LXVIII. A NOTE ON THE REPRECIPITATION OF THE ANTISCORBUTIC FACTOR FROM DECITRATED LEMON JUICE.

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In a recent paper [Zilva, 1930] it was pointed out that when the active precipitate obtained by precipitation with lead acetate at p_H 7.2 from decitrated lemon juice, previously cleared with neutral lead acetate, was dissolved in acetic acid and the reaction brought up with ammonia to p_H 7, no precipitate was formed at that hydrogen ion concentration. Only when the p_H was raised to 8 a precipitate began to appear and the maximum precipitation was obtained between p_H 8 and 9 only. This fraction was found to be totally inactive in doses equivalent to 5 cc. of the original juice. The observation was not in accord with that made by Grettie and King [1929] and Sipple and King [1930] (the latter cleared their decitrated juice in the first place with alcohol) who obtained a second precipitate at p_H 7.2–7.4 which, they claimed, showed very little loss in activity after the reprecipitation.

In dissolving the first precipitate in the above experiment an excess of 10 % acetic acid, namely a volume equal to that of the original juice from which the precipitate was obtained, was used and this yielded a very clear solution. It has been since found that if less acetic acid is used the p_H at which the second precipitate begins to appear is considerably lowered. Thus, with a certain juice it was possible, by using the least quantity of 10 % acetic acid necessary to dissolve the precipitate (about 10 cc. per 50 cc. of the original juice), to lower the p_H at which the precipitate first appears to 6.2–6.9 (average 6.4) instead of 8 previously observed, and consequently a considerable precipitate was obtained when the p_H was further raised to 7.4. The first precipitate began to appear at p_H 5.5–6.0 (average 5.7). On testing the second precipitate for its antiscorbutic potency, it was found that an equivalent of 5 cc. of the original decitrated juice gave very nearly the same degree of protection as an equivalent of 1.5 cc. of the first precipitate; in other words, a loss of rather less than 66 % was incurred in the reprecipitation. In estimating the dry matter in 7 samples of the first and second precipitates, decomposed with $MgSO_4$, during the progress of the biological test, it was found that the loss of solids varied from day to day but that the average amount

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of inactive material removed by the second precipitation amounted to only 33 %, a lower figure than that of the loss incurred in antiscorbutic activity. No purification was therefore effected in this case.

It must be further pointed out that the reducing capacity for phenolindophenol of the first precipitate of this batch averaged 11 cc. of 0.02 % phenolindophenol per equivalent of 5 cc. of the original juice, the highest figure so far obtained by the author. In the second precipitate there was a loss of 40–50 % of this reducing capacity. With the average preparation, however, such a loss would have brought the reduction down to a level which experience has shown to be characteristic of inactive fractions, and a risk is thus run of totally inactivating the fraction by a second precipitation, even when it is brought down at about p_H 6.4. This point will be dealt with in detail in a subsequent publication.

REFERENCES.

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