Tissue distribution and metabolism of newly absorbed vitamin A in the rat

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SUMMARY Chylomicrons containing newly absorbed labeled vitamin A were injected intravenously into normal intact rats, and the tissue distribution of radioactivity was observed for several days. Chylomicrons were used so that the vitamin could be administered physiologically, in the form in which it is normally absorbed. The total recovery of lipid-soluble radioactivity in the entire animal varied from 92% after 17 min to 63% after 24 hr to 56% after 6 days. At all times approximately two-thirds of the recovered radioactivity was found in the liver. Substantial amounts of radioactivity were also found in the kidneys and in the total depot fat, and small but significant amounts of labeled vitamin A were found in the plasma, small intestine, lungs, and adrenals. After 8 hr, labeled retinyl esters predominated in all tissues except plasma, with small amounts of labeled retinol also being present. Quantitatively significant amounts of labeled retinal or retinoic acid were not observed. The composition of labeled retinyl esters was remarkable in showing a consistent predominance of saturated esters in all tissues. Marked differences were, however, seen in the relative amounts of labeled retinyl palmitate and stearate in different tissues.

During the first 24 hr 3.7% of the injected radioactivity was excreted as expired CO_2, 3.5% was excreted as water-soluble compounds in the urine, and 8.7% was excreted in the bile. The biliary metabolites apparently consisted of a heterogeneous mixture of polar compounds, some of which were present as glucuronic acid conjugates.

KEY WORDS vitamin A · retinyl esters · retinol · chylomicrons · liver · kidney · adrenals · rat · metabolism · excretion · bile metabolites · plasma transport

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DIETARY VITAMIN A and β-carotene are absorbed into rat intestinal lymph almost entirely in the form of retinyl esters[1] (2–6). Recent studies have demonstrated that the composition of rat lymph retinyl esters is remarkably constant, regardless of the fatty acid composition of the test meal, and regardless of whether the retinyl esters are derived from retinol or from β-carotene (6). Retinyl palmitate was consistently found as the predominant ester, and saturated retinyl esters (palmitate + stearate) comprised two-thirds to three-fourths of the lymph esters. The retinyl esters (and other vitamin A derivatives) were mainly transported in lymph chylomicrons.

Very little detailed information is available on the metabolic history of newly absorbed vitamin A. Willmer and Laughland indirectly studied the metabolism of vitamin A, by examining the tissue distribution of radioactivity for up to 28 hr after the oral administration of C^{14}-labeled β-carotene to vitamin A-deficient rats (7). The total radioactivity recovered was greatest in the liver, followed by the adrenal glands, intestine, kidney, and blood. The highest concentration of radioactivity, per gram of tissue, was seen in the adrenals. Data on the metabolism of vitamin A in the intact rat have been reported by Wolf, Kahn, and Johnson (8). Twenty-four hours after the intraperitoneal injection of a Tween emulsion of retinol-C^{14} considerable amounts of C^{14} were found in the liver and carcass. Expired CO_2 contained 5.2% of the injected C^{14}, and 4–12% of the radioactivity was found in water-soluble compounds in the urine. Information is available on the distribution and storage of vitamin A in various organs and tissues (9). In mammals, vitamin A is stored mainly in the liver, where it is found largely as retinyl palmitate (10, 11). Retinyl

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

palmitate has, in fact, been reported to be the only ester which accumulated in rat liver after intracardiac injections of Tween emulsions of a variety of retinyl esters (12). Significant amounts of vitamin A have also been detected in other tissues, including kidney, lung, adrenals, plasma, and retina (9).

In the present study, chylomicrons containing newly absorbed labeled vitamin A were injected intravenously into intact rats, and the tissue distribution and metabolism of the labeled vitamin A were observed for several days. Since the vitamin was injected in the form in which it is normally absorbed, after either dietary vitamin A or β-carotene, the results should reflect the actual metabolism of newly absorbed vitamin A in the rat.

EXPERIMENTAL PROCEDURES

Retinol-15-C^{14} (62 μc, specific radioactivity 29.6 μc/mg) was dissolved in 0.4 ml of an equal mixture of corn and olive oil containing 1% α-tocopherol, and were given by gastric intubation to two rats whose cisternae chyli had been cannulated the previous day. Chyle was collected for 8 hr and was then layered under isotonic saline and centrifuged at 25,000 rpm for 25 min. The washed chylomicrons contained 17.4 μc of C^{14}, 93% of which resided in retinyl esters (see below).

Portions of the washed chylomicron suspension were injected into the tail vein of each of 7 male Sprague-Dawley rats (designated A through G) weighing 160–175 g, which had been fasted for 10–12 hr. Six rats each received 1.5 ml of chylomicron suspension, containing 54 μg of labeled vitamin A. This dose represents approximately one-tenth the usual amount of vitamin A found in rat liver (9). Rat A received a smaller dose. The rats continued to fast for 8 hr and were then fed laboratory chow. At specified time intervals the animals were anesthetized with ether, and blood (5–7 ml) was withdrawn from the abdominal aorta into heparinized syringes. Organs and tissues were removed as rapidly as possible in the following order: liver, epididymal fat bodies, kidneys, adrenals, small intestine, spleen, right adductor muscle, heart ventricles, and lungs. The tissues were rinsed, blotted dry, weighed, and extracted in ethanol–acetone 1:1 (v/v) (13, 14). The carcass and remaining viscera were ground and similarly extracted. The blood was immediately chilled with ice, and was then centrifuged. The plasma was removed, the red cells were washed twice with isotonic saline, and plasma and washed red cell samples were extracted with ethanol–acetone. At the time of extraction a mixture of unlabeled carriers consisting of 120 μg of mixed retinyl esters, 80 μg of retinol, 8 μg of retinol, and 8 μg of retinoic acid was added to each tissue sample.

Portions of each tissue and carcass extract were chromatographed on columns of deactivated alumina (Woelm, grade III) as described previously (6). The fractions were eluted as follows: 1, hydrocarbons (including β-carotene), eluted with 8 ml of hexane per 2 g of alumina; 2, retinyl esters (8 ml of benzene–hexane 3:17); 3, retinal (8 ml of benzene–hexane 1:1); 4, retinol (20 ml of benzene); 5, more polar compounds (8 ml of methanol). Retinoic acid was eluted as a sixth fraction (8 ml of methanol 25% acetic acid 3:1) or was extracted from aliquots of the total lipid extract with ethanolic KOH. Portions of each column fraction, and of each total lipid extract, were evaporated to dryness and the residue was dissolved in 15 ml of 0.5% diphenyloxazole in toluene and assayed for C^{14} in a Packard liquid scintillation counter.

The radioactive retinyl esters (column fraction 2) of 5 tissue samples (2 liver, 2 kidney, and 1 lung sample) were characterized by a series of chemical procedures involving saponification, oxidation, and acetylation (6). Radioactivity in the retinol fraction (column fraction 4) of 5 samples (1 liver, 2 kidney, and 2 plasma samples) was shown by similar procedures (6) to reside largely in retinol. All retinyl ester samples which were analyzed to determine their composition were also subjected to thin-layer chromatography (TLC) on alumina gel G (Brinkmann Instruments Inc., Great Neck, N.Y.), using benzene–hexane 1:3 as ascending solvent. This procedure separates long-chain fatty acid esters of retinol from retinyl acetate and from cholesterol esters (6). In every sample, almost all the radioactivity in the retinyl ester fraction resided in long-chain retinyl esters.

The composition of the labeled retinyl esters in the various retinyl ester fractions was determined by a combination of TLC on alumina gel G impregnated with silver nitrate, and reversed phase chromatography on silicone-impregnated paper, as described in detail elsewhere (6).

After the injection of chylomicrons, one rat (rat E) was confined to a metabolism cage which permitted the collection of urine, feces, and expired CO_2. The latter was collected by drawing a slow stream of CO_2-free air through the metabolism cage and then through a solution of 1 N NaOH. Portions of the urine and of the NaOH solution containing the expired CO_2 were added to the scintillation solution described by Bray (15) and assayed for C^{14}. Feces and urine were also extracted with ethanol–acetone and assayed for radioactivity.

Part of the plasma from rat G was adjusted to density 1.21 with a solution of KBr, and lipoproteins were separated as a single fraction by centrifugation for 30 hr at 40,000 rpm in the 40.3 rotor of a Spinco model L ultracentrifuge. The centrifuge tube was sliced in the clear zone below the lipoprotein layer, and the lipoprotein and "1.21 bottom" lipids were separately extracted with $CHCl_3$–CH_3OH 2:1 (v/v).

TABLE 1 RECOVERY AND TISSUE DISTRIBUTION OF C^{14} AT VARIOUS TIMES AFTER THE INJECTION OF CHYLOMICRONS CONTAINING NEWLY ABSORBED C^{14}-LABELED VITAMIN A

Rat*:	A	B	C	D	E	F	G	K
Time (hr):	0.28	1.00	3.00	7.75	23.5	72.	143.	26.5
Total Tissue Recovery (% of Dose):	92.0	78.8	79.2	80.7	63.6	59.8	55.7	61.7
				% distribution of recovered C^{14}				
Tissue								
Liver	67.81	76.30	67.35	72.39	66.85	72.51	78.73	58.41
Fat	2.58	9.33	3.39	6.73	9.57	8.13	8.96	7.62
Kidneys	0.42	1.14	3.57	4.04	9.28	12.16	4.20	16.04
Adrenals	0.053	0.19	0.13	0.13	0.17	0.12	0.26	0.05
Small intestine	0.68	1.90	2.74	2.20	1.62	0.71	0.76	2.09
Spleen	0.20	0.83	0.18	0.16	0.09	0.04	0.04	0.14
Skeletal muscle	3.60	6.55	7.36	4.74	6.19	4.09	5.13	4.49
Heart ventricle	1.29	0.26	0.19	0.07	0.08	0.04	0.03	0.03
Lungs	0.66	0.51	0.86	0.55	2.52	1.24	0.92	0.69
Plasma	20.0	2.83	3.75	2.07	1.87	0.94	0.95	1.42
Red cells	0.10	0.15	0.07	0.06	0.04	0.01	0.02	0.00
Rest of carcass	2.6	0	10.4	6.9	1.7	0	0	8.8

* Rat A was given 0.95 μc and rats B–G were given 1.58 μc of C^{14}-labeled chylomicrons (preparation number 1). Rat K was a bile fistula rat which was given 0.79 μc of C^{14} as chylomicrons (preparation number 2).

In a later study, a second preparation of washed chylomicrons was obtained after the administration of 19 μc of retinol-15-C^{14} (+ 2 mg of α-tocopherol) in 0.3 ml of an equal mixture of corn and olive oil to a rat with a lymph fistula. The composition of labeled retinol derivatives was almost identical in the two chylomicron preparations. Some of this chylomicron suspension (2 ml) was injected into the tail vein of each of three rats (designated K, L, M) in which polyethylene cannulae had been implanted in the common bile duct the previous day. Serial samples of bile were collected for 24 hr after which the tissues of one of the bile fistula rats (rat K) were removed, extracted, and analyzed as described above. Portions of bile were assayed for C^{14}. Other portions were extracted with 24 volumes of $CHCl_3$–CH_3OH 2:1, followed by the addition of 5 volumes of 0.05% H_2SO_4 in order to split the extract into a lower $CHCl_3$ and an upper CH_3OH phase. The two phases were separated and assayed for C^{14}.

Portions of whole bile and of methanol extracts of bile were exposed to strong acid (1 N HCl at 100° for 1 hr), or were incubated with β-glucuronidase (37° for 4 hr). Two preparations of β-glucuronidase were employed: one from beef liver (Ketodase, Warner Chilcott, Morris Plains, N.J.) and one from bacteria (Sigma Type II). Similar incubations were also carried out with a sulfatase preparation (Sigma Type III, from limpets). The incubation mixtures were extracted with $CHCl_3$–CH_3OH, the extracts were split into two phases, and each phase was assayed for C^{14}.

The tissue distribution of radioactivity in each animal was calculated. Depot fat was considered to comprise 7.08% and skeletal muscle 45.5% of the body weight of each rat (16). The plasma volume was taken as 3.5% of the body weight and the red cell volume determined from the measured hematocrit. Tissue recoveries were corrected for the amount of blood expected to be present in each tissue sample, using the same values employed in a previous study (14).

The sources of all materials and compounds used in this study were described in the preceding paper (6).

RESULTS

The observed total recoveries of C^{14} in all the tissue extracts of each rat, for time intervals varying from 17 min (0.28 hr) to 6 days, are presented in Table 1. After 17 min, 92% of the injected radioactivity was recovered as lipid-soluble C^{14} in the tissue and carcass extracts, whereas after 1–8 hr the recovery of C^{14} was only approximately 80%. After 24 hr 63%, and after 6 days 56% of the injected C^{14} was recovered as lipid-soluble radioactivity.

The tissue distribution of radioactivity in each rat is also listed in Table 1. In all animals, approximately two-thirds of the total recovered C^{14} was found in the liver. Substantial amounts of C^{14} were also found in the kidneys and in the total depot fat. The amount of radioactivity in the kidneys increased steadily for the first 24–72 hr and then declined, whereas the amount of radioactivity in the depot fat remained relatively constant after the first hour. Plasma contained 20% of the recovered C^{14} at the earliest interval studied (17 min), but only 1–4% thereafter.

The distribution of radioactivity in each tissue extract was determined by column chromatography. In all tissues studied, quantitatively significant amounts of C^{14} were found only in the retinyl ester and retinol fractions, which together contained more than 90% of the C^{14} in

almost all samples (Table 2). Small amounts of radioactivity were also recovered in column fractions 1, 3, and 5. Except for the carcass samples, these latter three fractions contained 0.4–2.4%, 0.4–4.4%, and 0.8–8.1% of the recovered C^{14}, respectively. Most (or all) of this small amount of radioactivity probably represented slight and variable overlapping of chromatographic fractions. Comparable recoveries of C^{14} in fractions 1, 3, and 5 were seen after chromatography of standard mixtures of labeled retinyl esters + retinol. Somewhat larger amounts of radioactivity were found in fractions 1, 3, and 5 of the carcass extracts, probably because of the larger chromatographic loads employed with these samples. The retinyl ester + retinol fractions contained 80–90% of the C^{14} in the carcass samples. Only very small amounts of C^{14} were recovered in any of the retinoic acid extracts.

Table 2 presents the percentage of recovered C^{14} present as labeled retinyl ester and labeled retinol in the tissues and carcass of each rat. After 7 hr labeled retinyl esters predominated in all tissues examined, with the exception of plasma. In plasma, labeled retinyl esters predominated only in the earliest sample (rat A), and labeled retinol comprised 85–95% of the plasma radioactivity at 3 hr and beyond. The labeled retinyl esters present in the plasma of rats A and B undoubtedly resided in chylomicrons which had not been cleared from the circulation; this interpretation is supported by the almost identical composition of labeled retinyl esters in the plasma of rat A and in the injected chylomicrons (see Table 3).

Results obtained with different tissues varied considerably. At all time intervals, retinyl esters comprised 80–90% of the radioactivity in liver and in adrenal glands, and 50–65% of the radioactivity in the carcass samples. In contrast, during the first 24 hr the ratio of labeled retinyl esters to retinol changed markedly in the kidney and lung samples. Labeled retinol predominated in both lung and kidney at 1 hr, and continued to predominate in the kidney up to 3 hr. The ratio of retinyl esters to retinol increased progressively between 1 and 24 hr. Retinyl esters contained 65–85% of the label by 24 hr and thereafter, in these two tissues.

The compositions of the labeled retinyl esters present in the various tissue and carcass extracts are presented in Table 3. Saturated retinyl esters predominated in all tissues, particularly in the liver, where saturated esters comprised 85–90% of the retinyl esters in all later samples. Saturated esters comprised approximately 75–80% of the labeled esters in adrenal glands and in the lungs, and approximately 70% of the labeled esters in kidneys and small intestine. A somewhat lesser preponderance of saturated esters (50–70% of the labeled esters) was seen in the carcass samples.

Despite the consistent preponderance of labeled saturated esters in all tissues, great differences were seen in the relative amounts of labeled retinyl palmitate and stearate in the different tissues. In the liver, although the earliest sample (rat A, 17 min) reflected the composition of the injected chylomicrons, this composition changed markedly during the first 3 hr. In all later liver samples (3 hr and later) retinyl palmitate was 5–6 times as abundant as retinyl stearate. Retinyl palmitate was also the predominant labeled ester in the small intestine, lung, and carcass samples, although this predominance was much less striking than in liver. In contrast, a marked predominance of retinyl stearate appeared in the adre-

TABLE 2 PERCENTAGE OF RECOVERED C^{14} PRESENT AS RETINYL-C^{14} ESTER AND AS RETINOL-C^{14} IN THE TISSUES AND CARCASS OF EACH RAT

Rat	Time of Sacrifice	Plasma		Liver		Kidneys		Adrenals		Lungs		Carcass*	
		R.† Ester	Retinol	R. Ester	Retinol	R. Ester	Retinol	R. Ester	Retinol	R. Ester	Retinol	R. Ester	Retinol
	hr	%		%		%		%		%		%	
A	0.28	91	4	85	8	76	15	87	5	75	17	61	26
B	1.00	24	72	82	13	19	70	83	11	41	50	52	33
C	3.00	4	93	82	12	35	58	79	12	73	19	55	29
D	7.75	1	96	87	7	57	38	—	—	76	17	54	28
E	23.5	5	87	85	11	75	19	87	5	86	6	61	20
F	72	2	94	87	7	83	10	—	—	85	10	52	27
G	143	3	86	90	6	64	28	92	3	87	8	63	19
K‡	26.5	5	84	85	6	80	11	87	7	69	17	64	23

In the chylomicrons injected, 93% of the C^{14} resided in retinyl esters (fraction 2) and 4% in retinol (fraction 4).

The values listed are the percentages of recovered C^{14} found in fractions 2 and 4 after alumina column chromatography. The values listed for lungs and carcass have been corrected for the amounts of labeled retinyl ester and retinol contributed by the plasma contained within the samples. This correction was small in every case. The plasma content of the liver, kidney, and adrenal samples contributed less than 1.3% of the C^{14} in each of these samples.

* The carcass samples represent almost all the depot fat and skeletal muscle, as well as the "Rest of Carcass" (see Table 1).

† R. = retinyl.

‡ In the small intestine of this rat the values were: 77% retinyl ester and 12% retinol.

nals, which contained after 24 hr up to 5 times as much labeled retinyl stearate as palmitate. Approximately equal amounts of labeled retinyl palmitate and stearate were observed in the kidneys.

The metabolic fate of the radioactivity not recovered as lipid-soluble C^{14} in the tissue extracts was investigated by examining the urine, feces, and expired CO_2 of one rat (rat E), and the bile of two bile fistula rats (rats L and M, whose tissues were not studied). Expired CO_2, collected for 23.5 hr after chylomicron injection, contained 3.67% of the injected C^{14}; most (51%) of this $C^{14}O_2$ was excreted during the first 8.25 hr. Urine excreted during the same period (23.5 hr) contained 3.46% of the injected C^{14}. Radioactivity in urine was almost entirely present in water-soluble compounds, since less than 1.5% of the urinary C^{14} could be extracted with ethanol–acetone. Very little radioactivity (0.21% of the injected C^{14}) was recovered in an ethanol–acetone extract of feces. Chromatography of this fecal radioactivity on an alumina column showed that less than 10% of it was eluted in fractions 1–6, most being retained on the column.

Significant amounts of C^{14} were recovered in the bile of rats L and M. The bile collected for 24 hr from these two animals contained 8.92 and 8.56% of the injected C^{14}. Approximately 70% of the biliary excretion of radioactivity occurred during the first 7 hr. Extraction of portions of bile with $CHCl_3$–CH_3OH, followed by the splitting of the extract into two phases, resulted in the recovery of less than 10% of the radioactivity in the $CHCl_3$ (lipid) phase. Portions of bile and of the residue from the CH_3OH phase were subjected to acid and enzymatic hydrolysis. After acid hydrolysis, or after incubation with β-glucuronidase, 40–50% of the biliary radioactivity became lipid-soluble (i.e., was found in the $CHCl_3$ phase of a $CHCl_3$–CH_3OH extract). Only small amounts of C^{14} were rendered lipid-soluble by incubation with

TABLE 3 Composition of Labeled Retinyl Esters in Rat Tissue Extracts

		% Distribution of Labeled Retinyl Esters				% Saturated Esters	Ratio 16:0/18:0
		16:0	18:0	18:1	18:2		
Chylomicron Preparation No. 1		43	22	22	13	66	1.95
Chylomicron Preparation No. 2		44	23	21	12	67	1.88
Tissue	Rat						
Plasma	A	42	25	22	11	67	1.70
Liver	A	43	24	22	10	67	1.76
Liver	B	60	16	15	8	76	3.72
Liver	C	71	14	8	6	85	4.98
Liver	D	76	13	7	5	89	5.91
Liver	E	74	15	7	5	89	5.01
Liver	F	76	14	6	3	90	5.31
Liver	G	76	12	6	6	88	6.12
Liver	K	71	13	11	5	84	5.29
Kidneys	D	40	34	15	11	74	1.17
Kidneys	E	35	40	15	10	75	0.88
Kidneys	F	37	31	19	12	68	1.19
Kidneys	G	33	37	19	11	70	0.91
Kidneys	K	31	26	26	17	57	1.21
Adrenals	A*	—	—	—	—	—	1.44
Adrenals	B*	—	—	—	—	—	0.76
Adrenals	C*	—	—	—	—	—	0.68
Adrenals	D + E†	24	51	15	9	75	0.47
Adrenals	F + G†	14	65	14	7	79	0.22
Adrenals	K	13	67	14	6	80	0.19
Lungs	C	48	31	14	8	79	1.56
Lungs	E	44	37	11	8	81	1.25
Lungs	G	41	32	19	8	73	1.28
Lungs	K	48	21	19	12	69	2.24
Small intestine	K	51	21	17	11	72	2.41
Carcass‡	A	38	29	22	11	67	1.28
Carcass	C	44	26	18	12	70	1.68
Carcass	E	32	22	24	22	54	1.54
Carcass	F	30	21	27	22	51	1.45
Carcass	G	34	23	25	18	57	1.48

* The adrenal retinyl esters of rats A, B, and C were subjected to TLC on $AgNO_3$-impregnated alumina, after which the saturated esters were eluted, and each eluate was used for reversed phase paper chromatography. These analyses hence defined the relative amounts of labeled retinyl palmitate and stearate in each sample, but not the complete retinyl ester compositions.

† Combined samples.

‡ See footnote * of Table 2.

sulfatase. Incubation with β-glucuronidase followed by chromatography of the lipid-soluble material on an alumina column resulted in the retention of more than half the radioactivity on the column, with less than 10% of it eluted in fraction 4 and less than 30% of it eluted in the combined fraction 5 + 6. Portions of the methanol extract of bile were also chromatographed on thin-layer plates of DEAE-cellulose as described by Oertel, Tornero, and Groot (17). This resulted in the separation of radioactive compounds into two bands: (*a*) a fairly narrow band with R_F 0.85, containing about one-third of the total C^{14}; and (*b*) a much broader band of R_F 0.3–0.4, containing a similar amount of C^{14}. The bands were scraped off and eluted with methanol. Incubation of the eluate from the first band with β-glucuronidase rendered 58% of the C^{14} lipid-soluble. Incubation of the eluate from the second band with β-glucuronidase or with sulfatase had very little effect.

After centrifugation of the plasma of rat G (6 days) at density 1.21, 94% of the plasma radioactivity was recovered in the "1.21 bottom" fraction, and only 6% was found in the upper lipoprotein fraction. Column chromatography indicated that virtually all of the radioactivity found in the "1.21 bottom" fraction was present in retinol, whereas approximately half of the small amount of C^{14} found in the lipoprotein fraction was present as retinyl ester and half as retinol.

DISCUSSION

The results presented here confirm and illustrate the major role of the liver in the metabolism of newly absorbed vitamin A. At least two-thirds of the retinyl esters (and retinol) in the injected chylomicrons were removed from the vascular compartment by the liver. In the liver, the composition of the labeled retinyl esters changed considerably during the first 3 hr, from a composition reflecting that of the injected chylomicrons to one with a much greater preponderance of saturated esters (85–90% of the labeled esters), and, in particular, of retinyl palmitate (70–75% of the labeled esters). The composition of labeled retinyl esters in liver did not change after 3 hr. This constancy in composition beyond 3 hr suggests that the labeled esters had equilibrated with the entire liver pool of retinyl esters, and that the final composition of the labeled esters reflected the total composition of retinyl esters in liver. A retinyl ester composition in rat liver similar to that observed here has recently been reported by Futterman and Andrews (11). The fairly rapid change in composition seen in the first 3 hr suggests that liver retinyl esters turn over rapidly, although selective metabolism and turnover of retinyl esters other than palmitate might also have occurred. There is no information available concerning the normal turnover of retinyl esters in liver. In this regard it should be noted that the ability of liver to esterify retinol is well established (11, 18, 19), and that the hydrolysis of long-chain retinyl esters by liver has recently been demonstrated (20).

Other than the liver, the kidney contributed most to the metabolism of newly absorbed vitamin A. Although the kidneys apparently played a negligible role in the initial uptake of labeled vitamin A from the circulation, they contained progressively greater amounts of labeled retinyl esters and retinol during the first 24 hr. Initially, labeled retinol accumulated in the kidneys much more rapidly than labeled retinyl esters. After 3 hr, however, the accumulation of labeled retinyl esters was more rapid, and retinyl esters predominated by 8 hr and thereafter. The initial more rapid accumulation of labeled retinol suggests that label first appeared in kidneys by the uptake of labeled retinol from the plasma, followed by esterification in situ. It is possible that these processes reflected continuing equilibration and turnover of plasma and renal pools, rather than a net uptake of vitamin A by the kidneys. The retinyl ester composition in kidneys differed markedly from that in liver and consisted of approximately equal amounts of labeled retinyl palmitate and stearate, with saturated esters comprising approximately 70% of the retinyl esters.

Beyond 8 hr, 10–15% of the labeled vitamin A in the animal was found in the total body depot fat plus skeletal muscle. Small but significant amounts of labeled vitamin A were also found in the plasma, small intestine, lungs, and adrenal glands. Labeled retinyl esters predominated in all tissues except plasma, with small amounts of labeled retinol also being present. Quantitatively significant amounts of labeled retinal or labeled retinoic acid were not observed in any tissue at any time. The composition of labeled retinyl esters in the various tissues was remarkable in showing a consistent predominance of saturated esters in all tissues. Marked differences were, however, seen in the relative amounts of labeled retinyl palmitate and stearate in the different tissues. Retinyl palmitate was more abundant than retinyl stearate in the small intestine, in the lungs, and in the depot fat plus skeletal muscle ("carcass") samples. In contrast, a striking predominance of retinyl stearate was seen in the later samples from the adrenals. After several hours, a consistent and characteristic composition of labeled retinyl esters was seen in each tissue. As already discussed, it seems likely that these compositions reflected the total composition of retinyl esters in each tissue.

Willmer and Laughland have reported the presence of large amounts of radioactivity in the adrenals after the oral administration of β-carotene-C^{14} to rats (7). After saponification, 92% of this adrenal radioactivity was shown to reside in vitamin A (7). In the present study, only small amounts of radioactivity were found in the adrenals at any time. The possibility that the findings of Willmer and Laughland resulted from their use of vitamin A-deficient rats should be explored.

The fatty acid composition of retinyl esters in rat lymph is remarkably similar to the composition of the fatty acids present in the α'-position of lymph lecithin (6). In addition, it is well known that lecithin from practically all sources shows a striking predominance of saturated fatty acids at the α'-position. The present finding of predominantly saturated retinyl esters in all tissues thus continues the analogy between the fatty acid composition of retinyl esters and that of the α'-position of lecithin. The reason for this similarity is not known, but may involve the mechanism of formation of retinyl esters in the different tissues (see reference 6).

During the first 24 hr a considerable portion of the radioactive vitamin A was metabolized and excreted. Excretion of radioactivity as expired CO_2 represented 3.7%, and excretion of radioactive water-soluble compounds in the urine 3.5%, of the injected radioactivity. The identity of the compounds in the urine is not known. The urinary excretion of slightly greater amounts of radioactivity after the oral (21) or intraperitoneal (8) administration of retinol-C^{14} to rats has been reported by others. Even larger amounts of radioactive metabolites of vitamin A were excreted in the bile. The biliary metabolites apparently consisted of a heterogeneous mixture of polar compounds, some of which were present as glucuronic acid conjugates. The latter were not simply conjugates of retinol or retinoic acid with glucuronic acid, although small amounts of these particular conjugates (together comprising no more than 25% of the total biliary radioactivity) may have been present in bile. The excretion of polar derivatives of retinol and retinoic acid in bile, after the injection of a Tween suspension of labeled retinol or retinoic acid into the portal vein or duodenal sac of a bile fistula rat, or after liver perfusion with a retinol suspension, has been reported by Zachman and Olson (19, 22). It has also been recently reported that retinoyl glucuronide is the major biliary metabolite of retinoic acid in the rat (23).

After 24 hr only 63% of the injected radioactivity was recovered in lipid-soluble compounds in the entire animal. Excretion of radioactivity in expired CO_2, urine, and bile accounted for an additional 16% of the injected radioactivity. The metabolic fate, during the first day, of approximately 20% of the labeled vitamin A was therefore not defined. It is possible that much of the radioactivity not accounted for was present in the tissues in water-soluble or protein-bound metabolites which were not recovered in the ethanol–acetone extracts. Beyond 24 hr the rate of disappearance of lipid-soluble radioactivity from the entire animal was quite slow, and by 6 days 56% of the injected radioactivity was still present in this form.

After 6 days almost all the radioactive vitamin A in plasma was present as retinol, which was found associated with a protein of density greater than 1.21. The association of plasma retinol with a transport protein of density greater than 1.21 has been reported by others (21, 24, 25). This protein differs from serum albumin in man (24) and has been reported to be an α_1-globulin in the rat (21). In the present experiments, a very small amount of labeled retinyl ester, associated with the plasma lipoproteins, was also observed. The possible physiological significance of this finding is not known.

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