OXIDATIVE DECOMPOSITION OF VITAMIN A
I. STABILITY OF VITAMIN A TOWARDS OXIDATION AND IRRADIATION

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In recent years a considerable amount of attention has been centered about the stability of vitamin A towards the effects of solvents, irradiation, and oxidation. Many statements made in this connection are contradictory to the findings of some workers. Such apparent discrepancies may be reconciled, however, when the source of the vitamin in terms of natural protective substances as well as the purpose and method of the experiment is considered.

The present investigation is designed to throw further light on the stability of vitamin A towards oxidation and irradiation. The nature of the solvent and the concentration of the vitamin are taken into account in order to correlate the data on a more concrete basis than has been published hitherto.

EXPERIMENTAL

Equipment and Reagents—The polarograph, employed to determine the oxidative strength of the solvent by the ferrous-ferric ion method, was constructed according to the direction of Koltchoff and Lingane (8). The instrument was equipped with a compensating circuit so that galvanometer readings would be directly proportional to the ferric ion concentration.

A Beckman quartz prism spectrophotometer, model D360, equipped with the ultraviolet attachments, was employed to determine the concentration of vitamin A at 323 mμ. We can be certain that carotenoids other than vitamin A are absent from soup-fin shark liver oils. Unsaturated glycerides and other substances do not markedly affect the shape of the absorption curves of high potency oils (2). If low potency oils had been used in these experiments, correction factors would have been necessary to give values truly representative of the vitamin A content of the oils.

Soup-fin shark livers (100,000 to 500,000 I.U. per gm. of oil) obtained from the California Division of Fish and Game, Bureau of Commercial

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Fisheries, were frozen in dry ice at the time of catching and kept in this condition until ready for use. The livers were then ground, while frozen, in a meat chopper and homogenized in a blender under a stream of nitrogen. An excess of sodium chloride was added to salt-out the tissue. After storing at 10° for 2 days under nitrogen, the clear supernatant oils were decanted and used as such.

Crystalline vitamin A acetate (Distillation Products, Inc.) was dissolved in triacetin (Eastman Kodak Company) to give a 0.5 per cent solution by weight.

Mixed tocopherols (40 per cent) (Distillation Products, Inc.) were employed as the antioxidant.

Peroxide-free diethyl ether was prepared by shaking C.P. diethyl ether with a 20 per cent solution of sodium bisulfite, then with dilute sodium hydroxide, and finally with water until the washings were alkali-free. The ether was then tested with potassium iodide and sulfuric acid to ascertain the absence of peroxides. In certain cases the ether was purified by shaking it with 5 per cent ferrous ammonium sulfate dissolved in 3 per cent hydrochloric acid and an equal volume of concentrated hydrochloric acid, then with water until the washings were free of acid.

Decomposition under Forced Aeration—Various soup-fin shark liver oils as well as 0.5 per cent crystalline vitamin A acetate dissolved in triacetin were employed to study the effect of forced aeration at temperatures ranging between 70-100°.

About 20 ml. of oil were placed in a Pyrex test-tube provided with a two-hole stopper. A capillary whip, inserted into one of the holes, extended to the bottom of the test-tube, while the other hole was connected to the suction pump. The test-tube was then immersed in a constant temperature water bath and air was aspirated through the oil at a rate of about 2 liters per minute. Aliquots were drawn off from time to time, weighed, and diluted with isopropanol. The optical density of the resulting solutions was measured at 328 mμ.

The time required to decompose 50 per cent of the vitamin was arbitrarily taken as a measure of the stability of the vitamin in the oil under the particular conditions of the experiment. Typical decomposition curves have been presented in previous papers (1, 2). As a result of a similar study on three different oils decomposed at various temperatures, the temperature coefficient of the reaction was calculated. The average value for every 10° rise in temperature in the range studied was 2 and varies from 1.6 to 2.2. Wokes and Willimott (12), working with cod liver oil, found a temperature coefficient of 1.4 to 1.5 per 10° rise in temperature.

Aeration of soup-fin shark liver oils resulted in a long induction period followed by a rapid oxidation of vitamin A, in agreement with the findings
of Chevalier et al. on vitamin A esters (3). The time of the induction period decreased as the temperature of the aeration increased.

Stability values obtained on various soup-fin shark liver oils varied between 52 and 175 minutes. The variation in the values appears to be characteristic of the particular oil, since all samples had been kept under dry ice until used and no significant oxidation could have taken place prior to the test. This observation suggests that the oils from the livers of individual sharks contain varying amounts of antioxidants. Other investigators (5–7, 9) working with other oils have reported that the addition of mixed tocopherols to an oil increases the stability of the oil to a marked extent; this is also true in the case of soup-fin shark liver oils (2), but tocopherols do not prevent the ultimate decomposition of the oils. The addition of 0.5 per cent mixed tocopherols to an oil which had been allowed to stand at room temperature in an open bottle restored a large portion of the stability of the vitamin (Fig. 1). Curve 1 was obtained on the fresh oil, Curve 2 was obtained on the same oil after it was allowed to stand 2 months in an open bottle in the laboratory (the sample was shaken daily), and Curve 3 demonstrated the effect obtained when 0.5 gm. of mixed tocopherols was added to 100 gm. of the partially decomposed oil.

The high degree of stability of vitamin A in soup-fin shark liver oils indicated by these experiments is not to be misinterpreted. Even though the oils had been drastically treated and showed no loss of vitamin A in

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**Fig. 1.** Influence of mixed tocopherols on the rate of decomposition of a partially oxidized soup-fin shark liver oil. Curve 1 was obtained on fresh oil; Curve 2 was obtained after the oil had been allowed to stand 2 months at room temperature; Curve 3 demonstrates the regenerative effect of tocopherols on the antioxidant activity of the oil. The decomposition was initiated 1 hour after the addition of tocopherols.
the initial lag period of the decomposition, the oils had lost the greater part of their antioxidant activity and had become rancid. This has been more clearly shown in Figs. 2 to 5 of previous publications (1, 2). The results showed that, although the potency remained the same over a period of time, the stabilities of the vitamin towards aeration decreased as much as 80 per cent. Sanford and Harrison\(^1\) reported similar results on the stability of vitamin A in dogfish liver oils.

Decomposition of Dilute Solutions—A number of soup-fin shark liver oils and of vitamin A ester concentrates were dissolved in diethyl ether and in methylene chloride. One-half of the resulting solutions were placed in the dark; the other half were kept in diffused light. Aliquots were drawn at various intervals, the solvents removed \textit{in vacuo}, and isopropanol added as required. The optical density at 328 m\(\mu\) of the resulting solutions was determined in the Beckman quartz prism spectrophotometer. In all cases the percentage decomposition was greater in the presence of light and in the more dilute solutions (Fig. 2). Thus a 1 per cent solution of a soup-fin shark liver oil in methylene chloride showed only about 4 per cent decomposition in the course of 12 hours when exposed to diffused daylight, while a 0.002 per cent solution of the same oil in methylene chloride was 98 per cent decomposed in the same period.

The addition of mixed tocopherols to fresh dilute solutions (Fig. 3) markedly retarded the rate of decomposition, suggesting that the decomposition is of an oxidative nature. Similar results were obtained with

\(^1\) Sanford, F. B., and Harrison, R. W., Technological Laboratory, United States Department of the Interior, Seattle, Washington; personal communication.
diethyl ether as the solvent (1). That the decomposition is the result of oxidation is further borne out in the following experiment. Treatment of the above solvents with ferrous ammonium sulfate resulted in a rapid oxidation of ferrous to ferric ion, as determined polarographically. The solvents thus reduced were not as effective as oxidants for ferrous ion (2) and for vitamin A (Fig. 4) as were the untreated solvents. That the solvents retained a fraction of their oxidizing power was undoubtedly the result of dissolved oxygen.

**Fig. 3.** Effect of mixed tocopherols on the irradiative decomposition of a soup-fin shark liver oil dissolved in refrigeration grade methylene chloride. The time of irradiation was 4 hours for Curve 1 and 22 hours for Curve 2. The oil concentration was 0.03 per cent.

**Fig. 4.** Effect of purification of methylene chloride on the rate of decomposition of dilute vitamin A solutions. Curves 1 and 3 were obtained on solutions kept in the presence of light, while Curves 2 and 4 were obtained on solutions kept in the dark. The solvent employed for Curves 1 and 2 was untreated, while that used for Curves 3 and 4 had been previously treated with ferrous ion in the presence of dilute hydrochloric acid.

The dilute vitamin A solutions contained the equivalent of 3 to 4 \( \gamma \) of vitamin A per ml. The solubility of oxygen in the solvents is undoubtedly great enough to oxidize vitamin A to a noticeable extent in such dilute solutions.

**DISCUSSION**

Evers (4) states that vitamin A in cod liver oil is destroyed by light and oxidation and that the chief cause of the decreased potency of such oils is light. He remarks also that the addition of 0.05 per cent hydroquinone does not prevent the destructive effect of light. It is shown here that heat and light catalyze the oxidative decomposition of vitamin A
and that mixed tocopherols have good antioxidant properties. In this connection we must remember that antioxidants do not prevent the ultimate oxidative destruction of a compound, but merely retard the rate of the decomposition.

Smith et al. (10) irradiated alcoholic solutions of vitamin A with ultraviolet light of a wave-length exceeding 300 m\(\mu\). According to these authors this treatment resulted in a progressive decrease in the \(E\) value at 328 m\(\mu\). The more intense the irradiation, the more rapid was the destruction of the 328 m\(\mu\) band. When these irradiated solutions were allowed to stand in the dark, the \(E\) value at 328 m\(\mu\) increased towards the original value. The greater the "decomposition," the less complete was the recovery, which indicates, according to these authors, that vitamin A undergoes a reversible photochemical isomerism accompanied by an irreversible destruction. We did not notice any restoration of the \(E\) value at 328 m\(\mu\) in our samples subjected to this treatment. Instead we found a further though less marked decrease in the \(E\) value at 328 m\(\mu\) which we attributed to dissolved oxygen. Supplee and Odessa (11) found that irradiation of milk, either dry or liquid, did not result in the destruction of vitamin A or in the production of toxic symptoms when such milk was fed to rats.

From the above it is quite certain that in any statement regarding the stability of vitamin A the ratio of the vitamin to dissolved oxygen or peroxides and the presence and nature of antioxidants must be taken into consideration. Light and heat catalyze the oxidative decomposition of vitamin A, the former probably through the intermediate formation of an activated vitamin A molecule.

**SUMMARY**

Various soup-fin shark liver oils and vitamin A preparations have been subjected to forced aeration at various temperatures. The stability of vitamin A, defined as the time required to decompose 50 per cent of the vitamin under aeration at 100°, varies between 52 and 175 minutes for soup-fin shark liver oils. The temperature coefficient of the reaction was found to be close to 2 for every 10° rise in the temperature range from 70–100°. The length of the induction period varied inversely with the temperature of aeration.

Dilute solutions of vitamin A on standing at room temperature show a greater percentage decomposition than do the more concentrated solutions. The decomposition seems to be an oxidation that is catalyzed by light and heat.

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OXIDATIVE DECOMPOSITION OF VITAMIN A: I. STABILITY OF VITAMIN A TOWARDS OXIDATION AND IRRADIATION
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