TABLE II

Standard Deviations and 95% Confidence Limits on Individual Samples

	Standard Deviation				95% Confidence Limits			
	Within labs.		Between labs.		Within labs.		Between labs.	
	Okla.	Calif.	Okla.	Calif.	Okla.	Calif.	Okla.	Calif.
Meat scraps	.14	.17	.17	.21	.40	.47	.48	.57
Yeast	.49	.51	.99	.89	1.36	1.41	2.76	2.47
44% S.B.O.M.	.15	.32	.23	.39	.43	.89	.62	1.09
Cottonseed meal	.24	.31	.36	.45	.65	.87	1.00	1.25
Mixed feed	.15	.12	.18	.21	.40	.34	.50	.57
Alfalfa meal	.48	.51	.61	.59	1.34	1.42	1.68	1.62
All samples	.32	.36	.52	.51	.87	.99	1.43	1.42

TABLE III

95% Confidence Limits Within and Between Laboratories on All Samples

	1959				1960	
	Modified Official	Okla. Screen	Buechner Funnel	Purdue Shimer	Okla. Screen	Calif. Buechner
Agreement within laboratories						
All samples	1.11	0.91	0.62	0.89	0.87	0.99
Less alfalfa	0.86	0.62	0.71	0.79	.....	.....
Less alfalfa and yeast	.....	.....	.....	.....	0.48	0.69
Agreement between laboratories						
All samples	1.58	1.41	1.27	1.27	1.43	1.42
Less alfalfa	1.28	0.93	1.08	1.26	.....	.....
Less alfalfa and yeast	.....	.....	.....	.....	0.68	0.92

tion that affects the accuracy of the crude fiber determination. The committee agreed that this apparent asbestos "blank" should be investigated and, if possible, eliminated. Rather than conduct collaborative work at this point, it was decided that investigational work by individual laboratories would be more fruitful. The following assignments were made: a) survey of asbestos used by the Liaison Committee members, by R.E. Anderson of the Archer-Daniels-Midland Company; b) survey of asbestos in crude fiber determination by F.W. Quackenbush of Purdue University; and c) effect of solvents on bound moisture in asbestos by J.P. Hughes of the Southern Utilization Research and Development Laboratory. These men have completed their assignments, and the results were reported at the October 1960 meetings of the A.O.A.C.

It is evident from the last two collaborative studies conducted by the Liaison Committee that little, if any, improvement can be expected in the precision of the Crude Fiber Method beyond what has been accomplished to date. We believe the committee has sufficient data at hand to write a method that will prevent the wide discrepancies in results which were noted by R.T. Doughtie Jr. in the A.O.C.S. Smalley Check Sample Program. We do not believe however we shall ever be able to attain a precision that will permit commodity trading on the basis of .1 or .2% crude fiber.

K.E. HOLT, chairman

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Some Effects of *y*-Radiation or Linoleate Peroxidation on *a*-Tocopherol[1]

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When *a*-tocopherol was irradiated in isooctane, the main product appeared to be a 5-exo-methylene tocopher-6-one derived by the abstraction of two hydrogen atoms from tocopherol. When tocopherol was irradiated in tributyrin, transesterification was found to be a major reaction. Results with three solvents show that the irradiation products of tocopherol are complex and dependent on the solvent.

In peroxidizing linoleic acid, *a*-tocopherol was oxidized to *a*-tocopheryl quinone, but no radical-tocopherol addition products were detected.

SOME of the most important reactions of tocopherols, the major lipid antioxidants in nature, are those with free radical intermediates of lipid peroxidation. When biological systems are subjected to ionizing radiations, there are similar reactions between free radicals and tocopherols. An important part of the damage of ionizing radiation to living organisms (7) and to food products (17), especially meats (6), is *via* free radical lipids. Besides its importance in protecting against radiation damage, tocopherol is the most labile of the fat-soluble vitamins (12).

There is little information available on these reactions between tocopherol and free radicals. One of the most significant studies is that of Inglett and Mattill (9,10). They reported on the products which they isolated after reaction of *a*-tocopherol (Ia in Figure 1) and 2,2,5,7,8-pentamethyl-6 hydroxychroman (Ib) with the relatively stable benzoyloxy and *t*-butoxy radicals. Most of these could be explained as addition products of the chromanoxy free radical (II) or the rearranged radical (III). They postulated that tocopherylquinone arose in their system through hydrolysis of the benzoyloxyl adduct of II,2,a. One product which Inglett tentatively charac-

[1] Supported in part by the Quartermaster Food and Container Institute for the Armed Forces.

[2] Abstracted from the Ph.D. thesis of F. W. Knapp, University of California, September, 1960. Present address: Department of Food Technology and Nutrition, University of Florida, Gainesville.

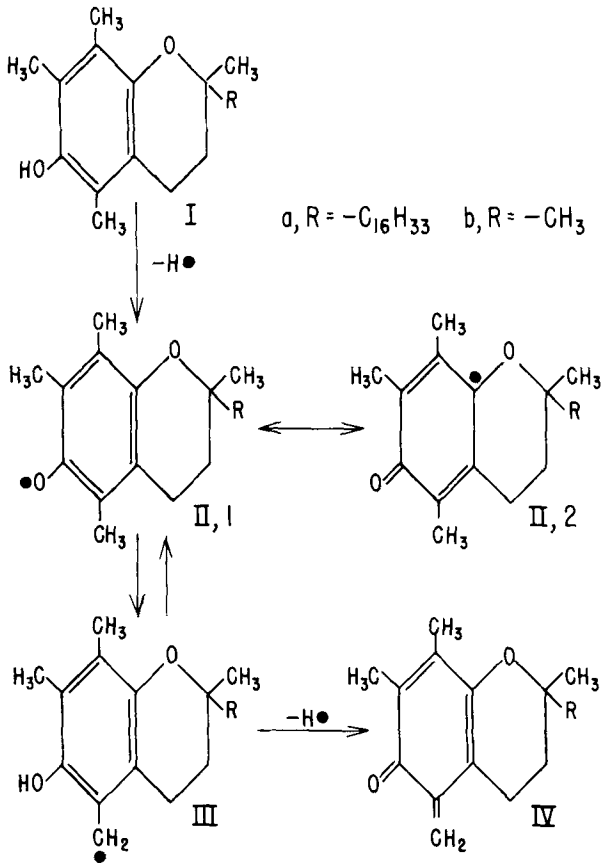

FIG. 1. Oxidation products of α-tocopherol.

terized as IVa, could have been formed by the abstraction of a second H atom from the rearranged radical IIIa.

One of the aims of our investigation was to determine if the action of radiation and peroxidation-induced radicals on α-tocopherol led only to dehydrogenation (and/or oxidation) or whether stable radical-tocopherol addition compounds were also formed. Another aim of this research was to study the products of tocopherol irradiated anaerobically in media of increasing complexity (isooctane, tributyrin, lard) or reacted with peroxidizing linoleic acid. A comparison of these products might yield a generalized picture for the reaction of tocopherol with relatively short-lived free radicals.

Experimental

Irradiation of Tocopherol. Solvents were deoxygenated by bubbling N_2 gas for one-half hour, α-tocopherol was added, and the solutions were poured, with bubbling N_2, into 160-ml. screw-cap glass bottles. When full, the bottles were quickly capped. The bottles were protected by being sealed in No. 2 cans. Some solutions of tocopherol in isooctane or tributyrin were filled directly into cans, which were gassed with N_2 and sealed. The solutions were held at −20°C. while at this laboratory and were kept cold by solid CO_2 during shipment by air to and from the irradiation site, the Materials Testing Reactor, Arco, Idaho. *Gamma* irradiation, at an average energy level of 1 mev. and at rates of $2–6 \times 10^6$ rad per hour, was carried out at 21°C. Dosimetry was accurate to ±10% at can centers.

Before adding tocopherol to tributyrin, the solvent was extracted with water to remove free glycerol and butyric acid. The water was then removed by heating to 125°C. until bubble formation ceased.

The lard (prime steam lard, stripped of the most volatile 10%) was melted by heating to 40°C. The solutions of 9.4×10^{-3}M α-tocopherol were prepared and treated as described except that a melting period was allowed previous to irradiation.

Separation and Analysis of Irradiation Products. For isooctane the solvent was removed from the irradiation products by a rotating evaporator at 70–80 mm. of pressure and 85°C. Final concentration was carried out under vacuum with a fine stream of N_2. Subsequent storage was under N_2 at −20°C. The irradiation products were fractionated by column chromatography on anhydrous $MgSO_4$ powder. Petroleum ether (BP 30–60°C.) was used as eluant, with 5–20% $CHCl_3$ and suction as needed. Column development was followed by fluorescence under ultraviolet (365 mμ). Fractions were collected according to this fluorescence, and the eluates were concentrated by evaporation.

For tributyrin, saponification was carried out in ethanolic KOH (3), followed by extractions with petroleum ether (twice with 10–20 ml. per original ml. of tributyrin). The combined extract was washed twice with 0.5% KOH and four times with water and dried with anhydrous $MgSO_4$; the solvent was removed in the usual way. Column chromatography was carried out as described above, with certain exceptions. Silicic acid was used as absorbant. The petroleum ether was purified by passing it through activated silica gel. Flow rate and column development were aided by 1–2 lbs. of pressure and 0.1 to 1% acetone. The most tightly-absorbed fraction was eluted with pure acetone. Final solvent-removal from fractions of eluate took place from tared 30-ml. beakers, making it possible to obtain the weight of each fraction.

All reaction products of all systems were subjected to paper chromatography. Paper chromatography was carried out on vaseline-treated paper (2), both ascending and descending. Solvent systems most often employed were (v/v) 75% ethanol, 95% methanol, 87% isopropanol, and methanol:isopropanol:water (82:14:4). Reducing spots were revealed by spraying with $FeCl_3$-1, 1'dipyridyl, or methanolic $AgNO_3$. Unsaturation was detected by dipping in $KMnO_4$ (13). Standards of known concentration were run on all papers for comparative purposes.

Ultraviolet spectra were recorded with the aid of Beckman DU and DK-2 spectrophotometers. The spectrum of each unsaponified solution was recorded, using the corresponding solvent, irradiated and diluted the same as the solutions for reference. Infrared spectra were recorded by means of a Beckman IR-5 infrared spectrophotometer with CCl_4 solvent and NaCl cells of 0.1-mm. spacing. Tocopherol determinations were carried out by the $FeCl_3$-1, 1'-dipyridine method (4), with reaction time shortened to 1.5 min. One to two ml. of acetone were included in each tube when needed to prevent phasic separation.

Reaction of Tocopherol in Peroxidizing Linoleate. This reaction was carried out in an atmosphere of O_2, using 2.5% tocopherol in linoleic acid with an equal volume of aqueous 1×10^{-4}M hemin. This mixture was held at 37°C. and shaken at 180 cycles per minute.

Oxygen uptake was measured manometrically. Reactions were run for periods of 4 to 23 hrs. At the end of the reaction period the material was transferred, with ethanol, to a separatory funnel, made alkaline, and extracted three times with petroleum ether and then with diethyl ether. The combined petroleum ether extracts and the ether extract were each washed, concentrated, and chromatographed as described for the unsaponifiables of tocopherol in tributyrin. According to the similarity of their ultraviolet spectra, neighboring fractions were combined.

When the experiment was conducted, using linoleic acid-1-C^{14}, the activity of the latter was 3.17×10^5 cpm per g. The groups of fractions separated on silicic acid were taken up in toluene with scintillation phosphors ("PPO" and "popo") and counted on a Packard Tricarb scintillation counter. The intensely yellow quinone fraction was bleached with ultraviolet illumination before being taken up in toluene.

Results and Discussion

Irradiation. Tocopherol in Isooctane. The α-tocopherol remaining after 40×10^6 rad was slightly less than 5% of the original amount. As a function of increasing irradiation, solutions of tocopherol exhibited decreasing absorbance at 298 mμ, with a progressive shift of the maximum to 291–283 mμ and increasing absorbance at 340 mμ. This ultraviolet spectrum (A, Figure 2) persisted in solutions irradiated to relatively high dose levels, indicating that the substance(s) responsible has (have) considerable radiation stability. Infrared spectrum A, Figure 3, is like-

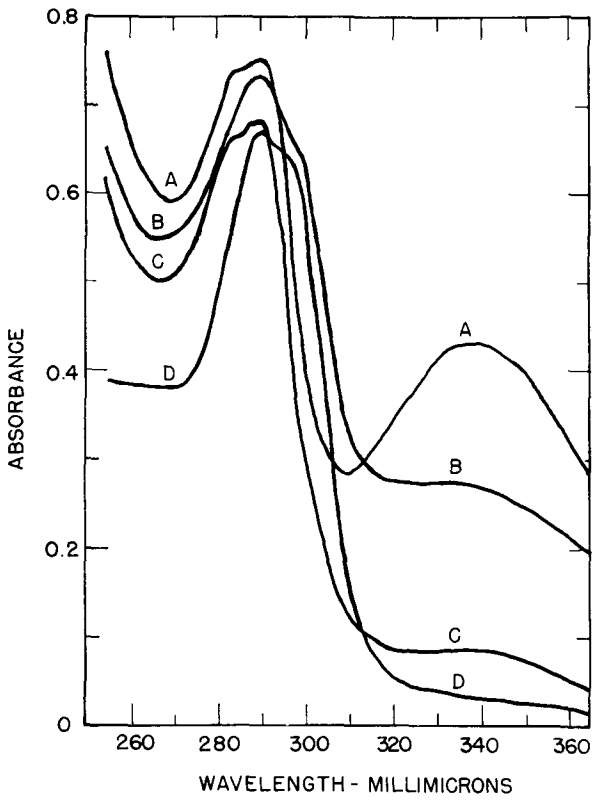

FIG. 2. Ultraviolet spectra.
A. Products of tocopherol irradiated in isooctane —N_2 at 40×10^6 rad.
B. After bromination.
C. After reduction with H_2-Pt O_2.
D. After reduction with HI.

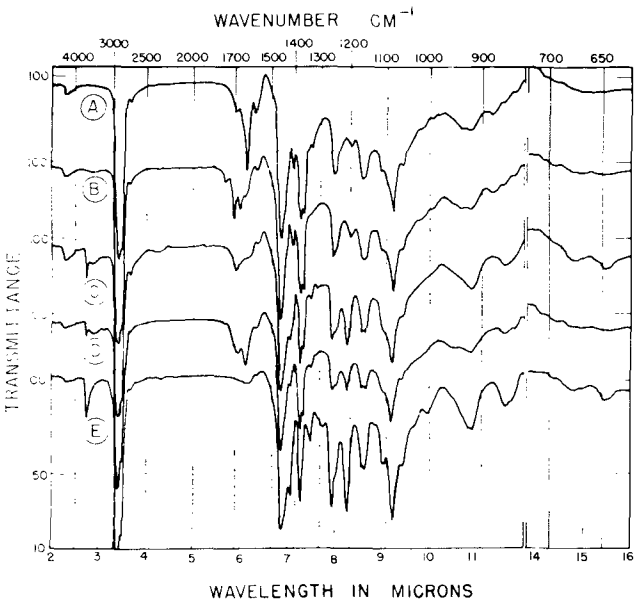

FIG. 3. Infrared spectra.
A. Unfractionated products of tocopherol irradiated as 1% solution in isooctane —N_2 at 40×10^6 rad.
B. After reduction with H_2-Pt O_2.
C. After reaction with HI.
D. After reaction with Br_2.
E. α-Tocopherol.

wise typical of tocopherol irradiated at much higher doses (75×10^6 rad).

Chromatography of these irradiation products on $MgSO_4$ revealed more than 10 bands which fluoresced under ultraviolet illumination (365 mμ). Since it must be assumed that many of the products would only absorb at wavelengths much shorter than this, it must be assumed that a large number of molecular species were present. However most of the fractions separated had their absorbance maxima at 283, 290–291, and/or 340 mμ. Apparently there were only a limited number of changes which involved the chroman nucleus but perhaps a large variety of alterations to the aliphatic side-chain. The latter might lead to considerable chromatographic inhomogeneity without changing the ultraviolet spectrum. However the most important fraction has ultraviolet and infrared spectra closely resembling those of the unfractionated irradiation products (spectra A, Figure 2 and A, Figure 3, respectively). As might be expected from a system in which there was no ready source of oxygen, most of the products of tocopherol irradiated anaerobically in this hydrocarbon were less polar than the vitamin. This was shown by both paper and column chromatography.

Examination of infrared spectrum (A, Figure 3) of these products indicates loss of the OH function (3650 and 1210 $cm.^{-1}$) as well as loss of the phenolic C-O bond at 1260 $cm.^{-1}$. The strong band at 1640 $cm.^{-1}$ indicates a highly conjugated ketone. This band is very characteristic of tocopherylquinone (cf. spectrum D, Figure 5). However the bands at 1308, 1265, and especially the strong band near 710 $cm.^{-1}$ seen in the latter spectrum are lacking here. The spectrum of the products shows absorption at 1210 and 1083 $cm.^{-1}$, indicating an intact chroman ring (9,10). The relatively high degree of carbonyl conjugation is also indicated by the ultraviolet absorbance at 340 mμ (A, Figure 2). Although tocopherylquinone is readily re-

duced by Zn-acetic acid or $Na_2S_2O_4$, stirring this material with these reagents was ineffective. The above evidence is consistent with the presence of compound IVa and is further supported by the following.

Hydrogenation at room temperature and atmospheric pressure with PtO_2 catalyst resulted in the spectral changes shown in Figure 2C and Figure 3B. From these it is evident that the amount of carbonyl conjugation is strongly reduced. The carbonyl region of the infrared spectrum shows bands at 1710 (saturated ketone) and 1665 cm.$^{-1}$ (α,β-, or α,β-γ,δ-unsaturated ketone). They could well be in 6-membered rings and would be the respective products of complete and partial hydrogenation of IV. The ketonic oxygen should not be reduced under these conditions (14), and indeed the product shows no OH absorption. The failure of the cyclohexanone to react as it should with 2,4-dinitrophenylhydrazine and semicarbazine must be ascribed to steric hindrance of carbon atom 6 as well as the inductive effect of the adjacent methyl groups.

Refluxing for 5 hrs. with 47% HI brought about the spectral changes shown in Figures 2D and 3C. The conjugated-carbonyl band at 1640 cm.$^{-1}$ disappeared, and the whole infrared spectrum came closely to resemble that of α-tocopherol in many particulars, including the hydroxyl absorption at 3650 and 1210 cm.$^{-1}$ and the phenolic C-O band at 1260 cm.$^{-1}$. As seen in Figure 2D, a shoulder appeared at 298 mμ, the wavelength of maximum absorbance of α-tocopherol. The result of 1,4 addition to the conjugated carbonyl system of IV would give 5-iodomethyl-7,8-dimethyltocol.

Although these irradiation products would not react with Br_2 in CCl_4 at room temperature, refluxing of this mixture for 1 hr. brought about reaction. The spectra of the resulting products are shown in Figure 2B and Figure 3D. That the reaction was not complete may be inferred from the persistence of absorbance at 340 mμ and 1640 cm.$^{-1}$. The location of the cyclohexanone carbonyl band at 1680 cm.$^{-1}$ is close to that (1685 cm.$^{-1}$) expected of an α,α'-dibromocyclohexanone. In other particulars, similar to those seen after reaction with HI, these spectra show evidence for the appearance of 5-methyl-substituted α-tocopherol. The HBr required for hydrobromination probably arose upon substitution of Br_2 into some other species among the irradiation products.

The presence of intramolecularly associated OH absorption at 3530-3510 cm.$^{-1}$ after reaction with both HI and Br_2 tends to substantiate the conversion of compound IV (Figure 1) to 5-halomethyl-7,8-dimethyltocol. Br on the 5-methyl group would be expected to bond more strongly to the hydroxyl H atom than would iodine in the same position. That such is the case may be seen by comparing spectra C and D, Figure 3.

These reactions with H_2, HI, and Br_2 lend support for the presence of compound IV as a major irradiation product of tocopherol in isooctane. Since a compound of this type would be expected to be quite reactive, its stability must be ascribed to steric hindrance by the methyl groups and the fused ring. It must be assumed that the remaining ultraviolet peak at 290 mμ and the carbonyl bands near 1700 cm.$^{-1}$ are related to other molecular species of unknown character and quantitative importance.

α-Tocopherol in Tributyrin. Table I gives the retentions of tocopherol in tributyrin after graduated doses of radiation. In most cases tocopherol in tributyrin is only slightly more stable to γ-radiation than in isooctane (12). As can be seen, the amount of

TABLE I

Irradiation of α-Tocopherol in Tributyrin $-N_2$

Dose 10^6 rad	mg. Tocopherol per ml. of tributyrin			$\frac{(2)-(1)}{C_0}$
	C_0	(1) Before saponification	(2) After saponification	
0	3.43	3.43	3.41	.....
1	3.43	2.62	2.75	0.04
5	3.43	1.00	1.33	0.10
10	3.43	0.11	0.52	0.12
10	4.36	1.13	1.10	0.13
15	4.31	0.035	0.58	0.12
15	4.36	0.84	1.29	0.10
20	4.31	0	0.37	0.09
25	3.43	0	0.32	0.09

reactive tocopherol increased as a result of saponification. Since this observation was somewhat surprising, possible sources of error were carefully scrutinized. Paper chromatography of the unsaponifiables showed that α-tocopherol was present in approximately the amounts calculated from direct assay. The absence of any other reducing spot tended to rule out the presence of pyrogallol (left from the saponification step). Saponification with N_2 instead of pyrogallol also gave a large increase in reducing ability. Irradiated tributyrin solutions of α-tocopherol added to unirradiated tocopherol depressed the amount and/or the rate of color development by the $FeCl_3$-1, 1'-dipyridyl reagent. But extrapolation to zero time and to zero amount of irradiated solution indicated that the error so introduced was quite small in proportion to the total quantity of tocopherol present.

Consequently it must be concluded that 10–12% of the original tocopherol was protected from irradiation damage by an alkali-labile group. Esterification would seem the most likely possibility. Keller and Weiss (11) ascribed the formation of cholesteryl acetate during irradiation of cholesterol in acetic acid to the action of acetyl radicals and ions. However, in view of the exceedingly short life of acyl radicals (1), a more likely process here would be radiation-catalyzed transesterification between the tributyryl ion and tocopherol. Mechanistically this might be quite similar to acid-catalyzed transesterification.

The ultraviolet spectrum of these tocopherol products resembled that from irradiation in isooctane (Figure 2A) in the 283–290 mμ region. A low broad maximum was also present in the 340 mμ region but disappeared upon irradiation at doses above 5×10^{-6} rad. A strong, sharp maximum at 240 mμ persisted to the higher radiation doses but disappeared during saponification. On the basis of their infrared spectra the products of tocopherol irradiated in tributyrin and chromatographed on silicic acid could be divided into five main groups. The first-eluted material had an infrared spectrum indicative of hydrocarbons only. Residual tocopherol constituted a high proportion of the next more polar group of products. This also had traces of ester carbonyl absorbance at 1746 cm.$^{-1}$ (tributyrin) and 1759 cm.$^{-1}$ (probably tocopheryl butyrate; tocopherol acetate abosrbs 1756 cm.$^{-1}$). The other fractions exhibited a variety of bands indicative of associated hydroxyl absorbance, carbonyl groups, and ethylenic conjugation. Persistence of absorbance

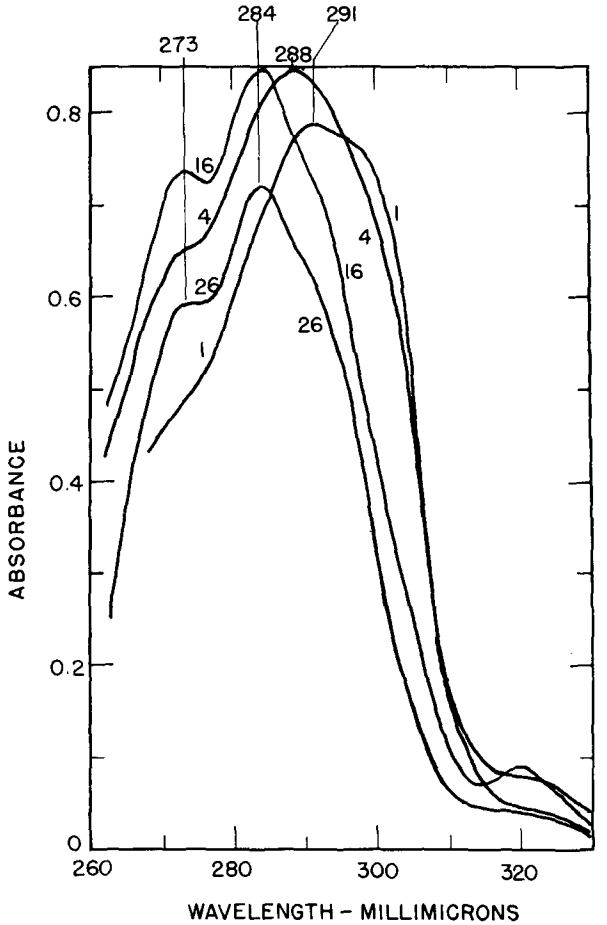

FIG. 4. Ultraviolet spectra of α-tocopherol irradiated in lard under N_2 at doses indicated, in 10^6 rad.

in the 1000–1200 cm.$^{-1}$ region, similar to that seen in Figure 3A, indicated that the chroman ring remained intact in many of these products. However it was not found possible to relate the observed spectral characteristics to any likely series of reactions involving α-tocopherol. There was no spectral evidence for the presence of tocopherylquinone.

α-Tocopherol in Lard. The stability of α-tocopherol irradiated in lard has been shown to be twice as great as when tributyrin was the solvent (12). No radiation-induced esterification of tocopherol could be demonstrated in lard. These differences may be ascribed to the presence of substances in the lard which might compete for free radicals and some of which (*e.g.*, sterols) might transesterify with the lard triglycerides more readily than tocopherol could. Figure 4 shows the shift in ultraviolet absorbance maxima. The peak at 291 mμ, similar to that seen in the other solvents, was replaced by absorbance at 284 and 273 mμ. The latter peak does not appear at all in the spectra from the irradiations in isooctane and tributyrin. From these spectral and other differences it seems that there may be important differences between the products of tocopherol irradiated in lard and in the other solvents. Because of the complex composition of lard and the low yields of tocopherol products when irradiation was carried out with lard as solvent, no further investigations were attempted with this system.

Reaction of α-Tocopherol with Peroxidizing Lipid. Oxygen uptake began almost at once and continued at a steady rate for 4 hrs., at which time slightly more than 1 mole of O_2 had been taken up per mole of tocopherol. After this time the reaction rate gradually decreased. After 4 hrs. of reaction the petroleum ether extract (90–95% of the total extracts) contained 5% residual tocopherol plus additional materials equivalent to another 8–10% of tocopherol in $FeCl_3$-reducing ability. The latter material was somewhat more polar than tocopherol but less polar than the quinone fraction, as judged by both paper and column chromatography. When separated on silicic acid, it was found that 30–40% of the petroleum ether-extractable material was concentrated in this less polar fraction, which appeared as a series of brightly fluorescing bands on the silicic acid column. The infrared spectrum (Figure 5B) of this less polar material showed the lack of hydroxyl absorption. Bands at 1736 and 1685 cm.$^{-1}$ probably represented carbonyl groups. But these failed to give a positive ferric hydroxamate test for ester or to form 2,4-dinitrophenylhydrazones or sodium bisulfite addition compounds. No feature of the infrared spectrum was sufficient to relate this material to α-tocopherol. Careful rechromatography of these fractions resulted in their resolution into a large number of fluorescent bands. From the small size of all fractions eluted, it was concluded that none was large enough to warrant further investigation. While some of this material may have been derived from tocopherol, it seems that a large part must have arisen by fragmentation and/or polymerization of the oxidizing linoleic acid. The low radioactivity of this fraction, 60–80 c.p.m. per mg., could have been caused by some extraction of the carboxyl-labelled linoleic acid out of the reaction mixture.

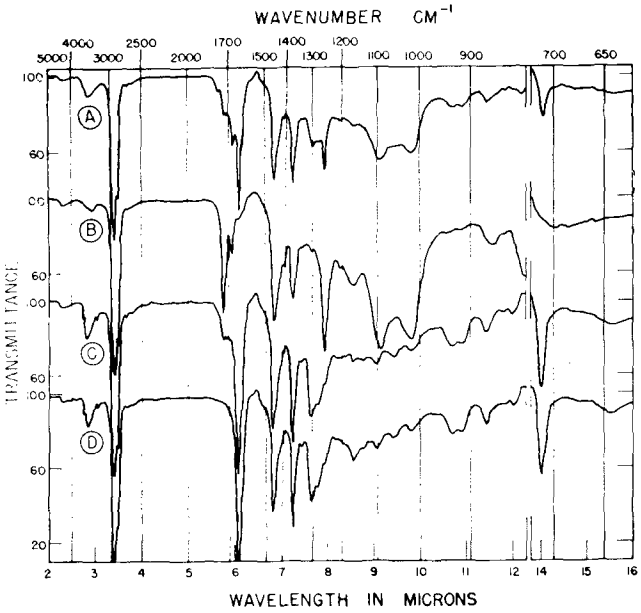

FIG. 5. Infrared spectra of products of α-tocopherol.
A. Unfractionated extract of α-tocopherol-peroxidizing linoleate reaction mixture.
B. First fluorescent bands from extract chromatographed on silicic acid.
C. Quinone band separated by chromatography.
D. α-Tocopherylquinone.

Infrared spectrum C, Figure 5, is that of the only quantitatively-important product isolated. This spec-

trum may be compared with that of authentic tocopherylquinone (spectrum D, Figure 5). The identity of this fraction with α-tocopherylquinone was further substantiated by paper chromatography, employing a variety of solvent systems as presented in Table II.

TABLE II

Chromatographic Comparison of α-Tocopherylquinone and a Tocopherol Oxidation Product

Developing system	Rf Values		
	Authentic α-tocopherylquinone	Largest spot of unfractionated extract	Largest fraction from column
65% isooctane, ascending	0.67	0.66	.....
60% isooctane, ascending, paper 1	0.61	.....	0.59
60% isooctane, ascending, paper 2	0.62	0.63	.....
75% ethanol, ascending	0.44	.....	0.46
80% ethanol, ascending, paper 1	0.75	0.75	0.75
80% ethanol, ascending, paper 2	0.66	0.66	0.69
Ethylene glycol,monoethyl ether, n-propanol, methanol, water (35:10:30:25), descending	0.1	0.12	.....
Methanol, n-butanol, water (86:6:8) descending	0.70	0.68	0.69

The quinone fraction equalled 40–45% of the petroleum ether extract and was not more than 80% pure on the basis of $E^{1\%}_{1\,cm.}$ of 435 (15). On the basis of recovery values the amount of tocopherylquinone may be calculated as representing approximately 35% of the original weight of α-tocopherol. This is only slightly higher than the values of 25–30% reported by others (5,16). The presence of α-tocopherylquinone as the only major product of tocopherol oxidation is also in agreement with these authors.

The radioactivity of the quinone fraction, 23 c.p.m. per mg., was too low to be attributed to anything other than contamination by linoleic acid. The low level of radioactivity found in all the materials isolated indicates that any radicals which may have been incorporated into the postulated tocopherol-radical addition products were only fragments (without the labelled carboxyl group) of linoleic acid. An alternative explanation for the low level of activity would be based on the suggestion by Harrison and co-workers (8) that linoleic acid-tocopherol addition products might not be extractable from alkaline solution with organic solvents.

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New Information on the Morphology of the Gossypol Pigment Gland of Cottonseed

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Exploratory electron microscope studies of the cottonseed pigment gland demonstrate a complex internal structure in which discrete particles ranging in size from one micron to less than 0.2 micron diameter are held within a membranous mesh-like network. This structure is extremely sensitive to water, and it may be the rapid swelling of the network which results in the explosive release of pigment particles from the gland on exposure to moisture. Pigment particles are spherical, of a wide range of diameters, and exhibit no birefringence in the gland or when extruded. Calculations of specific surface based on sizes of particles and density of purified gossypol (1.34) indicate surface area per gram of gossypol particles of the order of 8 square meters. Further work is indicated to determine details of the structure of the platelets, which constitute the wall of the gland, and to establish the relationship of gossypol and gossypurpurin to morphological features within the gland.

THE TOXICITY of gossypol is still the subject of research in spite of major progress in the processing of cottonseed meal and oil to remove this deleterious component. To obtain a better understanding of the mode of occurrence of the pigment within its morphological reservoirs in the seed, a brief study of the internal structure of the gland has been made with the electron microscope.

Previous work by Von Bretfeld (12), Hanausek (8), Stanford and Viehoever (11), and Boatner and coworkers (4,5,6) had shown that the pigment glands are profusely distributed in the cotyledons and hypocotyl of the cottonseed; the sizes of glands vary considerably with different varieties of cotton. The glands are highly colored, ranging from yellow through orange and red to dark purple. When exposed to water, they immediately rupture and expel a stream of minutely divided particles, exhibiting Brownian movement, and leave a nearly transparent structure in the shape of the original gland. The smaller glands are usually spherical whereas larger glands are ovoid, measuring from 100 to 400 microns on the long axis. The rigid thick gland wall consists of from 5 to 8 irregularly shaped curved platelets,

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