LIV. THE ALLEGED CONTAMINATION OF CAROTENE BY VITAMIN A.

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THE claim of Euler, Euler and Hellström [1928] that carotene can restore growth in rats receiving diets deficient in vitamin A has now been supported by various workers [Moore, 1929, 1, 2; Collison, Hume, Smedley-MacLean and Smith, 1929; Kawakami and Kimm, 1929]. Negative results, however, were obtained by Dulière, Morton and Drummond [1929, 1, 2], and it was suggested by these workers that pure carotene was inactive, and that samples found to be active must have contained traces of vitamin A as impurity. Recently Hume and Smedley-MacLean [1930] have shown that the real cause of this failure did not lie in the superior purity of the carotene used, but was in all probability traceable to the use of ethyl oleate as a solvent for the test doses. Nevertheless the criticism that even the purest samples of carotene might still contain traces of the familiar vitamin A of cod-liver oil has remained incompletely answered.

In the present note this problem has been approached by a comparison of the ultra-violet absorption spectra of carotene and cod-liver oil concentrate. It is well established that cod-liver oil and its concentrates are characterised by an absorption band at $328\mu\mu$, and this band is attributed with some confidence to the presence of vitamin A [Takahashi *et al.* 1925; Morton and Heilbron, 1928; Drummond and Morton, 1929]. In an examination of carotene samples of varying degrees of purity, however, Dulière, Morton and Drummond have failed to detect this band, and have suggested that the absorption of carotene in this region may be sufficiently dense to obscure completely any maximum due to the vitamin. From the experiments to be described below a different conclusion has been reached, and it will be shown that the activity of carotene cannot be correlated with sufficiently intense absorption at $328\mu\mu$ to account for its physiological activity on the basis of the presence as an impurity of the same factor as is responsible for the absorption band of cod-liver oil.

EXPERIMENTAL.

The carotene used was prepared from carrots by Dr T. Moore, and had been purified to m.p. 178° (uncor.) by 12 recrystallisations from cyclohexane. In biological experiments the minimal daily dose necessary to promote growth in rats had been found to be about 0.002 mg. The cod-liver oil concentrate

was also supplied by Dr Moore, and had been found to promote growth regularly at 0.01 mg., with one isolated response at 0.0033 mg.

The solutions were made up in dry ether and examined in 1 cm. cells in a Hilger sector photometer used in conjunction with a Hilger E₃ quartz spectrograph, a condensed copper spark being employed as a light source. The absorption curves obtained are shown in Fig. 1.

Fig. 1. Full curve: 0.005 % cod-liver oil concentrate.
Broken curve: 0.006 % carotene.

DISCUSSION.

From Fig. 1 it will be seen that the absorption of carotene at 328 μμ was considerably less than the absorption of the cod-liver oil concentrate dissolved at the same concentration. Now since the carotene, weight for weight, had been found to be rather more active than the concentrate it would have been expected, if the vitamin A factor of the pigment were present in the same form as in cod-liver oil, that the absorption at 328 μμ would at least be equal in intensity, even in the improbable event of pure carotene being perfectly transparent in this region. Indeed if the carotene were much less active than was found, a maximum should still be apparent from the intensity of the band shown by the concentrate.

It is, therefore, obvious that the biological activity of carotene cannot be explained by the presence of vitamin A as characterised by a band of 328 μμ. Unless it be assumed that the close association between this band and the

vitamin A of cod-liver oil is purely fortuitous it seems necessary to assume that vitamin A can exist in at least two forms, only one of which absorbs strongly at $328\mu\mu$ or that vitamin A can be synthesised *in vivo* from carotene, as has been suggested by Moore [1929, 3], or from some impurity still associated with the carotene.

SUMMARY.

1. The intense vitamin A activity of carotene is not accompanied by the absorption band at $328\mu\mu$ which is considered characteristic of the vitamin A of cod-liver oils.

2. The absorption of carotene (M.P. 178°) in this region is insufficiently intense to obscure the absorption at $328\mu\mu$ which would be entailed by the presence of the cod-liver oil factor in an amount sufficient to account for the physiological activity of the pigment.

3. Unless the close relation between the vitamin A activity of cod-liver oil and the $328\mu\mu$ band is deceptive it is essential to assume that vitamin A can exist in at least two forms, or that carotene behaves as provitamin A.

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