CXXV. THE VITAMIN C OF LEMON RIND.

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IN continuation of our study of the vitamins in the peels of *Citrus* fruits, we have investigated the antiscorbutic potency of the flavedo of the lemon by feeding an alcoholic extract as the sole source of vitamin C. According to Hess and Unger [1918], and to Harden and Zilva [1918], vitamin C is soluble in alcohol of all strengths. The use of 90 % alcohol avoids the possibility of extracting from the peel the oxidising enzymes, which we have found it to contain in large amount.

Details of the method of preparing the extract are given in a previous communication [Willimott, 1926]. Estimation of the total solids in samples taken at intervals from the extraction mixture showed that at the end of 48 hours extraction was practically complete. However, it was continued for not less than 7 days, when the mixture was filtered, and 1 cc. of the filtrate was considered to contain the vitamin C from 0.5 g. of rind. Preliminary feeding experiments having shown that a large dose of this filtrate was necessary in order to obtain antiscorbutic action, and bearing in mind the danger that the ingestion of large quantities of alcohol might vitiate the experiments, we prepared a stronger extract. To each cc. of the 1 in 2 extract another 0.5 g. of fresh peel was added and extraction continued for another 7 days. The filtrate thus obtained was considered to be a 1 in 1 extract, each cc. containing the vitamin C from 1 g. of rind. The 1 in 2 extract had the s.g. 0.905 and contained 3.4 % total solids, and the 1 in 1 extract had s.g. 0.959 and contained 6.3 % total solids. To prepare 100 cc. of this 1 in 1 extract, 13 or 14 ripe Palermo lemons are required.

Of the two methods available for testing for vitamin C, the superiority of the preventive method over the curative has been emphasised by Chick and Hume [1917], who found recovery in the latter to be uncertain and to lead to confusion in the interpretation of the results. The preventive method was therefore adopted. The basal ration of two parts of bran to one of oats was supplemented by 50 cc. per head *per diem* of fresh milk autoclaved at 15 lb. pressure, for 1 hour, as recommended by Chick, Hume and Skelton [1918]. Milk thus prepared contains no vitamin C and is a supplement to the basal ration rich in vitamin A and in calcium.

Four groups, each containing four healthy young guinea-pigs, were arranged so as to show correspondence as far as possible in sex and weight.

The sexes were segregated. The groups were given the following doses of the 1 in 1 extract per head *per diem*:

Group I. 0.5 cc., equivalent to 0.5 g. rind.

Group II. 0.75 cc., equivalent to 0.75 g. rind.

Group III. 1.0 cc., equivalent to 1.0 g. rind.

Group IV (Control). None.

The doses were administered orally by means of a hypodermic syringe. Considerable difficulty was experienced in inducing the animals to take the necessary dosage; dilution with water did not appear to render the extract any more acceptable. Goss [1925], working on a concentrated lemon juice, encountered a similar difficulty.

Growth effects.

In Group IV (Controls) there was no growth after the first week. The first death occurred on the 19th day, and the remaining three animals died in 6 days, the maximum period of survival being 25 days. Autopsies in each case showed typical signs of severe scurvy.

In Group I (on 0.5 g.) there was no growth after the second week, and the maximum period of survival was 31 days. The group as a whole showed severe scurvy.

In Group II (on 0.75 g.) there was no growth after the third week, the maximum period of survival was 37 days, and scurvy was confirmed in each case.

In Group III (on 1.0 g.) there was no growth after the third week, the maximum period of survival was 39 days. One animal which died unexpectedly on the 21st day was found to have impaction of the intestine. The symptoms of scurvy were not yet obvious, and it was difficult to say what was the cause of death. Two of the others died later of scurvy, and the fourth animal of the group was chloroformed on the 30th day, when in an advanced scorbutic condition.

Towards the end of the experiment (on the 22nd day), when it could be seen that the animals were not receiving their full antiscorbutic requirement, an attempt was made to increase the dose. As mentioned above, we found considerable difficulty in inducing the animals to take large doses of alcoholic extract, and were not able to raise the dose above 1.25 cc., which failed to cure. We then took 100 cc. of the extract, and concentrated it rapidly *in vacuo* at a temperature below 35° until practically all the alcohol had been driven off. The aqueous residue was made up to 50 cc. with distilled water, yielding a solution of which 1 cc. was considered to contain the vitamin C from 2 g. of lemon rind. To the two surviving animals in the control group we were able to give 2.5 cc. doses of this solution, equivalent to 5 g. of rind. Unfortunately, it appeared that scurvy had advanced too far for a cure to be effected, both the animals dying in 3 days when only two or three doses had been administered.

From our experimental results it would seem that the flavedo of ripe lemons contains only small amounts of vitamin C. Hess and Unger [1918], working on whole orange peel (flavedo *and* albedo), found that guinea-pigs given 0.5 g. of dried orange peel developed scurvy after 50 days, and were able to cure two out of three of the scorbutic animals by substituting for the dried peel an equivalent quantity of fresh orange peel. From our own data regarding the moisture content of orange and lemon peels, we conclude that the amount of fresh orange peel equivalent to 0.5 g. of dried peel would be from 1.5 to 2.0 g. But in the Report of the Accessory Food Factors Committee [1924, p. 69], it is stated, on the basis of Hess and Unger's work, that the protective dose of fresh orange peel is 5 g. There appears to be some discrepancy. From our work we conclude that the protective dose of fresh lemon flavedo is larger than that found for fresh orange peel by Hess and Unger. If this is so, it confirms the view put forward by Morgan and Chaney [1924] that the peel of the orange contains a higher proportion of vitamins than the peel of the lemon.

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