XCVII. VITAMIN A OF BUTTER.

By RICHARD ALAN MORTON AND ISIDOR MORRIS HEILBRON.

From the Department of Organic Chemistry, the University of Liverpool.

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It is now clear that the physiological action of the vitamin A of liver fats can be reproduced, or at any rate closely simulated, by the plant pigment carotene [Euler, Euler and Hellström, 1928; Euler, Euler and Karrer, 1929; Moore, 1929, 1, 2; 1930; Collison, Hume, Smedley-MacLean and Smith, 1929], and it therefore becomes important to ascertain

- (a) whether butter contains either or both of these substances;
- (b) the relative importance of these substances, if both are present;
- (c) whether the quantities of either or both vary with seasonal, dietary or other conditions.

In the present paper an account is given of a preliminary survey of this question wherein we have applied spectrographic methods as a means of arriving at a decision concerning the above points.

Before dealing with the absorption spectra of the butter extracts themselves it is necessary to recall that both carotene and the vitamin A of liver fats can be recognised in extracts of non-saponifiable matter by their absorption spectra and also by the spectral location of the principal absorption band in the blue solutions obtained by the well-known antimony trichloride reaction.

Absorption spectra.

A solution of carotene in chloroform shows maxima at 492, 462, 436, 348 and 279 $\mu\mu$, whilst in alcohol the visible bands appear at 480, 451 and 427 $\mu\mu$. Whereas the ultra-violet absorption of carotene is weak relatively to the visible absorption [Dulière, Morton and Drummond, 1929], liver oils and non-saponifiable extracts on the other hand show negligible absorption in the visible, but in the ultra-violet a strong band with a maximum near 328 $\mu\mu$, the intensity of absorption at the head of the band affording a trustworthy measure of the vitamin A potency [Morton and Heilbron, 1928, 1930].

The non-saponifiable matter from a sample of New Zealand butter exhibited clear maxima at 492, 462 and 432 $\mu\mu$ when the solvent was chloroform and at 480, 452 and 427 $\mu\mu$ when alcohol was used. Comparison of the intensity of absorption for pure carotene and for the butter extracts indicated that the latter contained about 1 part in 200 of the pigment. The butter extract is, however, sharply differentiated from the absorption spectrum of carotene

itself by the fact that it exhibits rapidly increasing intensity of absorption on the ultra-violet side of $380\mu\mu$ (Fig. 1). The absorption curve shows a marked

Fig. 1.

inflection at $310\text{--}340\mu\mu$, and there can be little doubt that the substance responsible for this is identical with the vitamin A of liver oils. In addition to this, however, another substance which shows a clear maximum in the neighbourhood of $272\text{--}275\mu\mu$ (Fig. 3) is also present in the butter extract. The observed ultra-violet absorption of the butter extract is therefore a summation of three separate effects:

- (1) the ultra-violet absorption of the carotene, the intensity being deducible from the visible absorption of the butter extract by utilising the known absorption curve for pure carotene;
- (2) the ultra-violet absorption of the unidentified substance characterised by a maximum near $272\mu\mu$;
- (3) the ultra-violet absorption of the distinct vitamin A which has hitherto been associated mainly with fish-liver oils.

The impossibility of distinguishing quantitatively between the two types of ultra-violet absorption restricts the interpretation of the absorption spectrum of butter extracts in the ultra-violet to fixing an upper limit to the potency of the vitamin A. Calculations indicate that the non-saponifiable matter from the New Zealand butter contains, in addition to about 0.5 % of carotene, sufficient vitamin A to make its potency not more than about six times that of a good cod-liver oil¹.

An examination of the non-saponifiable matter of Danish butter (commercial sample) provides evidence of a much smaller carotene content (Fig. 2), this being of the order of 0.1–0.15 %; as regards the vitamin A content, the upper limit does not appear to be very different from that for the New Zealand

¹ This figure is arrived at by comparing the intensities of absorption at $328\mu\mu$ for equal concentrations and all thicknesses of cod-liver oil and butter concentrate.

butter. On the other hand the concentration of the material responsible for the band near 272μ is very definitely greater (Fig. 3), and this last observation would tend to show that the true vitamin A content of the Danish butter is

Fig. 2.

lower than that of the New Zealand butter, the high concentration of the unidentified substance being probably responsible to some extent for the intensity of absorption near 320μ .

Fig. 3.

Antimony trichloride reaction.

The blue colour obtained with pure carotene is characterised by a well-defined band at 591μ [Dulière, Morton and Drummond, 1929], whilst that obtained with non-saponifiable matter from liver oils shows a sharp maximum at 624μ [Morton and Heilbron, 1928, 1930]. Using the New Zealand extract both these maxima are shown clearly at 624 and 592μ . The intensity of the 624μ band was about five times that associated with an equal weight of a good cod-liver oil, whilst the less intense band at 592μ indicated a maximal carotene content of 2 %. This figure is undoubtedly too high, as the liver oil concentrates (in which carotene is certainly not present in any significant amounts) often give high, if somewhat unselective, absorption at 592μ .

The non-saponifiable matter from Danish butter gave a good colour with antimony trichloride, the tint being definitely more green than blue. A very sharp band at 620–622 $\mu\mu$ was seen but no band at 592 $\mu\mu$ could be recognised. This was not surprising as the spectrographic data had already proved that the carotene content was relatively low. The intensity of the 620–622 $\mu\mu$ band indicated that the potency of the Danish butter extract was about 2.45 times that of cod-liver oil.

It will thus be seen that the direct absorption spectra data and the study of the antimony trichloride blue colours give a consistent account of the properties of the non-saponifiable matter from butter.

The recognition of carotene together with the distinct vitamin A of liver fats in a product derived from milk is of importance and lends support to Moore's view [1930] that carotene can be converted *in vivo* to vitamin A.

It should be emphasised that the butters used were not chosen as representative of their respective classes, and many more samples will need to be studied before any generalisations can be made.

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SUMMARY.

The non-saponifiable matter from butter has been shown spectroscopically to contain carotene, vitamin A, and an unidentified substance characterised by selective absorption at 272 $\mu\mu$. The carotene and vitamin A potency can be estimated with some degree of accuracy.

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