CCLI. SOME REMARKS CONCERNING THE NEW COLOUR REACTION OF VITAMIN A.

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ANDERSEN AND LEVINE [1935] studied the new colour test of vitamin A described by us [1934, 1, 2] and came to the conclusion that the reaction takes the same course if the test-tube is simply warmed without the addition of catechol or guaiacol, and that therefore these substances are superfluous.

It is well known that the blue colour of the Carr-Price antimony trichloride test for vitamin A is not stable on standing, it slowly changes into violet, red, then yellowish red and finally becomes turbid. This colour change may be hastened by warming the test-tube, the shade of the warmed solution, however, differing from the colour which results when the colour change occurs without warming: a purplish hue is produced which subsequently turns into brick-red. This colour did not prove to be stable enough, and, in addition, the shade itself was found to be unsuitable for colorimetric comparison.

We therefore searched for substances capable of retarding or preventing this undesirable change of colour. Among the substances tried for this purpose at first catechol seemed to be the most suitable. In the course of spectrophotometric measurements, however, it soon became evident that the colour thus produced is not constant enough, as the maximum of its absorption is not stable (see Fig. 1). This change renders colorimetric estimation very difficult. Besides this, we found other drawbacks of the catechol method, which have been commented upon in a detailed manner in our second publication. We therefore made experiments with other phenols and their derivatives and found that guaiacol was the most appropriate for our purposes. The colour of the guaiacol test has been found extraordinarily stable and this has been verified by spectrophotometric measurements [Rosenthal and Weltner, 1935].

Thus guaiacol—and to a less extent catechol—has an inhibiting effect upon the reaction, as has also been stated by Andersen and Levine. In all our measurements we used washed absolute chloroform, as, according to our experience, water and alcohol have an unfavourable effect upon the reaction. The practice of Andersen and Levine of using chloroform not free from alcohol, therefore, cannot be endorsed.

As to carotene, it has been stressed also by us that the blue colour produced with antimony trichloride with the addition of catechol or guaiacol shows some

Fig. 1. Absorption curves of the new colour test for vitamin A performed with catechol: I, II, III, IV, absorption curves of the first, second, third and fourth 10-minute periods.

fading. This peculiarity of the reaction proved to be highly advantageous when the vitamin A content of blood had to be determined in the presence of carotene [Rosenthal and Szilárd, 1935]. The carotene content of blood, in the presence of catechol or guaiacol, does not give rise to a blue colour, whereas the pale violet-red colour yielded by vitamin A can be measured without difficulty.

We willingly agree with Andersen and Levine in that if we aim only at qualitative differentiation of vitamin A from carotene, the use of either catechol or guaiacol is superfluous; in this case, however, no warming is necessary either. The reaction mixture containing vitamin A will change its colour also if left to stand in the cold, whereas the blue shade of carotene produced by antimony trichloride is stable. Warming becomes necessary only if we wish to demonstrate vitamin A in the presence of carotene. However, as soon as our purpose is quantitative determination of vitamin A, in the interest of the possibility of exact colorimetric reading, the addition of guaiacol becomes advisable. The more so, as the shade produced without guaiacol is different from that given with added guaiacol. This has also been stated by Andersen and Levine. In the presence of guaiacol the colour is bluish and identical with that of a freshly prepared dilute solution of potassium permanganate. The wave-length of the typical absorption maximum of this colour exactly tallies with the wave-length of the yellowish green colour filter (No. 7) of the Leitz absolute colorimeter, which fact renders accurate measurements very easy.

It is on account of the facts mentioned that we recommend that the new colour test for vitamin A should be carried out with addition of guaiacol instead of by simply warming the test-tube.

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