SPECIAL ARTICLES

VITAMINS A, D AND E AND THE OXIDATION OF FATS AND OILS

THE potency of oils and fats in vitamin deficiency work is closely related to their unsaturation. Evans and Burr[1] have shown that the compound concerned in vitamin E contains double bonds; and Hess and coworkers,[2] Rosenheim and Webster[3] and Holtz[4] have shown that the compound concerned in vitamin D formation is associated with ergosterol—a triunsaturated sterol. Vitamin A is known to be similar to some degree in this respect.

Beyond the knowledge of association of unsaturation with the vitamins little is known. Nothing is known concerning the activating mechanism. The works of Mattill,[5] Hess and coworkers,[2] Anderegg and Nelson,[6] Evans and Burr[7] and others show, however, that oxidation is a serious factor in vitamin destruction. This is of natural consequence since when active oxidation begins in the oil or fat the unsaturated compounds associated with vitamin activity, like all the unsaturated compounds present, would themselves be involved.

Irradiation catalyzes autoxidation[8] and hence promotes the onset of the active oxidation period. However, with irradiation, active oxidation does not begin immediately but passes through an induction period during which there is practically no oxygen absorption.[8] One must not assume, however, that during this period no reaction occurs. Some reaction must occur to increase the oxidizing intensity to the point where the double bonds are attacked, and then active autoxidation begins.

The ease with which fats are attacked depends upon the type of unsaturated compound present as well as upon the presence of compounds containing hydroxy groups.[8] Ergosterol, a highly unsaturated compound, should respond readily to any treatment tending to alter its susceptibility to oxidation. Cholesterol, on the other hand, is very resistant to oxidation and responds to irradiation only when this treatment is prolonged at higher temperatures.[9]

The induction period is of special interest in susceptibility studies and from the author's point of view should be of interest to workers in the field of nutrition. Irradiation shortens the induction period,[8] or increases susceptibility to oxidation, and during this irradiation the vitamin D content increases. Prolonged irradiation induces active oxidation with simultaneous destruction of vitamins A, D and E.

A correlation of published data of work in nutrition and fat oxidation suggests that vitamin D and perhaps also vitamins A and E may be closely allied with changes occurring to vary susceptibilities to oxidation, or, in other words, may be allied with intermediate oxidation reactions. If slight oxidation is involved it should be possible to obtain vitamin D activity with other unsaturated compounds very similar in their chemical nature to ergosterol and stabilized by the presence of hydroxy groups, but as heretofore suggested irradiation would have to be adjusted to the stability of the compounds to oxidation.

Rosenheim and Webster[3] assert that the OH group is necessary to the mechanism concerned in vitamin D formation. It seems unnecessary to assume at present that it actually enters into combination to form the active (vitamin) compound. Unsaturated compounds containing OH groups (ricinoleic acid) oxidize slowly[8] and it is probable that the action of these groups is merely one of retarding the oxidation which destroys the "vitamin." The hydroxy groups may, therefore, be present as part of the compound concerned or as a constituent group of another compound. The latter case seems to have been shown by the experiments of Mattill,[5] who postulates also that the OH group is perhaps an important constituent of a fat in the prevention of the destruction of vitamins A and E by oxidation.

Another explanation of protection seems possible. If the constituents of an admixed oil or fat oxidize at a lower intensity level than do the compounds responsible for vitamin activity, it seems probable that oxidation may in some cases, especially those wherein water is present, proceed to some extent without involving the "vitamin" parent substance.

That *vitamins are labile states and not stable entities* seems to follow from a consideration of their properties. *Oxidation may, however, be involved in their formation.* Hart, Steenbock, Kleitzein and Scott's[10] experiments upon vitamin D may bear directly upon this point. Corn oil and the non-saponifiable fraction of cod-liver oil, neither potent when fed to goats, showed potency when mixed and fed.

Irradiation may be viewed as a promoter of oxidation and the extent to which it can be carried depends

[1] Evans, H. M., and Burr, G. O., Univ. of California Memoir No. 8 (1927).

[2] Hess, A. F., *J. Am. Med. Assn.*, 1927, LXXXIX, 337.

[3] Rosenheim, O., and Webster, T. A., *Biochem. J.*, 1927, XXI, 389.

[4] Holtz, F., *Klin. Wochenschr.*, 1927, VI, 535.

[5] Mattill, H. A., *J. Am. Med. Assn.*, 1927, LXXXIX, 1505.

[6] Anderegg, L. T., and Nelson, V. E., *Ind. and Eng. Chem.*, 1926, XVIII, 620.

[7] Evans, H. M., and Burr, G. O., *J. Am. Med. Assn.*, 1927, LXXXIX, 1587.

upon the resistance of the oil used to oxidation. Irradiation in vacuum must be carefully controlled in order to yield infallible results. Few oils are entirely free from loosely bound oxygen and even after exhaustive evacuation at low pressures they contain enough oxygen to autoxidize actively when sealed in vacuum.[8] Ethyl ether, due to the presence of peroxides, is not a safe reagent to use in work upon vitamins. Water and alcohols have a protective action to autoxidation[11, 12] and have been shown to have a protective action upon vitamins in diets.[6]

In view of vitamin destruction through oxidation the practice of administering materials to be tested admixed with easily oxidizable oils is apt to yield inconsistent and unreliable results.

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MODIFICATIONS IN CHILODON UNCINATUS PRODUCED BY ULTRA-VIOLET LIGHT

USING a Cooper-Hewitt mercury vapor quartz lamp, run on three and a half amperes, rapidly dividing and conjugating cultures of *Chilodon uncinatus* were exposed to ultra-violet light. The distance from the light to the top of the cultures was twenty-two centimeters. The cultures were exposed for two minutes at intervals of two and three days, depending upon the state of the cultures. There were eight exposures in all.

One ex-conjugant, not fully reorganized, was used to start a culture, and eight subcultures were made from this, two being used as controls, and six exposed to ultra-violet light.

In five of the cultures, no important changes were observed. In the sixth, a culture in which an epidemic of conjugation was occurring at the time of the last two exposures, many abnormalities were found, and three distinctly different types of animals.

(1) The normal *Chilodon uncinatus*. This type has four chromosomes in the diploid count, easily determined during conjugation. The controls show this same number.

(2) A larger animal having the same features as the controls, but which, when isolated and cultivated, was found to have eight chromosomes. This has been checked up through three conjugation epidemics. The form is, therefore, a tetraploid form.

(3) The third type of animal is very different from the other two, as it shows characteristics of both *C. uncinatus* and *C. cucullulus*. The macronucleus has moved from the posterior end of the animal to the middle. In appearance, this macronucleus is much more like that of *C. cucullulus* than *C. uncinatus*, the shape being elliptical, and the portion surrounding the endosome is much less granular. The micronucleus, as in *C. cucullulus*, is not in the posterior portion of the macronucleus, but on the left side near the anterior end.

One vacuole has changed position. In *C. uncinatus*, there is a vacuole on the left side near the margin at the anterior end, and one on the right side near the margin about one fourth of the distance from the posterior end. In the new form, this last vacuole has moved to the posterior end in the center.

The pharyngeal basket is shorter, and is more anterior. The average number of trichites seems to be twelve.

In general shape, the animal looks more like *C. cucullulus*, but the ciliation is more like *C. uncinatus*, the only difference being that here the usual short marginal rows are a little longer than in the original.

Though this animal has been kept alive in pure cultures since the last week of August, 1927, it has never conjugated. In cultures where the animals are fairly abundant, encystment has occurred regularly, the whole culture sometimes encysting within a few hours. The length of time elapsing before animals are recovered from the cysts is very variable. Seven days is the shortest time in which they have been observed to emerge, and three to four weeks is more usual.

A more detailed description, with figures, will appear in a later paper.

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[8] Holm, G. E., Greenbank, G. R., and Deysher, E. F., *Ind. and Eng. Chem.*, 1927, XIX, 156.

[9] Striteskey, J., *Biochem. Z.*, 1927, CLXXXVII, 388.

[10] Hart, E. B., Steenbock, H., Kleitzein, S. W., and Scott, H., *J. Biol. Chem.*, 1927, LXXI, 271.

[11] Holm, G. E., and Greenbank, G. R., Proc. World's Dairy Congress, 1923, II, 1253.

[12] Greenbank, G. R., and Holm, G. E., *Ind. and Eng. Chem.*, 1924, XVI, 598.

THE NATIONAL ACADEMY OF SCIENCES

THE National Academy of Sciences will hold its annual meeting in Washington on April 23 and 24. The following papers will be presented:

MONDAY, APRIL 23

Morning, 10:00

WILLIAM DUANE: *X-radiation from Mercury Vapor* (illustrated).

EDWIN H. HALL: *Electron Free Path and Supra-conductivity in Metals* (illustrated).

W. A. NOYES: *Reactions of Compounds having Odd Electrons; Nitric Oxide and Nitrogen Trichloride.*

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