L. A NOTE ON THE PRECIPITATION OF THE ANTISCORBUTIC FACTOR FROM LEMON JUICE.

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THE method of fractionation of the antiscorbutic factor introduced by the author [1924, 1925, 1] is based on the facts that the active principle is precipitated by basic lead acetate and that inactive impurities after the removal of the acids from the juice are eliminated by precipitation with neutral lead acetate and alcohol. The main objection to this procedure is that the addition of basic lead acetate in excess brings the reaction of the resulting solution to the alkaline side of neutrality and thus a certain risk of inactivation is incurred. Evidently the stage at which the vitamin is precipitated by the lead acetate is controlled by the hydrogen-ion concentration of the medium [Zilva, 1925, 2], and it is now found that the major part of the activity is in the fraction precipitated at about p_H 7. This is seen from the following experiment. Lemon juice was neutralised with calcium carbonate and allowed to stand for about an hour, after which time it was filtered. An excess of neutral lead acetate was then added to the filtrate, and the reaction adjusted by the addition of dilute ammonia to p_H 7 (phenol red). The precipitate was finally centrifuged and decomposed in the usual way. This preparation was tested out on guinea-pigs (250–300 g.) by the daily administration of freshly prepared doses equivalent to 1.5 cc., 3 cc. and 5 cc. of the original lemon juice, three animals being used for each dose. On the lowest dose only one animal survived the test term of 60 days, showing scorbutic signs at the autopsy after being chloroformed; of the other two animals one died after 56 days and the other after 52 days. The guinea-pigs on the 3 cc. and 5 cc. doses were chloroformed after 60 days, and only in the former group were *very* slight signs of scurvy found at the *post-mortem* examination. It is, therefore, seen that the best part of the active principle was precipitated by the lead acetate at p_H 7. Traces of the antiscorbutic factor could be demonstrated in the filtrate by testing the precipitate obtained on raising the p_H of the solution to 7.5.

Further investigation has shown that most of the antiscorbutic factor is brought down by lead acetate within the p_H range of 5.4–7.2. Decitrated lemon juice as prepared above was treated with an excess of neutral lead acetate. After centrifuging the precipitate, the p_H of the supernatant solution

(5.4) was raised by the cautious addition of dilute ammonia to p_H 7.2. A further precipitate much smaller in bulk was formed, which, on being decomposed, was tested for its activity in the same doses and on the same number of animals as above. The activity was found to be of the same order.

As will be seen from the following table, in spite of the undiminished antiscorbutic activity there is a great reduction in the total solids and in the sugar-content of this preparation. This fraction which is quickly and conveniently prepared can therefore be utilised as a suitable starting-point for further purification.

Table I.

Fraction composed of the total precipitate at p_H 7		
No.	Dry matter (%)	Reducing power as glucose (%)
1	1.610	0.580
2	2.432	0.930
3	2.136	0.800

Corresponding fraction obtained by precipitation between p_H 5.4-7		
No.	Dry matter (%)	Reducing power as glucose (%)
1	0.1278	0.041
2	0.1800	0.070
3	0.199	0.053

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