linoleic acid in promoting the growth of rats, but not in preventing dermatitis (*6*), also supports clonal growth in the absence of serum albumin (Table 1). The usable concentration range is again very limited, and the overall results are not quite as good as with linoleic acid. Highly purified oleic acid shows only a trace of activity. Ethyl linoleate and methyl linolenate are far less effective than the free acids.

An edible corn oil, claimed to be high in polyunsaturated fats, also supports satisfactory clonal growth of strain CHD-3 in the absence of serum albumin. Once again, the useful concentration range is very narrow. No data are available on the percentage of free fatty acids in this preparation.

Erratic clonal growth of strain CHD-3 in the absence of added albumin or fatty acids is obtained at higher concentrations of fetuin (*1*). However, at a fetuin concentration of 10 μg/ml little or no growth is obtained without added albumin or albumin-replacing fatty acids (Table 1). The growth at higher fetuin concentrations is thought to represent a small contamination of fatty acids or albumin with bound fatty acids in the fetuin preparations tested.

Neuman and Tytell have described a fatty acid requirement for growth of massive populations of mammalian cells in a serum free medium (*7*). They also found the range of useful concentrations to be narrow, and best growth occurred at concentrations somewhat higher than those which support clonal growth of strain CHD-3. This may have resulted from the lessening of toxic effects by large populations of cells (*8*), and from their use of esters, which in our experience are less effective than the free acids. They also found oleate to be active, although it was required in larger amounts than the other fatty acid esters. This could represent trace impurities of more active fatty acids in the oleate, or less efficient utilization of oleic acid, which cannot be detected in the clonal growth system because of greater sensitivity to the toxicity of oleic acid, or a difference in the fatty acid metabolism of the cell strains used.

The conclusion that linoleic acid will replace serum albumin is based entirely on 10-day clonal growth experiments with Chinese hamster strain CHD-3 cells (*9*).

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Vitamin E Oxidation with Alkaline Ferricyanide

Abstract. *Oxidation of* dl-α-*tocopherol or its model, 6-hydroxy-2,2,5,-7,8-pentamethylchroman, with alkaline ferricyanide yields a variety of products. In addition to the known dimeric keto ether, two new products, compound A, a trimer, and compound B, an isomer of compound A, were ioslated. Ultraviolet and infrared absorption spectra and paper chromatography indicate that compound A might be identical with liver metabolite "O" which results from the addition of* α-*tocopherol to the diet.*

Oxidation of *dl*-α-tocopherol (*1*) with alkaline ferricyanide has been reported to yield a yellow oil with a strong ultraviolet (UV) absorption band at 235 to 236 mμ and with a weak band at 300 mμ (*2*). The results of acid cleavage excluded structure I (see Fig. 1 for structural formulae) analogous to the product (II) of the alkaline ferricyanide oxidation of methyl substituted hydroquinone monoethers. Our studies with Stuart atomic models have shown that structure I is sterically strained while structures of type II are not.

Draper, Csallany, and Shah (*3*) reported a UV absorption maximum at 295 mμ for this ferricyanide oxidation product and postulated that it was identical to liver metabolite "O" described by Alaupovic *et al.* (*4*).

Recently Nelan and Robeson (*5*) reinvestigated this oxidation and isolated a yellow oil absorbing at 300 mμ with a weaker band at 337 mμ. Chemical studies on this oil indicated that it was a keto ether and structure III was proposed. However, no mention was made of the other three possible isomers of compound III, namely compounds V, VI, and VII.

In our studies of the alkaline ferricyanide oxidation of both *dl*-α-tocopherol and its model, 6-hydroxy-2,2,5,-7,8-pentamethylchroman, we have found by the use of silica-gel thin-layer chromatography that a variety of products are produced. Since many of these products are isomeric, some being dimers and some trimers, elementary analysis is not a criterion for establishing purity. Thin-layer chromatography has proven extremely valuable for separation of these products in pure form. In addition to the keto ether (III or IV), we have isolated two new products, compounds A and B. The ratio of these various products is somewhat dependent upon the time allowed for the oxidation to take place. After 6 minutes no tocopherol remains. The yield of yellow dimeric oil (III) is 65 to 75 percent; that of compound A, a colorless wax, 2 to 3 percent; and that of compound B, 2 to 3 percent. The remainder is composed of compound IX, trace amounts of other oxidation products, and some decomposition products.

Parallel studies with the model compound, 6-hydroxy-2,2,5,7,8-pentamethylchroman, yielded crystalline products. The time required to oxidize this chroman completely was greater than in the case of *dl*-α-tocopherol. A yellow crystalline keto ether (IV) was isolated free from compound A by silica-gel chromatography. Its melting point was 77° to 79°C, its composition $C_{28}H_{36}O_4$, its molecular weight in benzene by the vapor pressure osmometer method (Mechrolab) was 433 (calculated 436).

Nelan and Robeson (*5*) reported a melting point of 126° to 127° for this compound. We have found it difficult to separate compound IV from compound A, which is higher melting, except by repeated chromatography and fractional crystallization. The infrared- and ultraviolet-absorption characteristics of compound IV agreed with those reported by Nelan and Robeson and with those of the tocopherol analog

(III) which we obtained as an analytically pure, yellow oil. The nuclear magnetic resonance spectrum of this keto ether did not allow distinguishing between structures IV, Va, VIa, or VIIa.

Treatment of this oxidation product with maleic anhydride yielded a colorless, crystalline adduct, mp 143° to 145°C. The nuclear magnetic resonance spectrum of this adduct showed the presence of a single methyl group on an isolated double bond (τ, 8.38) indicating that the structure of the keto ether is either IV or Va since VIa or VIIa would form an adduct having no methyl group on an isolated double bond.

Very recently, Schudel, Mayer, Rüegg, and Isler (*6*) offered an unequivocal proof of the structure of yellow, crystalline keto ether by degradation studies and the use of nuclear magnetic resonance spectroscopy on the degradation product. They reported the structure for the keto ether from the model compound to be IV and III for the oxidation product of tocopherol.

In our laboratories, compound A from the model compound has been isolated as a colorless, crystalline solid; mp ≅ 230°C; composition, $C_{42}H_{54}O_6$; ultraviolet maximum in isooctane, 294 mμ; extinction coefficient, $E^{1\ \text{percent}}_{1\ \text{cm}}$, = 85.0; molecular weight in benzene by vapor pressure osmometer (Mechrolab), 659 (calculated 654). The corresponding trimer from the oxidation of *dl*-α-tocopherol was a colorless wax, $C_{87}H_{144}O_6$, ultraviolet maximum in isooctane, 294 mμ, $E^{1\ \text{percent}}_{1\ \text{cm}}$, 59.0, molecular weight in benzene by vapor pressure osmometer (Mechrolab), 1200 (calculated 1284). The infrared absorption spectra of these trimers show the absence of hydroxyl (3.00 μ) and the presence of carbonyl (5.92 μ), aryl (6.10 μ), and chroman (C-O-C) (9.15 μ). By refluxing equimolar amounts of III and *dl*-α-tocopherol or IV and the model chroman in toluene, a mixture of the trimer, compound B, and the dihydroxy dimer (IX, wax or IXa, mp 187°C) was obtained. Compound IX also could be obtained by reducing compound III with $LiAlH_4$ and compound IXa by reducing IV with ascorbic acid. Refluxing either compound III or IV alone in toluene induced no change. Upon standing, at room temperature, mixtures of compound IV and the model chroman produced some dihydroxy dimer IXa, and some trimer, compound A, which was demonstrated by silica-gel thin-layer chromatography.

Treatment of the model chroman with azobisisobutyronitrile in refluxing benzene yielded the dihydroxy dimer IXa, mp 187°C; the melting point with a sample prepared by the ascorbic-acid reduction of IV showed no depression. Oxidation of IXa with alkaline ferricyanide under the conditions used by Martius (*2*) yielded IV, mp 77° to 79°C.

Although the structures of the trimers, compound A, are not yet unequivocally known, by analogy with the trimerization of orthoquinone methides, (*7*) structures VIII or VIIIa or those where the newly formed ring has the oxygen and methylene groups reversed are proposed. The strong carbonyl band at 5.92 μ in the infrared indicates α,β-unsaturation. However, the ultraviolet absorption spectrum, infrared absorption spectrum, and paper chromatogram of VIII rather than III resemble that of metabolite "O" reported by Alaupovic *et al.* (*4*).

Compound B has ultraviolet and infrared absorption spectra that are almost identical to those of compound A. Its infrared absorption spectrum differs from that of compound A only in having a strong carbonyl band at

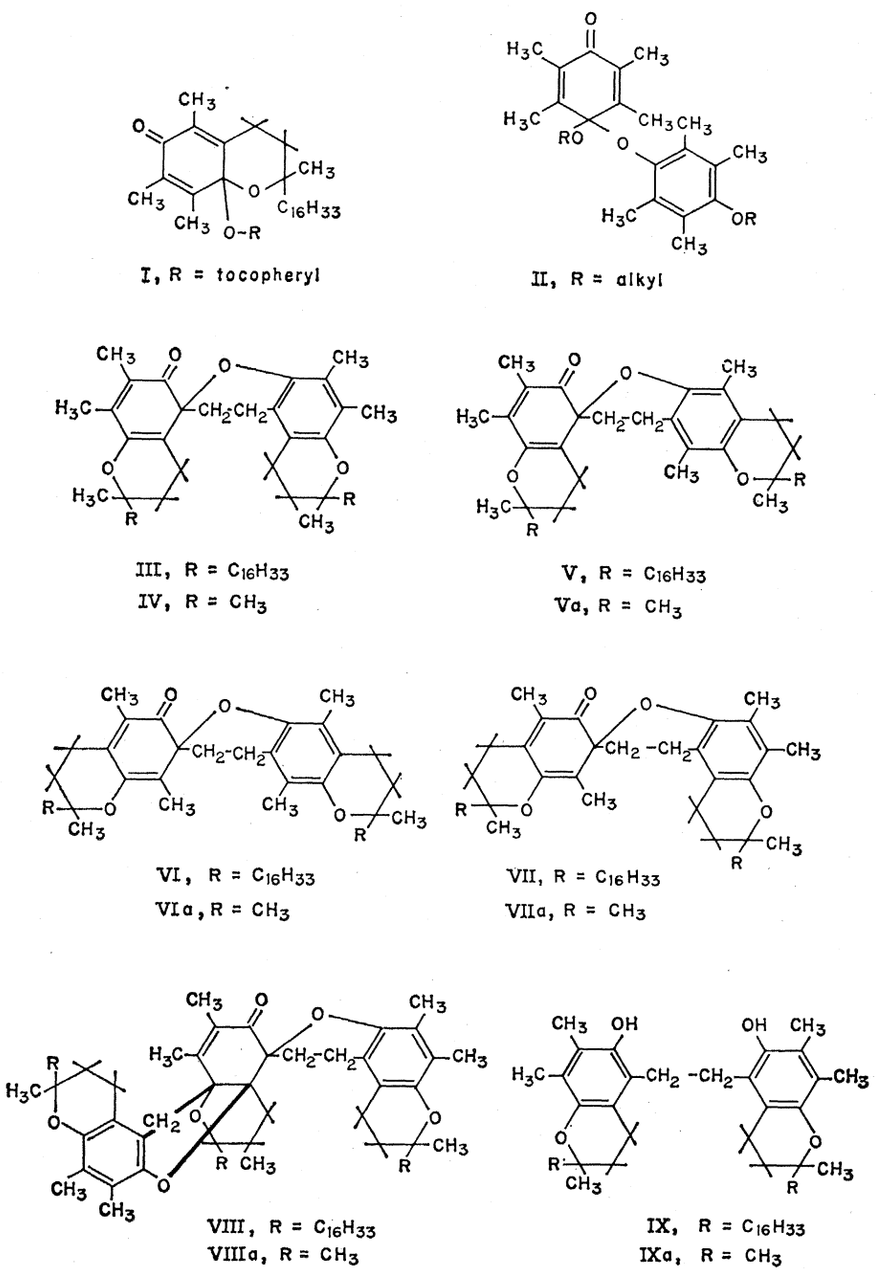

Fig. 1. Oxidation products of *dl*-α-tocopherol and 6-hydroxy-2,2,5,7,8-pentamethylchroman.

5.80 μ in addition to the band at 5.92 μ. Compound B moved more slowly on silica-gel thin-layer chromatography than did compound A when a mixture of hexane and chloroform (2:1) was used for development. In several other solvent systems on silica-gel thin-layer chromatography or on paper chromatography, compounds A and B were inseparable (*8*).

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Motor Responses of Moths to Low-Intensity X-ray Exposure

Abstract. *A brief burst of x-rays elicited flight activity in moths placed in a darkened x-ray exposure room. Wing-beat activity was recorded as an index of this behavior. Wing-beat activity could be initiated in resting moths or amplitude augmented in active moths by x-ray dose rates of 0.01 to 1.5 roentgens per second, with a latency of less than 1 second after onset of exposure.*

Prompt behavioral repsonses to ionizing radiation have been reported for a number of species. In the rat an arousal reaction from sleep was found to occur within seconds after the start of low-intensity exposure (*1*). Also, an immediate disturbance in lever-pressing behavior upon the onset of exposure has been reported when radiation was used as a conditioned stimulus (*2*). Hug (*3*) has identified some reflex-like reactions in marine invertebrates and insects which have involved tentacle retraction, shell closure, or migration from the exposure area with relatively low-intensity radiation exposure. Born (*4*) observed a closure response of the snail mantle cavity similar to the snail tentacle response described by Hug. With high-intensity beams, prompt behavioral disturbances have been reported in several organisms including daphnids (*5*), turtles (*6*), mosquitoes (*7*), and fish (*8*).

Recent experiments in this laboratory have indicated that the moth is remarkably sensitive to low-intensity radiation exposure. In the initial observations it was found that a brief burst of x-rays would elicit flight behavior in moths placed in a darkened x-ray exposure room. This behavioral response is readily accessible and can be measured by observations of wing-beat frequency. We now describe this reaction and its sensitivity to radiation.

Moths from eight species of Noctuidae and one species of Arctiidae were used in the study. The moths were collected in the San Francisco area and tested within 24 hours after capture. X-rays were generated by a General Electric Maxitron x-ray machine, operated at 250 kv (peak), 25 ma, with a beam half-value layer of 2.3 mm Cu. The exposure interval was controlled by a lead shutter operated manually from outside the x-ray room.

To record the wing beat, the moth was attached to a wire which was cemented to a ceramic crystal transducer (Electrovoice model 53-3). The signal from the transducer was amplified so that vigorous flight movement resulted in a $\pm$10-mm deflection on an oscillographic recording. To attach the moth to the motion transducer, we anesthetized the moth briefly with CO_2 and brushed the scales from the dorsal exoskeleton of its thorax. The moth was then joined to the wire of the transducer with heated Tackiwax (Cenco). The mounted preparation is displayed in Fig. 1.

In the test procedure, the animal preparation was mounted on a stand in the exposure room. The x-ray tube was turned on and the beam attenuated to a background radiation level by closing the shutter. The moth was allowed to dark adapt for 4 minutes prior to the exposure tests. Each animal was tested at least three times with

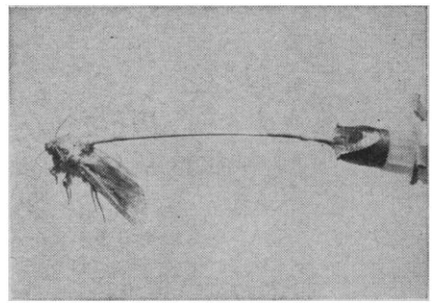

Fig. 1. Moth preparation for the study of activation of wing beat by low-intensity x-rays. The attachment of the subject to the crystal transducer can be seen.

dose rates of 0.33, 0.13, 0.10, and 0.07 r/sec. The stimulus intensity at the site of action is presumed to be related to the dose rate. For a closer determination of the minimum effective exposure intensity, additional dose rates were usually required. Differential dose rates were achieved by varying the distance from the subject to the source. The thimble chamber of a Philips dosimeter was placed adjacent to the moth to record the dose rate during exposure. The duration of the exposure varied from 1 to 15 seconds and the time between exposures varied from several seconds to 3 minutes.

To demonstrate that the moth was reacting to the x-ray beam and not to shutter manipulation or some other stimulus, several types of controls were used. (i) Each animal was given several "sham" exposures in which all conditions were the same except that the power to the x-ray tube was turned off. (ii) A tympanic nerve preparation, made according to the method of Roeder (*9*), was used to test for the presence of auditory stimulation from

Table 1. X-ray dose rates effective in eliciting prompt motor activity for each specimen tested. All species are classified in the family *Noctuidae* except the last specimen (family *Arctiidae*).

Species	Minimum effective dose rate (r/sec)
Crymodes devastator (Brace)	0.50
Protorthodes rufula (Grote)	0.37
Agrotis ypsilon (Rottemberg)	0.01
A. ypsilon (Rottemberg)	0.01
A. ypsilon (Rottemberg)	0.12
A. subterranea (Frabricuis)	0.20
Acronicta mormorota (Smith)	0.10
Catocala irene (Behr)	0.17
C. irene (Behr)	1.50
Prodenia praefica (Grote)	0.17
Mamestra configurata (Walker)	0.33
Halisidota maculata (Harris)	0.03