CVII. THE ALLEGED SPECIFIC COLOUR REACTION FOR THE ANTISCORBUTIC FACTOR.

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THE disadvantages which attend the biological methods employed in the detection of the vitamins have always kept in the foreground the necessity of devising a chemical means for their estimation. As long as the accessory factors remain chemically unidentified principles it is only possible to fall back on indirect methods, such as colorimetric methods, of estimation.

Funk and Macallum [1913] have demonstrated a certain parallelism between the colour reaction with the Folin and Macallum reagent for the determination of uric acid and phenol and the antineuritic activity of some products. Similarly, Drummond and Watson [1922] have drawn attention to the striking association of the fat-soluble A factor with a substance in oils giving the well-known purple coloration with sulphuric acid. Neither of the above reactions has, however, been found reliable enough to replace the biological methods of estimation of these vitamins.

Recently Bezssonoff [1922] devised a reagent—a modification of Folin's reagent for phenol—which he claimed gave a specific colour reaction for the antiscorbatic factor, because he found that a number of substances known to be antiscorbatically active produced a positive reaction with this reagent.

By employing this reaction on a great number of substances which have been simultaneously tested out on guinea-pigs we have come to the conclusion that, although there is a certain association between this colour reaction and the antiscorbatic activity of some substances, the relationship does not hold true in all cases. We have been able to find, on the one hand, some antiscorbatically active substances which fail to give the colour reaction, and on the other hand, inactive substances which produce the coloration in question.

The colour test was applied by us mostly to various chemical preparations which were tested out biologically in connection with an investigation in which one of us (S. S. Z.) is at present engaged. We soon became aware that although with the majority of the active substances a coloration of varying intensity could be obtained, there was no quantitative relationship between the two tests, and finally, as already mentioned, that it was possible to obtain a biologically active substance giving no coloration with the reagent.

Decitrated lemon juice was prepared in the usual way, namely, by precipitating the acids and other dissolved substances of the juice with excess of calcium carbonate and absolute alcohol, filtering, removing the alcohol in a vacuum at 40–50° in an atmosphere of carbon dioxide and bringing the solution up to the original volume. A daily dose of 1.5 cc. of such a preparation will keep a guinea-pig on a scorbutic diet alive for at least two months. This solution gave a very intense colour reaction. When it was adsorbed with “norit” (5 g. of “norit” to 100 cc. of solution) in a vacuum for 15 minutes (the utmost care having been taken that the adsorbent was introduced in a vacuum after all the dissolved air of the solution had been previously removed by exhaustion) the solution lost some of its antiscorbutic potency, since a daily dose of 1.5 cc. failed to protect a guinea-pig from scurvy; a daily dose of 3 cc., however, prolonged the onset of scurvy, whilst 5 cc. protected the animals for about 52 days, when the experiment had to be discontinued. Only slight scorbutic signs were observed at the autopsy. In spite of the diminished antiscorbutic potency this preparation was, therefore, definitely active, yet no coloration could be obtained with the modified Folin reagent as prepared by Bezssonoff. The above preparation was freshly made up every day before being administered to the animals. During the dosing periods it was tested colorimetrically on eighteen occasions before the animals were dosed and only on three occasions an extremely faint reaction was obtained. On the remaining days the preparation gave negative tests. The tests were carried out, as was done by Bezssonoff, with 1 cc. of the solution diluted with 1 cc. of distilled water to which five drops of the reagent were added. When no coloration was obtained within 30 minutes, undiluted solutions with varying quantities of the reagent were tried for confirmation purposes.

Further experiments showed the possibility of obtaining a positive colour reaction with antiscorbutically inactive substances, viz. yeast and yeast-extract. Brewer's top-fermentation yeast was washed with water and centrifuged. The washed yeast was then dried for 60 hours at 37°. The dried yeast was digested for 3 hours with about ten times its weight of water and centrifuged. On addition of six drops of the reagent to about 2 cc. of the extract a blue coloration was produced immediately with the formation of a precipitate. This precipitate was centrifuged off after standing with the reagent for 10 minutes and a clear blue supernatant solution was obtained.

Similarly, the yeast preparation “marmite” when treated with a few drops of the reagent is precipitated at first and on a further addition of the reagent an olive-green coloration is produced. When the blue coloration obtained with an active substance is compensated in a comparator with untreated marmite the same olive-green coloration is observed.

The above experiments are sufficient to show that the colorimetric test for the antiscorbutic factor suggested by Bezssonoff is not reliable enough to be of any use in the detection of the vitamin. One need only consider the investigation referred to above to see how wrong conclusions might have been arrived

at by depending on the colorimetric reaction alone. The fact that a great number of antiscorbutic substances also give the test is no proof of the specificity of the reagent for the detection of the vitamin.

It is of interest, however, to record that the substance which gives the blue reaction with the modified Folin reagent employed by Bezssonoff is like the vitamin destroyed by oxidation.

Decitrated lemon juice heated at 100° in an atmosphere of carbon dioxide gives the reaction, whilst a similar juice heated in air does not do so. Also a juice made *N*/20 alkaline and allowed to stand exposed to the air for 2 hours at room temperature no longer gives the coloration. This behaviour is similar to that observed [Zilva, 1922, 1923] in the inactivation of the antiscorbutic factor in decitrated lemon juice under the above conditions, which was shown to be due to oxidation.

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