respectively. The ratios of fraction 1:fraction 3 in the RBC were different from these ratios in plasma.

The β-carotene levels of plasma and RBC of three patients with EPP taking various amounts of β-carotene and α-tocopheryl acetate are presented in **Fig. 2**. It is seen that in all cases (Fig. 2, A–C) the plasma and RBC carotene levels do not parallel each other closely, e.g., the RBC show maximum carotene levels at a later time than the maximum plasma levels. RBC β-carotene concentrations were highest in September at the end of the summer, whereas plasma β-carotene concentrations were highest in the early part of the summer. The increases in plasma carotene concentration with carotene supplementation were also larger than the increases in RBC carotene concentration. Plasma carotene levels frequently increased by a factor of ten or more while RBC carotene levels increased usually by a factor of no more than two to five. When carotene therapy was discontinued in September the RBC carotene levels returned to normal more rapidly than plasma carotene levels; by November the RBC carotene levels had returned to normal but the plasma carotene levels were still above the normal plasma range (0.14–0.79 μg/ml).

TABLE 4. β-Carotene levels of plasma and RBC in unsupplemented human subjects

	Plasma Carotene[a] μg/ml	RBC Carotene μg/ml packed RBC
1)	0.146	0.028
2)	0.335	0.036
3)	0.500	0.009
4)	0.266	0.016
5)	0.278	0.018
6)	0.360	0.008
7)	0.536	*b*
8)	0.373	*b*
9)	0.793	*b*
Average ± SEM	0.399 ± .210	0.019
Range	0.146–0.793	0.008–0.036

[a] Carotene concentrations corrected to 100% by recovery of trace amounts of β-[^{3}H]carotene.
[b] Insufficient RBC for analysis.

In March and April, six months after discontinuing carotene, the plasma levels were within the normal range. Two additional patients with EPP were studied while on β-carotene therapy and the results were the same as described above for carotene transport.

The RBC and plasma tocopherol levels for these subjects with EPP during tocopherol therapy are also presented in Fig. 2. Average values for RBC and plasma tocopherol levels in our laboratory are 3 and 14 μg/ml, respectively. Tocopherol levels in blood are known to respond rapidly to ingested tocopherol (16). The rapid fall in elevated tocopherol levels when intake is decreased is illustrated in Fig. 2B, where both plasma and RBC levels are observed to return to normal two weeks (June 14–29) after stopping supplementary tocopherol intake. This is in contrast to the plasma carotene levels in Fig. 2A which are still elevated almost tenfold three weeks (June 1–20) after stopping supplemental carotene intake. In general it is observed that the highest RBC tocopherol levels occur with the highest plasma levels (Fig. 2, A–C), i.e., there is a parallel, though not exact, relationship between the increase in plasma tocopherol levels and RBC tocopherol levels upon tocopherol supplementation.

DISCUSSION

A number of studies on the absorption of tocopherol have been carried out in man (33) and other species. When chylomicrons and VLDL containing radioactive tocopherol are injected intravenously into rats, the radioactivity is rapidly distributed among all the plasma lipoproteins, RBC, and some tissues (14). The mechanism of incorporation of tocopherol from lipoproteins into tissues is not known. Tocopherol appears to be exchanged rapidly in a manner similar to the exchange of free cholesterol between plasma lipoproteins and RBC (17, 18), and this may be the basis for the rapid incorporation of radioactivity into RBC and tissues when these lipids are fed.

Tocopherol levels in plasma are increased as the total lipid content of human plasma increases (29, 30). Elevated levels have also been reported for disease states associated with elevated plasma lipids (34–36). Peake, Windmueller, and Bieri (14) found that the individual rat plasma lipoprotein tocopherol content was most closely correlated with the total lipid content of each lipoprotein. In the present work, plasma tocopherol levels are correlated more closely with plasma total lipid levels than with the levels of the individual major lipids. When each lipoprotein fraction (VLDL, LDL, and HDL) was examined separately, it was found that, overall, the distribution of tocopherol was slightly better correlated with the distribution of total lipid (0.94–0.96) than with the distribution of free cholesterol (0.92–0.96), although the correlation with free cholesterol was greater than that for the distribution of the other individual major lipids. Normal human subjects had the largest amount of plasma tocopherol in the LDL fraction, which also contains the largest amount of plasma total lipid. In several subjects with hypertriglyceridemia with elevated VLDL levels, both the largest amount of plasma total lipid and of tocopherol were found in the VLDL. In subjects receiving supplemental tocopherol orally the same correlations between tocopherol and lipoprotein lipids existed, so the elevated tocopherol concentration had no effect on the tocopherol–total lipid relationship.

The above observations suggest that tocopherol is in dynamic equilibrium between the individual plasma lipoproteins, and between those lipoproteins and RBC. It is possible that tocopherol may also be in dynamic equilibrium between the plasma lipoproteins and body tissues, since adipose tissue levels tend to parallel plasma levels of human subjects fed supplemental tocopherol (15) and, in rats fed orotic acid, changes in plasma and liver lipid levels are paralleled by similar changes in tocopherol levels (37).

In normal subjects and subjects with hyperlipemia there did not appear to be a close correlation between plasma carotene and lipid levels. In addition, in the individual plasma lipoproteins of these subjects β-carotene is predominantly associated with the LDL fraction, as has been previously

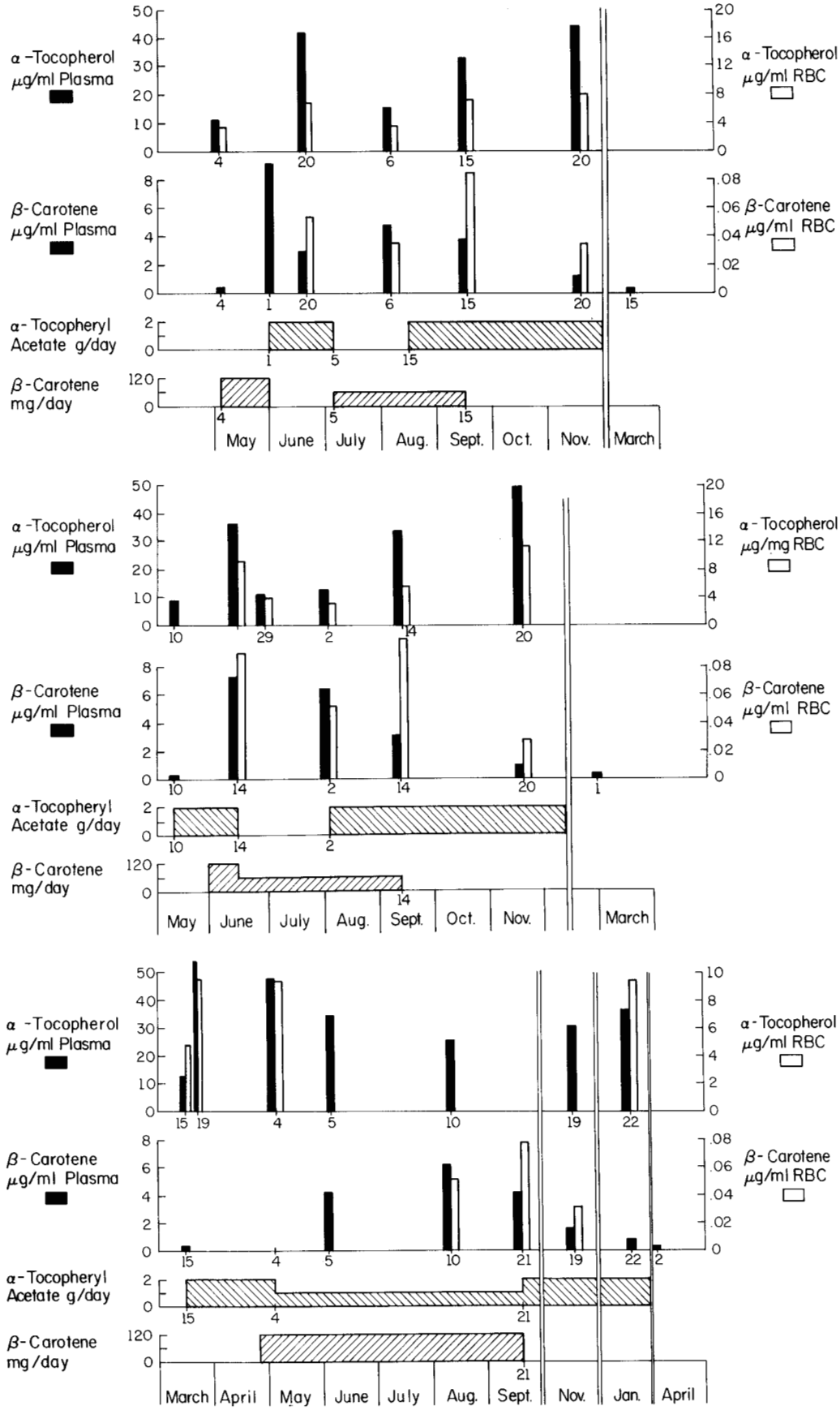

Fig. 2. α-Tocopherol and β-carotene levels in RBC and plasma of three patients with EPP ingesting various amounts of α-tocopheryl acetate and/or β-carotene. Top, J.L.; middle, L.L.; bottom, M.M.

observed (26). In patients with EPP receiving supplemental β-carotene and/or tocopherol the β-carotene–LDL relationship is unchanged, despite a tenfold elevation of plasma carotene. Similar observations were noted by Mathews-Roth and Gulbrandsen (38). The lipoprotein distribution of β-carotene and tocopherol are independent of each other, at least with respect to dietary supplementation.

It has been observed that plasma and xanthoma carotene concentrations rise after carotene supplementation (19, 20), but no data were available on RBC content of carotene. Using the extraction procedure developed in our laboratory for the quantitation of tocopherol in RBC, Mathews-Roth has confirmed the presence of β-carotene in RBC of normal and carotenemic subjects (39). Her report establishes that β-carotene is present in RBC ghosts, but does not provide any quantitative data. In our study of normal subjects the RBC carotene levels ranged from 0.008 to 0.036 μg/ml packed RBC, and for subjects on carotene therapy these values reached levels of 0.190 μg/ml packed RBC. In following plasma and RBC carotene levels with changing dietary carotene intake, it was found that these levels did not closely parallel each other. The maximum levels of carotene appeared more slowly in RBC than in plasma when supplemental β-carotene was administered and, when supplementation was discontinued, the RBC carotene levels returned to previous levels more rapidly than plasma. This suggests that carotene is not readily transferred from plasma lipoproteins to RBC and that the lipoproteins and RBC take up carotene from different pools. It is possible that after several months of carotene therapy a large pool of β-carotene develops that maintains elevated plasma levels for more than two months after therapy is stopped. Whether this is one pool or several and which tissues are involved are not known, although the liver is likely to be involved since it is a major site of synthesis and catabolism of the lipoproteins.

Recent evidence suggests that a suitable model for VLDL might consist of a surface layer of phospholipid, free cholesterol, and apolipoprotein surrounding a core of triglyceride and cholesteryl ester (40, 41). Whether this model is valid for the other lipoproteins, LDL and HDL, remains to be ascertained. The concept of surface and core components for lipoproteins could explain the observation that free cholesterol and phospholipid exchange readily whereas triglyceride and cholesteryl ester do not; i.e., the more polar lipids on the surface are available for exchange while the less polar lipids in the core are not (42).

β-Carotene could be located in the core of the lipoproteins, making it a nonexchangeable component, as are triglycerides and cholesteryl esters. The physico-chemical properties of β-carotene are consistent with this hypothesis; i.e., the molecule is very nonpolar and hence possesses no polar functional group to interact with water. It is possible that the rapid and parallel responses of RBC and plasma tocopherol levels to tocopherol supplementation reflect this molecule's ability to exchange rapidly, while the slower and less parallel responses of RBC and plasma carotene levels to carotene supplementation result from a less facile exchange, or nonexchange of β-carotene. This difference may also explain why the plasma carotene levels remain elevated for so much longer than those of tocopherol after stopping their respective supplementation, even though the amount of tocopherol ingested per day on therapy was more than sixteen times the amount of β-carotene. Alternative explanations involving different tissue pools or mechanisms of tissue storage are also plausible and further study, especially on the exchange or transfer of lipids from lipoproteins to tissues, is necessary for a definitive answer.

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