CXXVIII. SPECTROPHOTOMETRIC INVESTIGATIONS OF THE NEW VITAMIN A COLOUR TEST.

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COLORIMETRIC determination of vitamin A has frequently been based upon the Carr-Price reaction, namely the production of a blue colour when vitamin A is added to a saturated solution of antimony trichloride in chloroform, which shows two absorption maxima at $606 m\mu$ and at $572 m\mu$, respectively. This reaction has the following drawbacks: (1) the blue colour is not specific for vitamin A, being given also by carotene, (2) the colour shows a considerable change after the lapse of 30 seconds, rendering colorimetric estimation very difficult. In order to eliminate this difficulty Rosenthal and Erdélyi [1934, 1] devised a new colour test. In this the vitamin-containing material is warmed with a solution of antimony trichloride and catechol in chloroform. The resulting blue colour soon turns into a purplish red. This colour, quite similar to that of a dilute solution of potassium permanganate, is much more stable than the blue colour obtained with the Carr-Price reaction. No other carotenoids except vitamin A produce this colour; the blue colour obtained with other carotenoids does not change on warming. Rosenthal and Erdélyi emphasise the fact that the purplish red hue produced by catechol remains unchanged for some 5 to 10 minutes if fairly pure preparations of vitamin A are used. This fact made possible a more thorough spectrophotometric examination of this purplish red colour.

In our investigations we employed the König-Martens spectrophotometer at a layer depth of 0.2 cm., a Nitra bulb being used as a light source. The extinction coefficient has been calculated according to the following equation:

$$E = \frac{\log \lg \alpha_2 - \log \lg \alpha_1}{d \cdot c},$$

where α_2 and α_1 are the angles measured, d is the layer thickness and c the concentration. A vitamin A concentrate, 0.2 and 0.4 % solution in chloroform has been used.

If reading was performed within the first 10 minutes, two maxima were observed: a broad band at $545 m\mu$ and a narrower one at $475 m\mu$. If however the measurement was repeated during the second, third and fourth 10-minute periods, within the first broad maximum two new maxima and a minimum appeared, the latter corresponding to $545 m\mu$, the maxima corresponding to $535 m\mu$ and $552 m\mu$, respectively. At the same time the second maximum also showed a resolution into a lower maximum at $485 m\mu$ and a considerably higher one at $470 m\mu$. With the aid of chromatographic analysis Karrer and Morf [1933] recently prepared two fractions of vitamin A, called by the authors vitamin A α and vitamin A β , respectively. Van Eekelen *et al.* [1933] described also a separation of two fractions of vitamin A by fuller's earth. Further research is needed to determine which of these fractions is involved in the production of the above maxima observed by us.

The colour reaction with catechol being more stable is definitely superior to the simple antimony trichloride test; however, the spectroscopic data mentioned above show that the stability even of this colour is not sufficient. This fact induced Rosenthal and Erdélyi [1934, 2] to try to improve and to stabilise their colour test still further. They found that the purplish red hue could be obtained not only with catechol but also with quinol, guaiacol and veratrol. This test therefore can be considered as dependent on the presence of a polyhydric phenol. Other polyhydric phenols however are unsuitable for this test because of their insolubility in absolute chloroform. For quantitative determinations guaiacol has been found optimum among the substances mentioned. A 5 % solution of guaiacol in chloroform has been employed. This modification proved more favourable in several respects: the catechol solution has to be prepared fresh daily, whereas the guaiacol solution may be kept ready for use for days. The final colour of the reaction performed with catechol remains constant only for a short time (5–10 minutes), whereas the final colour of the guaiacol test is quite stable even for 1–2 hours. If very pure preparations are used, the colour does not change even after 24 hours. With the catechol test the fluid becomes turbid after a while, whereas the guaiacol modification has the important advantage in respect of spectrophotometric examination that the fluid remains perfectly transparent.

Fig. 1. Absorption curve of colour produced in the antimony trichloride-guaiacol test for vitamin A.

Owing to these circumstances we obtained a quite invariable and characteristic curve when examining the guaiacol reaction spectrophotometrically. It

has two maxima, a higher one at 545 $m\mu$, and a lower one at 478 $m\mu$, which are in good accordance with the characteristics of the curve of the catechol test read within the first 10 minutes (see Fig. 1).

As there was no pure vitamin A available, we were unable to determine the quantitative relations of this absorption curve.

SUMMARY.

The purplish red colour of the antimony trichloride-catechol test for vitamin A examined within the first 10 minutes shows two maxima at 545 $m\mu$ and at 475 $m\mu$, respectively. During the second, third and fourth 10-minute periods two other maxima appear.

The purplish red colour of the guaiacol modification of this test remains unchanged for several hours. Spectrophotometric examination of this colour showed two maxima at 545 $m\mu$ and at 478 $m\mu$, respectively.

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