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The absorption, storage, and metabolism of α-tocopherol-C^{14} in the rat and chicken

S. Krishnamurthy* and J. G. Bieri

Laboratory of Nutrition and Endocrinology,
National Institute of Arthritis and Metabolic Diseases,
National Institutes of Health,
Bethesda 14, Maryland

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SUMMARY

α-Tocopherol-C^{14} was administered orally to rats and chicks, and its distribution in the body and rate of excretion were determined at short intervals up to 24 hr and at longer intervals to 21 days. The radioactivity in all tissues was identified almost exclusively as unchanged α-tocopherol except after 21 days, when trace amounts of unknown compounds appeared in chick liver and kidney. No evidence was obtained for a significant accumulation of tocopheryl quinone or other metabolic products in either rat or chick tissues. α-Tocopherol in rat liver and intestinal mucosa cells was distributed about 50–60% in the mitochondria and 15–20% in the microsomal and supernatant fractions.

Relatively few detailed studies have been made on the metabolic fate of dietary α-tocopherol labeled with C^{14}. Johnson (1) reported briefly on the excretion by rats of injected labeled α-tocopheryl succinate, while Simon, Gross, and Milhorat (2) studied the absorption and excretion in rabbits. Sternberg and Pascoe-Dawson (3) investigated the distribution of radioactivity in tissues after administering α-tocopherol-C^{14}; however, no attempt was made to characterize the radioactivity, and the brevity of experimental details made interpretation of the data difficult. Martius and Costeli (4) found a radioactive quinone, which was not tocopheryl quinone, in liver of rabbits following the administration of α-tocopherol-C^{14}. Recently, several metabolites of α-tocopherol including α-tocopheryl quinone have been reported to be present in rat or pig tissues (5, 6, 7). With the advent of chromatographic procedures (8, 9), which permit the separation of α-tocopherol from most of the nonsaponifiable constituents in animal tissues, it was decided to reinvestigate the fate of this compound in the rat and the chicken.

METHODS

Crystalline d,α-tocopheryl-5-methyl-C^{14} succinate (specific activity 1.1 μc/mg)[1] was reduced with $LiAlH_4$ in diethyl ether (10), and the free tocopherol was subsequently purified by preparative chromatography on zinc carbonate-treated paper (8).[2] The purity of this preparation was established by column chromatography on a semimicro column of alumina and zinc carbonate (9). For administration to the experimental animals, the purified radioactive α-tocopherol was dispersed in water with 5% Tween 80 (polyoxyethylene sorbitan monooleate).

Weanling rats of the Sprague-Dawley strain were reared on a tocopherol-free diet containing 20% vitamin-free casein, 4% lard (stripped free of nonsaponifiable material by molecular distillation), 4% salt mixture, 72% sucrose, and all vitamins except E. The animals were used after three to four weeks when body weights were 120–180 g. Day-old, female chicks were fed a vitamin E-free diet containing 30% isolated soybean protein,[3] 4% stripped lard, 6% salts, 60% glucose, and all vitamins except E. In addition, 0.5 ppm selenium (as sodium selenite) was added to prevent exudates due to vitamin E and selenium deficiency. The chicks were used after four weeks. Under these conditions, the rats and chicks had very little α-tocopherol remaining in their livers (0–5 μg/g by chemical analysis [9]).

* Present address: Department of Pathology, Trivandrum Medical College, Kerala, India.

[1] Distillation Products Industries, Rochester, N. Y.

[2] One batch was found to contain about 10% of γ-tocopherol, apparently a result of incomplete methylation in the synthetic process. Only the α-tocopherol homologue was used for experimentation.

[3] Archer, Daniel-Midland isolated soy protein C-1, Cincinnati, Ohio.

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN THE TISSUES OF RATS AFTER A SINGLE ORAL DOSE OF 500 μG OF α-TOCOPHEROL-C^{14} (500,000 CPM)*

Tissue	% of Administered Dose in Whole Organ or Tissue		
	Hours After Dose		
	2.5	4.5	24†
Liver	6.40	5.40	5.53
Heart	0.06	0.06	0.37
Plasma‡	1.12	2.40	1.70
Erythrocytes	0.40	0.34	1.56
Small intestine	11.00	8.00	4.06
Kidneys	0.57	1.50	0.90
Brain	0.08	0.05	0.20
Spleen	0.11	0.14	1.16
Stomach	0.59	0.15	0.41
Lungs	0.15	0.32	0.84
Mesenteric fat (per g)§	0.12	0.31	0.88
Intestinal and stomach contents	27.0	8.12	1.05
Cecal contents and feces	6.80	28.20	39.82
Total	54.40	54.99	58.48

* Values are averages of results in two rats.

† Rats fitted with tail cups to prevent coprophagy.

‡ Total value calculated on the assumption that the rat contains 6% of its weight as blood.

§ This value expressed as percentage of dose per gram of tissue since no estimate of total mass of mesenteric fat was made.

Although partially depleted of their more labile stores of α-tocopherol, the animals were normal with respect to growth rate and appearance. By partially depleting the animals, it was hoped that the deposition of single doses of tocopherol would be enhanced compared with nondepleted animals.

The rats and chickens were fasted 24 hr and were given 0.5 ml of the α-tocopherol-C^{14} dispersion containing 500 μg (1 μg = 1,000 cpm) directly into the stomach (crop in the case of chicks) by a blunt needle and syringe. In some experiments, tail cups (11) were attached to the rats to prevent coprophagy. After the required time periods, the rats were anesthetized with light ether and killed by taking as much blood as possible from the heart with a heparinized syringe. Chickens were bled by heart puncture and subsequently killed by decapitation. Urine and feces of rats were separately collected using a metabolism cage, and the excreta of chickens were collected on polyethylene sheets. Tissues were removed immediately after death, weighed, and frozen at −20°. Intestinal and stomach contents were combined.

Isolation of the α-tocopherol from tissues and excreta prior to counting was performed by column chromatography after saponification and extraction as described previously (9). Urine samples were first hydrolyzed with 3 N HCl before extraction to free the tocopherol or possible metabolites from glucuronides (2). The feces, together with the colon and cecum and their contents, were homogenized in water in a blendor prior to saponification. Redistilled hexane was used in all extractions. The columns, containing a 1:1 mixture of alumina and zinc carbonate, were standardized with α-tocopherol, and only those eluate fractions corresponding to α-tocopherol were used for counting (α-tocopheryl quinone is retained on the column). Aliquots (10–100 μl) of the combined eluate fractions were spotted on a lens tissue in planchets (12) and spread uniformly to infinite thinness with the aid of 40 μl of 4% Tween 80 in ethanol. Radioactivity was measured in a windowless gas flow counter; a minimum of 1,000 counts was recorded except for very weak samples when 200 counts was the minimum.

Verification that the material in the column eluates that was counted was indeed α-tocopherol was made by chromatographing an aliquot of the pooled fractions on zinc carbonate-impregnated paper (8). The paper strips were sprayed with ferric chloride–bipyridyl reagent and then scanned with a radiochromatogram scanner. In all cases, only a single radioactive peak appeared corresponding to the colored zone with an R_F (0.4–0.5) identical to that of α-tocopherol.

RESULTS

The radioactivity in the nonsaponifiable extracts was accounted for almost entirely in eluate fractions 2 to 4 corresponding to α-tocopherol. In most tissue extracts, however, a small but significant amount (about 3–7%) of the radioactivity usually stayed at the origin of the paper or at the top of the chromatographic column and could be eluted with 95% ethanol. An aliquot of α-tocopherol-C^{14}, carried through the usual procedures of saponification, extraction, and chromatography, gave the same pattern as the tissue extracts, i.e., about 5% of the activity was very firmly adsorbed. It would appear, therefore, that some oxidation or degradation product of α-tocopherol always resulted from the process of saponification and extraction. Consequently, the presence of this small amount of non-α-tocopherol activity was considered to be of little significance.

The amount of radioactivity as α-tocopherol in the various tissues of rats killed 2.5, 4.5, and 24 hrs after a single oral dose of radioactive tocopherol is given in Table 1. α-Tocopherol-C^{14} appeared in all tissues after 2.5 hr, with liver and intestine having the most activity. The liver maintained a constant amount of activity from 2.5 to 24 hr, indicating that a steady state had been reached even within the first few hours.

TABLE 2. RECOVERY OF RADIOACTIVITY FROM THE RAT 24 HR AFTER A SINGLE ORAL DOSE OF 437 μG (437,000 CPM) OF α-TOCOPHEROL*

Tissue or Excreta†	Total Activity	% of Dose
	cpm	
Liver	10,500	2.4
Organs‡	5,000	1.1
Intestinal tract + contents	203,150	46.5
Carcass	110,000	25.2
Feces	57,400	13.1
Urine, ether-soluble	360	0.1
Urine, glucuronide	1,840	0.4
Respiratory CO_2	2,880	0.7
	391,130	89.5

* Rat fasted 4 hr prior to dosing, then allowed food for remaining 24-hr period.

† Liver, organs, and intestinal tract with contents were ground with anhydrous sodium sulfate and extracted with acetone for 3 hr by mechanical shaking. Carcass was autoclaved and homogenized in a blendor with water, and an aliquot was extracted with chloroform–methanol 2:1. Feces were extracted with a mixture of $CHCl_3$, MeOH, and acetic acid. Urine was extracted with diethyl ether, then hydrolyzed with HCl and reextracted with ether. Respiratory CO_2 was collected in 5 N KOH and precipitated as $BaCO_3$.

‡ Kidneys, testes, lungs, heart, thymus, and spleen.

Heart, brain, spleen, and lungs had their highest value at 24 hr, while kidney appeared to reach a maximum at 4.5 hr. Stomach appeared to lose tocopherol by 4.5 hr, then regained it by 24 hr. The plasma activity showed a maximum at 4.5 hr, which decreased only slightly by 24 hr. The erythrocytes had a much smaller amount of radioactivity for the initial 4.5 hr and reached the same concentration as in plasma in 24 hr, indicating an equilibrium between the plasma and red cell. The uptake by the brain was slow compared to other tissues, in agreement with the recognized slow passage of lipids across the blood-brain barrier. The intestine had a considerable amount at 2.5 hr, which decreased gradually in 24 hr. The activity in mesenteric fat increased markedly during the 24-hr period. The total recovery in all of these tissues, and including the unabsorbed tocopherol, was 54–59% of the dose.

An effort to account quantitatively for the total radioactivity administered was made with one rat, 24 hr after dosing (Table 2). Radioactivity extracted from the samples in this experiment was not characterized; i.e., the extracts were not chromatographed but counted directly. The intestine, its contents, and feces accounted for 60% of the dose and 25% was present in the carcass. There was more in the liver (2.4%) than in all other organs combined (1.1%).

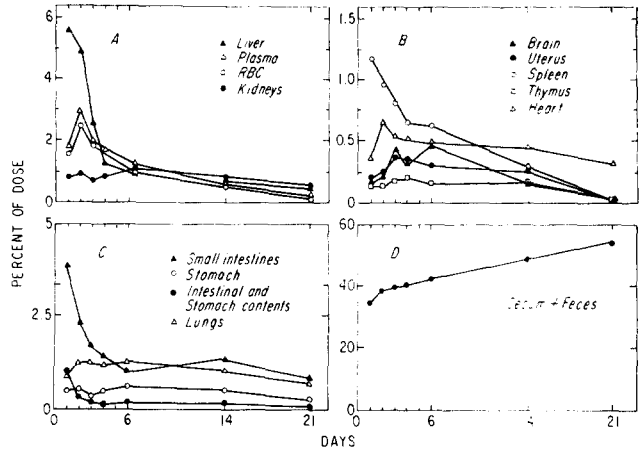

FIG. 1. Rate of depletion of α-tocopherol-C^{14} from rat tissues and the cumulative fecal excretion after a single oral dose of 500 μg. Points represent activity in the total organ or sample from two rats at each time interval.

Less than 1% was excreted in the respiratory gases and only 0.5% was in the urine, most of the latter activity being water-soluble.

In Fig. 1 are given the results of a longer time study of the rate of depletion of α-tocopherol-C^{14} after a single oral dose of 500 μg. Two animals were killed at the given time intervals and the mean value of the percentage radioactivity of the dose plotted for each tissue against time in days.

The uptake and depletion rate of α-tocopherol-C^{14} in the different tissues varied considerably. The plasma and erythrocytes followed a very similar pattern and by 21 days only negligible amounts of activity remained. In terms of absolute amounts, 3.6 μg of α-tocopherol was present in the total plasma and an almost equal amount in the erythrocytes. Although the initial uptake by liver was rapid, there was no appreciable retention. After six days, the amount of activity was only one-sixth that after one day. During the following two weeks, a slow rate of depletion occurred. The heart, kidneys, lungs, uterus, and thymus, however, maintained an almost constant amount for 14 days, then a gradual decrease followed. The curve for spleen resembled that for liver. It is interesting to note that both the small intestine and stomach retained a constant amount of tocopherol from the 14th to 21st days. The combined contents of the intestine and stomach had a small but definite amount of radioactive tocopherol at all time intervals. This activity in the intestinal tract was not due to recycling by coprophagy since the rats had tail cups. About 36–38% of the ingested dose was excreted within the first 24 hr in the feces, after which an almost constant amount of about 0.7–1% was excreted per day. The excreted radioactivity was completely accounted for as un-

TABLE 3. CONCENTRATION OF α-TOCOPHEROL-C^{14} IN RAT TISSUES AFTER AN ORAL DOSE OF 500 μg (500,000 CPM)*

Tissue	Days After Dose		
	1	14	21
	μg/g	μg/g	μg/g
Liver	7.4	0.6	0.4
Thymus	2.1	2.0	—
Stomach	3.3	3.5	2.1
Heart	3.4	4.6	3.0
Uterus	4.5	3.3	0.1
Kidneys	2.5	3.5	2.1
Lungs	4.6	5.0	4.4
Plasma (per ml)	3.2	0.7	0.1
Erythrocytes (per ml)	3.4	0.6	0.1
Small intestine	4.4	1.6	1.0

* Rats on experiment 14 and 21 days were fitted with tail cups to prevent coprophagy. Average of two rats at each period.

changed α-tocopherol, as determined by both paper and column chromatography. The radioactivity excreted in urine (not shown), which was essentially all water-soluble, was very low and reached a cumulative value of about 1% in 21 days.

Table 3 summarizes the amounts of α-tocopherol-C^{14} per gram of tissue 1, 14, and 21 days after dosage, as calculated from the radioactivity in the extracts. The concentration at the end of 21 days was highest in lungs, followed by heart, kidneys, and stomach. Liver, uterus, plasma, and red cells had very low concentrations, while the thymus had essentially none.

Intracellular Distribution of α-Tocopherol-C^{14} in the Liver and Intestinal Mucosa. An oral dose of 500 μg of α-tocopherol-C^{14} was given to two rats and after 6 hr the intestines and livers were removed. The intestinal mucosa was scraped off using a dull scalpel blade, and the mucosal cells and a piece of liver were homogenized separately in cold 0.25 M sucrose and fractionated by differential centrifugation (13). The cellular fractions and an aliquot of the whole homogenate were saponified, extracted, and counted after column chromatography. The results in Table 4 show that about 52 and 62% of the α-tocopherol is localized in the mitochondria of the liver and intestinal mucosal cells, respectively, while an appreciable portion is present in the microsomes and supernatant solution as well. Paper chromatography and radioactive scanning showed that all the fractions had only α-tocopherol.

Possible Metabolites in Liver. Two rats were each given 500,000 cpm of α-tocopherol-C^{14} by stomach tube and killed after 24 and 96 hr. One gram of liver was ground with anhydrous sodium sulfate and extracted with diethyl ether (fraction A). Another 1-g portion was homogenized in water and extracted with hexane (fraction B). To the aqueous layer was added an equal volume of 95% ethanol and the mixture reextracted with hexane (fraction C). The remaining aqueous layer was centrifuged to remove protein, and the clear supernatant solution (fraction D) was plated for counting. The radioactivity of each fraction, as well as the activity from the nonsaponifiable fraction of 1 g of liver, is shown in Table 5.

TABLE 4. INTRACELLULAR DISTRIBUTION OF α-TOCOPHEROL-C^{14} IN RAT LIVER AND INTESTINAL MUCOSA*

Fraction	Liver		Mucosa	
	cpm/g	%	cpm/g	%
Homogenate	8,800	100	20,388	100
Nuclei	571	6.5	1,800	8.8
Mitochondria	4,600	52.2	12,600	61.9
Microsomes	2,000	22.7	4,200	20.6
Supernatant solution	1,640	18.6	3,100	15.2

* 500 μg (500,00 cpm) α-tocopherol-C^{14} was given orally and the animals killed after 6 hr. Average of two rats.

Hexane alone extracted very little of the α-tocopherol from a water homogenate (fraction B), but, after denaturation of the protein with ethanol, 92–95% of the activity was fat-soluble (fraction C), indicating that the tocopherol is associated with the proteins. The aqueous layer remaining after extraction with ethanol–hexane contained about 1.5% of the total radioactivity, indicating that the amount of possible water-soluble metabolites is very small. The nonsaponifiable extract contained essentially the same amount of radioactivity as was obtained in the total lipid extraction with ether after grinding the tissue with sodium sulfate. Paper chromatography of the

TABLE 5. DISTRIBUTION OF RADIOACTIVITY FROM α-TOCOPHEROL-C^{14} IN RAT LIVER IN ETHER, HEXANE, AND WATER-SOLUBLE FRACTIONS*

Fraction	After 24 hr		After 96 hr	
	cpm/g	μg/g†	cpm/g	μg/g
A: Ether-soluble, from homogenate	7,250	—	3,960	—
B: Hexane-soluble, from homogenate	750	—	520	—
C: Hexane-soluble, after alcohol denaturation	6,830	—	3,630	—
D: Aqueous supernatant solution from C	120	—	60	—
Nonsaponifiable fraction	7,180	6.8	4,018	40

* See text for preparation of fractions. Male rats, depleted of vitamin E, were given 500,000 cpm (500 μg) of α-tocopherol-C^{14} orally and killed at intervals noted; one rat at each time.

† Determined colorimetrically.

TABLE 6. RADIOACTIVITY IN TISSUES OF CHICKS AFTER A SINGLE ORAL DOSE OF 500 μg OF α-TOCOPHEROL-C^{14} (500,000 CPM)*

Tissue	% of Administered Dose in Whole Tissue			Concentration of α-Tocopherol†		
	Day After Dose			Days After Dose		
	1	7	24	1	7	24
				μg/g		
Liver	1.90	0.52	0.10	0.70	0.40	0.05
Kidneys	0.20	0.14	0.04	0.50	0.37	0.02
Spleen	0.24	0.06	0.02	1.30	0.26	0.11
Heart	0.26	0.08	0.03	0.60	0.22	0.08
Plasma‡	4.00	1.92	0.03	1.00	0.50	0.07
Erythrocytes‡	3.20	1.20	0.03	0.86	0.34	0.08
Leg muscle	—	—	—	0.13	0.32	0.16
Abdominal fat	—	—	—	—	—	0.35
Excreta§	15.1	18.1	22.5	—	—	—

* Average values of two chicks at each period. Weights ranged from 300–400 g when dosed. Blanks indicate no estimation was made.

† Calculated from the radioactivity.

‡ Blood volume estimated as 10% of body weight.

§ Cumulative value for the entire period.

ether and hexane extracts indicated a single radioactive zone corresponding in R_F to α-tocopherol.

Chick Experiments. The distribution of radioactivity in some of the tissues of the chicken at intervals after a single oral dose of α-tocopherol-C^{14} is summarized in Table 6. Blood and liver had the greatest amount of total activity after one and seven days, but the rate of loss was remarkably similar in most tissues. Kidney appeared to have a relatively constant amount from days one to seven, while leg muscle accumulated tocopherol during this period. Of particular interest was the apparent rapid equilibrium between plasma and erythrocytes, which persisted even after one week. In terms of concentration, spleen was highest after one day followed by the blood, liver, kidneys, and heart. By seven days, the range of concentrations in all tissues was only about twofold. Very little activity remained in any tissue after 24 days; however, abdominal fat had a significant concentration.

The data for the chicks killed after one day show that the tissues analyzed accounted for only about 10% of the dose while 15% was recovered in the excreta. In view of the difficulties in collecting chick excreta quantitatively, it is probable that this latter figure is low. Paper chromatography revealed that only α-tocopherol was present in the extracts of excreta.

The radioactivity of all tissues obtained on the first and seventh days was due entirely to unchanged α-tocopherol as shown by counting the various fractions eluted from the alumina–zinc carbonate column. However, the 24th-day tissue samples (liver, kidneys, and plasmas) on column chromatography (Table 7) showed that the major fraction of radioactivity was not due to unchanged α-tocopherol, although the leg muscle and erythrocytes still contained most of their radioactivity as α-tocopherol. The activity in fraction 8 could be α-tocopheryl quinone since, with this column, the quinone is eluted with ethanol. The nature of the activity in fraction 6 is unknown.

In Vitro Incubation of α-Tocopherol-C^{14} with a Liver Extract. A 10% homogenate of liver from a vitamin E-depleted chick was prepared in 0.1 M phosphate buffer at pH 7.4 and centrifuged at 800 × *g* for 10 min. To 2 ml of the supernatant solution were added 3.25 ml of buffer, 0.5 ml of an emulsion of 80 μg of α-tocopherol-C^{14} dispersed in 5% Tween 80, and 0.25 ml of an emulsion of 500 μg ethoxyquin[4] in water as antioxidant. The flask was incubated in air at 37° for 3 hr. At 60-min intervals, 1 ml was removed, the protein precipitated with ethanol, and the mixture extracted with diethyl ether. The extracts were chromatographed on the column of alumina and zinc carbonate, and aliquots of the eluate fractions were counted. Other aliquots were chromatographed on zinc carbonate paper and subsequently scanned. More than 95% of the starting activity was recovered at each time interval, and all of the activity was accounted for as α-tocopherol. Under these conditions, the liver does not metabolize or degrade α-tocopherol.

TABLE 7. DISTRIBUTION OF RADIOACTIVITY IN CHROMATOGRAPHIC FRACTIONS OF NONSAPONIFIABLE MATTER FROM CHICK ORGANS AND TISSUES 24 DAYS AFTER A SINGLE DOSE OF 500 μG (500,000 CPM) OF α-TOCOPHEROL-C^{14}*

Eluate Fraction	Liver	Kidneys	Plasma	Erythrocytes	Leg Muscle
	cpm per g or ml				
1	1	0	0	0	6
2, 3	8	16	7	59	89
4	0	4	2	12	14
5	0	0	0	0	0
6	22	120	67	0	0
7	0	0	5	0	0
8	23	22	45	16	29
% Recovery†	108	105	111	105	98

* The column of alumina and zinc carbonate (8) was used and 1-ml fractions collected. Fractions 2, 3, and 4 represent unchanged α-tocopherol. Fractions 1–7 were eluted with 1 ml of 15% benzene in hexane, fraction 8 with 2 ml of 95% ethanol.

† Recovery calculated on the original counts of the samples.

[4] 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline ("Santoquin"), Monsanto Chemical Co., St. Louis, Missouri.

DISCUSSION

By using the oral route of administration and with a dose level approximating a reasonable dietary intake, the present experiments should reflect fairly accurately the normal distribution and fate of ingested α-tocopherol.

It is apparent from the experiments with both rats and chicks that different tissues of the body vary considerably in their rate of uptake and depletion. Although liver and blood accounted for most of the dose during the first several days, these tissues rapidly lost α-tocopherol and after three weeks the concentration was less than 1 μg/g (or milliliter). In contrast, several organs in the rat that do not build up relatively high concentrations appear to retain a constant amount for at least three weeks. Thus, α-tocopherol in lung, kidney, heart, and stomach had a slow turnover rate and significant concentrations (2–4 μg/g) persisted at 21 days. This suggests that these organs would be better criteria of the tocopherol status of an animal, from the biochemical viewpoint, than would blood or liver. The latter tissues, however, would reflect current nutritional status. Thymus and uterus, like liver, had concentrations of less than 1 μg/g after three weeks.

Several recent reports have appeared indicating the formation of various metabolites in the rat from α-tocopherol. Csallany et al. (7) have isolated tocopheryl quinone following the injection of d,α-tocopherol-C^{14}, and, in another communication (6), the same group reported a second oxidation product of α-tocopherol in rat liver. Simon et al. (2) showed that rabbits can oxidize the tocol portion of α-tocopherol to a quinone (tocopheronolactone), which is excreted in the urine as a glucuronide. We found barely detectable evidence for this compound in rats. The excretion of radioactivity in urine in our rat experiments was about 1% of the total dose in 21 days. Sternberg and Pascoe-Dawson (3) reported a considerable excretion (37%) via urine in 64 hr. However, our values are in agreement with the results of Johnson (1), who reported less than 1% excretion via urine in the first 48 hr in the rat.

Inasmuch as we found no tocopheryl quinone or the other oxidation product described by Draper and co-workers (6, 7) after *oral* administration of α-tocopherol-C^{14} in rats, it would appear that the quinone is a negligible metabolite by the normal pathway. Morton and Phillips (14) have found small amounts of tocopheryl quinone in some, but apparently not all, livers from normal rats. As pointed out above, a compound amounting to about 5% of the total activity is invariably present as an artifact in all tissue extracts carried through the procedures of saponification and extraction.

The activity due to α-tocopherol, which Csallany et al. (7) isolated from rat liver two days after injection of α-tocopherol-C^{14}, was only 25% of the total radioactivity. In contrast, we invariably found over 90% of the activity in the liver to be due to unchanged α-tocopherol after *oral* dosing, regardless of the time interval.

The distribution of α-tocopherol in rat liver cellular fractions confirms previous reports that vitamin E, as also vitamin A (15) and ubiquinone (16), is not confined to any one compartment. The amount we found in rat liver mitochondria, 52%, is about twice that in chicken liver mitochondria (14). No evidence was found for the presence in mitochondria of a quinone derived from α-tocopherol-C^{14} and suggested by Martius and Costeli (4) to be phyllobenzoquinone.

The results with chickens are in general agreement with those obtained for rats. The amounts deposited in tissues were, however, much lower, in accordance with the lower dosage as related to body weight. Twenty-four days after the administration of radioactive α-tocopherol, all the tissues were practically depleted of the compound; the only exception was abdominal fat, which still contained 0.25 μg/g. The small amount of activity (100 cpm/g) present in most tissues at this time was found by chromatography on the alumina–zinc carbonate column to contain one or two nontocopherol compounds. As described in Table 7, the consistent recovery of a marked amount of radioactivity in fraction 6 from several tissues (liver, kidney, and plasma) indicates the presence of a degradation product or a possible metabolite in these tissues. On the other hand, all the tissue nonsaponifiable fractions contained a strongly absorbing substance, elutable with ethanol (fraction 8), at a much higher percentage than was obtained with similar tissue extracts one and seven days after dosing. The chromatographic behavior of fraction 8 suggests that it could be tocopheryl quinone. Neither fraction 6 nor 8 could contain the dimer of α-tocopherol obtained by potassium ferricyanide oxidation (6, 17, 18). An authentic sample of this dimer, prepared by Dr. W. Durckheimer and L. A. Cohen in the Laboratory of Chemistry of this Institute, was found to be eluted in fractions 1 to 3. The dimer is nonreducing and has an R_F on the zinc carbonate paper considerably greater than that of α-tocopherol (0.95 vs 0.50). Thus, the dimer would not have been included in the colorimetric determination of α-tocopherol, nor would it have been overlooked in the radioactive scanning of the paper strips.

The absence of metabolites of α-tocopherol in the tissues of our rats and chicks (except for 24-day-old chicks) is at variance with the results of several investigators as noted above. Possible reasons for these discrepancies have already been indicated; namely, the intraperitoneal route may provide oxidative reactions that are either greatly diminished or absent when tocopherol is absorbed via the intestine. In addition, it is very probable that large, oral doses are partially shunted into pathways not used for smaller doses. This may be the explanation for the formation of tocopheronolactone; however, a species difference cannot be ruled out. A further variable might be the possible effect of tocopherol status; i.e., chronically deficient, depleted, or normal animals. Although there can be no question that α-tocopherol is degraded in the body, our study indicates that the molecule is relatively stable, and that, once degradative attack begins, there is not significant accumulation of intermediate products.

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