XXV. STUDIES IN THE STABILITY OF VITAMINS A AND D

III. THE EFFECT OF LIGHT ON VITAMIN A

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It is well known that the vitamin A in an oil or concentrate gradually disappears on aeration or storage. It has also been recorded that vitamin A is destroyed by irradiation [Chevallier & Choron, 1934; Chevallier, 1935; 1938; De, 1935; Morton & Heilbron, 1928; Morton *et al.* 1931; Zilva, 1919]. On the other hand, Boyle [1934] obtained a large increase in the value of $E_{1\text{ cm.}}^{1\%}$ 328 m μ and in the Carr-Price value of a turbot-liver concentrate when this was irradiated for 20 min. Such a large increase has not been observed by other workers, though small increases in the value of $E_{1\text{ cm.}}^{1\%}$ 328 m μ have been found to occur during storage of oils and concentrates for a short time, both by ourselves and by other workers [Gillam & Morton, 1931; Griffiths *et al.* 1933; Heilbron *et al.* 1931; MacWalter, 1934; Garratt, 1938]. The increase was in every instance slight and was always followed by a steady decrease. Chevallier & Choron [1934] claim that on irradiation of vitamin A a substance A¹ is first formed which is sparingly soluble in ethyl alcohol, but readily soluble in hexane, and has an absorption band with a maximum at 312.5 m μ ; further irradiation results in the formation of β -ionone.

We have ourselves confirmed the findings of the majority of workers that a decrease in the value of $E_{1\text{ cm.}}^{1\%}$ 328 m μ occurs when solutions of oils or concentrates containing vitamin A are irradiated, but we have encountered a new phenomenon, a brief report of which has already been published [Smith *et al.* 1938]. When a solution of vitamin A was irradiated and $E_{1\text{ cm.}}^{1\%}$ 328 m μ was measured immediately, and again after standing in the dark for a few hr., it was found that the latter value was greater than the former. Furthermore, after a brief irradiation, causing a relatively small decrease in $E_{1\text{ cm.}}^{1\%}$ 328 m μ , the value obtained after standing in the dark was found to be about the same as that of the unirradiated solution. More prolonged exposure to the irradiation was followed by a smaller degree of recovery on standing in the dark. As might be expected, the effect of irradiation was most marked with intense ultraviolet light, e.g. from a mercury vapour lamp, but a decrease in the value of $E_{1\text{ cm.}}^{1\%}$ 328 m μ was also observed after several hours' exposure to daylight. In order to bring about an increase of $E_{1\text{ cm.}}^{1\%}$ 328 m μ after intense irradiation it was not necessary for the irradiated solution to be kept in complete darkness; storage in daylight resulted in some recovery. Thus exposure to daylight brought about a decrease in $E_{1\text{ cm.}}^{1\%}$ 328 m μ if the solution had previously been kept in the dark, but it resulted in an increase in $E_{1\text{ cm.}}^{1\%}$ 328 m μ if the solution had previously been exposed to intense radiation.

EXPERIMENTAL

All absorption spectra were measured by means of a Hilger E2 Medium Quartz Spectrograph F_D 60 cm. with an H237 Spekker Photometer using tungsten-steel electrodes and a high-voltage spark. A range of exposures from

Fig. 1. Absorption of glass screen.

Fig. 2. Irradiation of fish liver oil No. 11/323. Curves 1-4 in cell with screen at following distances from mercury vapour lamp: (1) 20 cm.; (2) 26 cm.; (3) 35.5 cm.; (4) 45 cm. Curve 5, in diffused daylight, in specimen tube. Curve 6, in dark, in specimen tube.

0.5 to 25 sec. was normally used for each determination; the spectra were matched visually, and in every instance the accuracy of the match-points was checked by a second observer. The solution was transferred to the cell of the

spectrophotometer, and was irradiated either by exposure to the beam of light from the spark of the instrument, or by exposure to a mercury vapour lamp, after which the cell and contents were immediately transferred to the spectrophotometer for the absorption measurement. In order to minimize the effect due to light of very short wave-length, a glass microscope slide 1 mm. in thickness and having the transmission curve shown in Fig. 1 was interposed between the spark and the photometer in the first method and between the mercury vapour lamp and the cell in the second method. Fig. 2 shows a set of results obtained by irradiating an alcoholic solution of a fish liver oil with a mercury vapour lamp placed at different distances from the solution. It also shows the effect of

Fig. 3. Curve 1, irradiation of vitamin A concentrate No. 1/584 by tungsten-steel spark. Curves 2, 3 and 4, irradiation of same solution after recovery in the dark overnight.

Fig. 4. Irradiation (—) and subsequent recovery (---) of concentrate No. S.A.I.

diffused daylight (from a north window) on the same solution. The same solution stored in the dark for over a month showed no appreciable change in the value of $E_{1\text{cm.}}^{1\%}$, 328 m μ . Fig. 3 shows a set of similar results obtained by

irradiating an alcoholic solution of a concentrate with the spectrophotometer spark; it shows also the recovery to almost the original value that occurred on standing overnight in the dark, and the further decrease in $E_{1\text{ cm.}}^{1\%}$ 328 $m\mu$ that took place on re-irradiating. The amount of the subsequent recovery was very small.

Fig. 4 illustrates the effect of irradiating an alcoholic solution of a concentrate with a mercury vapour lamp and shows that the degree of recovery diminishes with the period of irradiation. Fig. 5 A shows similar results, a point of particular interest being that the concentrate showed a preliminary increase in the value of $E_{1\text{ cm.}}^{1\%}$ 328 $m\mu$ when its solution was allowed to stand in the dark.

Fig. 5. Irradiation and recovery (A) in dark (B) in daylight of concentrate No. S.A.I.
Irradiation —; Daylight — —; Dark ---.

It is believed that previous exposure to daylight had reduced the value of $E_{1\text{ cm.}}^{1\%}$ 328 $m\mu$ and that the reversible change had taken place when the concentrate had subsequently been dissolved in alcohol and allowed to stand. It is suggested that this is also the explanation of the slight increase in the values of $E_{1\text{ cm.}}^{1\%}$ 328 $m\mu$ on storage of concentrates referred to above. Fig. 5B illustrates the slow decrease in absorption of the same concentrate on exposure to daylight and the rapid further decrease on exposure to the mercury vapour lamp, followed by slow recovery in daylight.

Fig. 6 shows the effect of irradiation, under identical conditions, on solutions of a vitamin A concentrate of the same concentration in different solvents. For ease of comparison the ordinates represent percentages of the initial values of $E_{1\text{ cm.}}^{1\%}$ 328 $m\mu$, because these values varied slightly in accordance with the solvent effect noted previously [Smith *et al.* 1938]. The reduction in the value of $E_{1\text{ cm.}}^{1\%}$ 328 $m\mu$ on irradiation is also not independent of the nature of the solvent.

The effect of daylight may be of analytical importance, because errors may be introduced by exposure of the solutions to daylight during saponification of oils and extraction of the soap solutions. Table I shows the reduction in ultra-violet absorption when vitamin A solutions were allowed to stand in the light for a few hours. With some spectrophotometers long exposure of the vitamin

solution to the light from the spark or arc occurs during measurement of the absorption spectrum, and this may also cause errors, but the effect is negligible when only short exposures are given.

Fig. 6. Irradiation by mercury vapour lamp in various solvents.
■ Cyclohexane. • Ethyl alcohol. ▲ Ether.

Table I. *Keeping properties of vitamin A in daylight*

Kept in corked flask on bench by north-facing window

No.	Type of sample	Solvent	Month	$E_1^{1\%}$ 328 $m\mu$		Hr. exposure	% initial E
				Initial	Final		
1/243	Concentrate	Cyclohexane	January	467	467	6	100
12/136	Halibut liver oil	Hexane	December	41.2	38.4	6½	93
12/168	Concentrate	Chloroform	January	405	387	7	96
S.A.I.	Concentrate	Cyclohexane	February	1030	821	47*	80
S.A.I.	Concentrate	Ether	February	1090	950	47*	87
S.A.I.	Concentrate	Chloroform	February	975	758	2	78
S.A.I.	Concentrate	Chloroform	February	960	915	2	95
					588	5	61
					85	22†	9
4/110	Concentrate	Cyclohexane	April	67.1	64.8	2	96.5
4/104	Concentrate (mammalian)	Cyclohexane	April	195	181	5½	93

* Approximately 18 hr. of daylight.

† Approximately 8 hr. of daylight.

DISCUSSION

It is impossible at present fully to explain these experiments. The most plausible hypothesis appears to be that already advanced [Smith *et al.* 1938]. It seems likely that the vitamin A in an oil or concentrate exists as a mixture of geometrical isomerides, but that on irradiation energy is absorbed, causing a change in the proportion of isomerides. It is very probable, as pointed out by Morton [1938], that *cis-trans* isomerides of vitamin A would display different intensities of absorption. On removal from the radiation slow reversion to the equilibrium mixture occurs. It is evident that there is a simultaneous irreversible photochemical change of the vitamin A into some other substance, possibly bicyclic, with little absorption at 328 $m\mu$.

SUMMARY

Exposure to ultraviolet radiations of wave-lengths exceeding $300\text{ m}\mu$ approx. causes a progressive decrease in the value of $E_{1\text{ cm.}}^{1\%}$ $328\text{ m}\mu$ for vitamin A-containing concentrates or oils. This decrease is the more rapid the more intense the radiation, but even diffused daylight has some effect. When the irradiated solutions are allowed to stand in the dark (or in light of lower intensity) the value of $E_{1\text{ cm.}}^{1\%}$ $328\text{ m}\mu$ increases towards the original value. The further the value of $E_{1\text{ cm.}}^{1\%}$ has been reduced by irradiation, the less complete is the recovery in the dark. It appears that vitamin A suffers reversible photochemical isomerization. Simultaneously slow photochemical destruction of the vitamin occurs.

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