XIX. ABSORPTION SPECTRA IN RELATION TO VITAMIN A.

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(Received November 9th, 1929.)

THE object of this paper is primarily to remove the confusion which appears to have arisen in a recent note on the absorption spectrum of vitamin A by Rosenheim and Webster [1929].

Takahashi *et al.* [1925], in work on "biosterin," recorded the fact that concentrates from the non-saponifiable fraction of liver oils exhibit selective absorption near $320\mu\mu$ which was ascribed to vitamin A. Adverse criticism having made it clear that "biosterin" was very far from being pure vitamin A, it became uncertain whether the absorption band was due to the vitamin itself or to some other substance present in the concentrate.

Morton and Heilbron [1928] undertook to test the assumption that vitamin A exhibits selective absorption in the near ultra-violet, since the substance appears to undergo photochemical decomposition in sunlight. It was found that cod-liver oils, rich in the vitamin, exhibited a broad band in the region $300\text{--}350\mu\mu$ (max. near $328\mu\mu$), and that the material responsible for the band was concentrated in the non-saponifiable fraction. It was shown further that in all cases the intensity of the band was proportional to the intensity of the blue colour obtained with antimony trichloride. When the oils were irradiated by means of any light source emitting rays near $320\mu\mu$, and when the oils were treated with oxygen, the absorption band near $328\mu\mu$ and the capacity to give a blue colour disappeared at exactly the same rate. In all, the results for spectroscopic examinations both of blue colours and ultra-violet absorption for some 40 samples of fish oils, liver oils, concentrates and animal oils were observed without disclosing any departure from the strict parallelism between ultra-violet absorption, blue colour test, and, so far as was possible, growth-promoting action.

Drummond and Morton [1929] endeavoured to test the parallelism still more stringently. Six samples of cod-liver oil were examined, (a) fresh, (b) after storage for one year in darkness, (c) after storage for one year exposed to diffuse daylight. Throughout, the parallelism between the colorimetric and spectrographic tests was exceedingly close. Exhaustive biological tests on

large numbers of rats were carried out on these oils, and considering the great difficulties in the way of a quantitative assay by animal tests, the agreement with the two physical tests was highly satisfactory. Further tests on sheep-liver fat and oil obtained from marine animals or fishes gave consistent results. More important perhaps from the present point of view was the fact that the richest concentrates of vitamin A obtained from liver fats gave a satisfactory growth response at precisely the dosage predicted from the intensity of the ultra-violet band and the blue colour. There is, therefore, a not inconsiderable body of evidence for stating that the growth-promoting factor present in liver fats exhibits selective absorption near 328μ and possesses the power of giving a blue colour (which has been defined spectroscopically) with antimony trichloride.

It will be observed that the claims have not been extended outside their proper sphere. The growth-promoting factor present in substances of vegetable origin may or may not be identical with the material in liver fats; there is no satisfactory evidence available yet. It is not claimed that if a substance promotes growth it must necessarily give the precise colour reaction of cod-liver oil and the near ultra-violet absorption band. To do so would be to make the unwarranted assumption that the vitamin A of liver fats is unique in the sense that it alone can produce a complex physiological response. It is, moreover, not claimed that if a substance absorbs near $300\text{--}350\mu$ it must be vitamin A. If such a claim were pushed to a logical, if extreme, conclusion, such diverse substances as citral, benzaldehyde and xanthophyll would have to be reckoned with.

The work on the vitamin A of liver fats rests essentially on an analytical basis. The best way to dispose of either the blue colour test or the ultra-violet absorption band as properties of the vitamin would be to concentrate the vitamin still further than has yet been done and to show that the parallelism to which reference has already been made ceases to hold.

Rosenheim and Webster [1929] consider that they have subjected the claim that vitamin A has a band near 328μ to the experimental test by preparing dehydroergosterol, and (a) confirming the absorption curve previously obtained by Windaus and Linsert [1928], (b) observing that the material does not promote growth, nor (c) give a blue colour with antimony trichloride. It is stated that dehydroergosterol possesses intense absorption exactly in the same region as that claimed for vitamin A, and "that selective ultra-violet absorption at $320\text{--}328\mu$, at any rate by itself, cannot be taken as a criterion of vitamin A."

The criticism advanced by Rosenheim and Webster means that if a substance such as dehydroergosterol exhibits selective absorption similar to that found in liver oils and concentrates, then such absorption cannot be ascribed to vitamin A. Such criticism is only valid if it be shown quantitatively that the selective absorption of liver oils in the near ultra-violet is actually due to dehydroergosterol. Otherwise, fortuitous coincidences cannot

be ruled out. Whether the resemblance is indeed fortuitous is another matter; no relevant evidence has yet been brought forward.

In choosing dehydroergosterol as the reference substance it is conceivable that Rosenheim and Webster were acting on the suspicion that, since vitamin D is an ergosterol derivative, vitamin A might also be related to ergosterol. Accepting the idea as attractive if as yet but vaguely supported, let us submit that an argument valid for vitamin D might also be valid for vitamin A. Vitamin D and ergosterol appear to absorb selectively in roughly the same region of the spectrum. If, however, the criticism of Rosenheim and Webster with regard to vitamin A has any meaning, selective absorption at $260\text{--}300\mu\mu$ is not a test for vitamin D. Admittedly such an incomplete description of a physical measurement would include many compounds. The chance of coincidence is, however, greatly reduced when the intensity of absorption, say a 0.01 % solution and a 1 mm. layer, is specified.

Now in comparing the absorption curve for dehydroergosterol and vitamin A, Rosenheim and Webster neglect the intensity factor. We have conducted a very careful re-investigation of dehydroergosterol [cf. Windaus and Linsert, 1928] and find that $\log I_0/I = 1.0$ (*ca.*) with a 2 mm. layer of 0.02 %

Fig. 1.

— Absorption curve for dehydroergosterol; concentration 0.02 %; cell thickness 2 mm.
 - - - The same selective absorption as observed without resolution (cf. Windaus and Linsert).

solution. A sheep-liver fat concentrate containing at most 10 % of vitamin A (according to estimates supplied by Prof. Drummond) absorbed to roughly the same extent with a 3 mm. layer of a 0.02 % solution. Hence if the band shown by the concentrate is due to vitamin A, the absorption of dehydroergosterol must be of a lower order. Not only is this the case, but the general shapes of the respective curves for vitamin A and dehydroergosterol are entirely different. Whilst the curve we have attributed to vitamin A has not so far been resolved¹, that of dehydroergosterol shows three distinct bands

¹ Evidence of ill-defined resolution is now being obtained using very rich concentrates. (Note added February 3rd, 1930.)

at 342, 326 and 311 $\mu\mu$ and an inflexion at about 297 $\mu\mu$ (Fig. 1). In fact the only proved resemblance is that the two curves extend between roughly the same limits. In view of this startling discrepancy it will perhaps be worth while to append evidence that our material is indeed dehydroergosterol.

The dehydroergosterol was prepared by boiling an alcoholic solution of ergosterol with mercuric acetate in alcoholic solution acidified with acetic acid, under the exact conditions described by Windaus and Linsert [1928]. After removal of mercurous acetate, the product was repeatedly crystallised from chloroform-methyl alcohol and finally obtained in large plates melting at 146°. The melting-point of dehydroergosterol is, however, in itself no trustworthy criterion of purity, as impure samples containing unchanged ergosterol melt at the same temperature as the pure product. We have consequently followed the purification throughout by means of the polarimeter. The portion used for the spectrographic examination had the following constant rotation:

$$[\alpha]_D^{19} = +149.3 \text{ (} c = 1.59 \text{ in chloroform).}$$

Windaus and Linsert [1928] give

$$[\alpha]_D^{18} = +149.2 \text{ (} c = 1.21 \text{ in chloroform).}$$

Fig. 2. Curves illustrating the disappearance of the characteristic absorption of dehydroergosterol as a result of increasing periods of exposure to ultra-violet light. Initial concentration 0.02 %; cell thickness 2 mm. throughout.

In order further to characterise our product, dehydroergosterol was acetylated by boiling with acetic anhydride (10 parts) for 30 minutes. The material obtained after recrystallisation from absolute alcohol had physical properties identical with those described by Windaus and Linsert for dehydroergosterol acetate.

Found: m.p. 146°; $[\alpha]_D^{19} = +193.0$ ($c = 1.5$ in chloroform).

Windaus and Linsert give

m.p. 146°; $[\alpha]_D^{18} = +192.5$ ($c = 2.0$ in chloroform).

It is not difficult to realise how the finer structure of the dehydroergosterol curve could escape detection by Windaus and Linsert if a photoelectric

method were used. By this method the intensity of absorption is determined for selected wave-lengths of monochromatic radiation, *e.g.* 253-6, 265, 289, 303, 313, 336 $\mu\mu$, intermediate wave-lengths being neglected. With a photographic method, in conjunction with a perfectly continuous spectrum, the detailed structure of the absorption curve becomes apparent.

The photochemical decomposition of dehydroergosterol is a matter of some interest, and in Fig. 2 the result of exposure to the full radiation from a quartz mercury lamp using quartz test-tubes is shown. Among the products of the reaction is a substance exhibiting a broad band with a maximum near 247 $\mu\mu$. In the present preliminary work a current of nitrogen was kept bubbling through a 0.08 % solution in alcohol. The photochemical changes are being further studied under different conditions.

SUMMARY.

1. The criticism of Rosenheim and Webster [1929] that the selective absorption in the ultra-violet (broad band, max. 328 $\mu\mu$) of liver oils, in itself, cannot be taken as a criterion of vitamin A, is contested on general grounds.

2. The statement that dehydroergosterol exhibits an absorption band in the ultra-violet similar to that of vitamin A of liver oils is also contested, since this substance exhibits four narrow bands. The only similarity between the ultra-violet absorption spectra of dehydroergosterol and vitamin A is that they absorb in approximately the same region. Further, the intensity of absorption of dehydroergosterol is not greater than one-sixth of that shown by the absorbing constituent of liver oils.

In conclusion we desire to express our appreciation of the support given by the Department of Scientific and Industrial Research.

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