XLVI. THE INFLUENCE OF REACTION ON THE OXIDATION OF THE ANTISCORBUTIC FACTOR IN LEMON JUICE.

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THE earliest investigations of experimental scurvy have revealed that reaction influenced the stability of the antiscorbutic factor. Holst and Frölich [1912] were the first to draw attention to the fact that in acid solution the antiscorbutic potency displays greater stability than in alkaline solution. This observation has since been confirmed by various workers [Harden and Zilva, 1918; Hess and Unger, 1919; La Mer, 1921]. Further work on the stability of the antiscorbutic factor has shown that oxidation has also a destructive action on the potency of active solutions [Hess and Unger, 1921; Zilva, 1921; Dutcher, Harshaw and Hall, 1921].

This paper deals with the elucidation and correlation of the inactivating influences of reaction and oxidation. The experiments were instituted as a result of some suggestive information obtained in connection with another inquiry on adsorption, which is still in progress. Evidence is here produced which shows that in the case of decitrated lemon juice the destruction of the factor is a process of oxidation which proceeds rapidly in alkaline solutions and slowly in an acid medium. Under anaerobic conditions alkaline reaction seems to have no inactivating influence; in presence of air when the hydrogen ion concentration is raised the destruction of the antiscorbutic potency is delayed. An active juice made approximately $N/20$ alkaline (p_H 12.5) and kept at this hydrogen-ion concentration for 24 hours under strictly anaerobic conditions did not deteriorate to a marked extent, whilst the same juice and of the same reaction kept exposed to the air during the same period failed to protect or even to delay the onset of scurvy in guinea-pigs in a dose about 5–6 times larger than that which protected the animals from the disease when the juice was kept anaerobically. Furthermore, when the hydrogen-ion concentration was brought up to p_H 2.2 the inactivation by aeration at 100° was definitely delayed.

At p_H 12.5, in the presence of air and at room temperature, the destruction of the antiscorbutic factor proceeds fairly rapidly. After three hours it is almost completely destroyed whilst after half an hour the activity is diminished to a considerable extent.

EXPERIMENTAL.

All the preparations were tested out on guinea-pigs. The animals were kept on a scorbutic diet of oats, and bran supplemented by a daily ration of not more than 40 cc. autoclaved milk. On this diet guinea-pigs develop scurvy in about 10–15 days and succumb to the disease after about four weeks. On the other hand when this diet is supplemented by an adequate antiscorbutic dose the animals thrive well on it.

The doses to be tested were administered daily *per os* after the animals had been on the scorbutic diet for about 14 days. In the case of a positive result the duration of the experiment was two months, after which time the guinea-pigs were chloroformed and submitted to a post mortem examination.

The Influence of Alkalinity on the Antiscorbutic Factor under Aerobic and Anaerobic Conditions.

The citric acid was removed from the lemon juice by the addition of excess of calcium carbonate and two volumes of absolute alcohol. After filtration, the solution was concentrated in a vacuum at 40–50° and eventually brought up to the original volume of the juice. The reaction was then adjusted colorimetrically to p_H 6.6–6.8. In the aerobic experiments this adjusted juice was made approximately $N/20$ by the addition of normal sodium hydroxide (1.3 cc. of N NaOH to 23.7 cc. of juice) and allowed to stand in a conical flask in the laboratory for 24 hours before being administered to the experimental animals. Great precautions were taken in the anaerobic experiments to exclude air. The adjusted decitrated juice was introduced into bottles of small capacity (about 50 cc.) immediately after the addition of the requisite alkali for the production of a $N/20$ alkaline solution, the bottle was closed with a tight-fitting rubber stopper containing a glass tube with a tap and rapidly exhausted by means of an air pump. The bottle was then placed under a bell-jar over alkaline pyrogalllic acid which was in its turn exhausted. The hydrogen-ion concentration of samples of decitrated juice made alkaline was determined electrometrically both immediately after the addition of the alkali and after storage under anaerobic conditions. No significant difference could be detected in the reaction. The actual figures found in one of these cases were as follows:—immediately after the addition of alkali $p_H = 12.12$, the same juice kept under anaerobic conditions for 24 hours $p_H = 12.09$. When, however, the juice was made alkaline and allowed to stand exposed to the air the hydrogen-ion concentration gradually increased. I should like to take this opportunity of thanking Dr E. E. Atkin for having kindly carried out the electrometric estimations.

At the expiration of 24 hours the aerobic and anaerobic solutions were acidified and fed to the animals. These preparations were made daily. Doses of 1.5, 3, 5 and 7 cc. were given of each preparation. The aerobic alkaline solutions were found quite inactive, whilst the same alkaline solutions kept

anaerobically were found to be protective even in the lowest dose of 1.5 cc. Fig. 1 gives the weight curve of the animals receiving the highest dose.

It was further of interest to ascertain the time taken to bring about the total inactivation of the antiscorbutic factor in a solution of the above alkalinity when kept exposed to the air. For this purpose the decitrated lemon juice was made approximately $N/20$ alkaline as before and kept in small open conical flasks at room temperature for 0.5 hour, 1 hour and 3 hours. After the respective times of treatment, the solutions were acidified by the addition of citric acid (0.1 g. to 10 cc.) and administered to the animals the same day. The tests have disclosed that after keeping the decitrated lemon

Fig. 1.

juice alkaline for one hour a daily dose of 5 cc. showed no potency. The same dose of decitrated lemon juice kept alkaline for 0.5 hour was found to be active as will be seen from Fig. 2 although the animal receiving this dose was found to show signs of incipient scurvy when chloroformed after two months. Doses of 1.5 cc. and 3 cc. of the same preparation were however ineffective to prevent or even to delay the onset of scurvy. As the minimum protective dose of decitrated lemon juice is 1-1.5 cc., it seems that about 80 % of the antiscorbutic factor has been destroyed in half an hour.

*The Influence of Acidity on the Inactivation of the Antiscorbutic Factor
by Aeration at 100° C.*

In these experiments the lemon juice was prepared as in the previous experiments and adjusted to p_H 6.6-6.8. Some of the juice was brought to p_H 2.2-2.4 by the addition of citric acid. Both preparations were boiled under

a reflux condenser, air being aspirated through the boiling liquid. The decitrated lemon juice of p_H 6.6–6.8 was treated in this way for one hour. The acidified juice was aerated for one hour and for two hours.

Daily doses of 1.5, 3 and 5 cc. of the three preparations were tested out on animals. In all the three preparations 1.5 cc. of the solution failed to show any protective properties, the higher dose of 3 cc. failed to protect in the neutral preparation and in the acid solution aerated for two hours. This dose of the acid preparation which was aerated for one hour offered partial protection—the animal surviving for two months although it was in a declining and scorbutic condition at the end of that period. The difference in the degree of inactivation was best evident in the experiment in which the 5 cc. daily doses

Fig. 2.

were used. Fig. 3 gives a graphic presentation of the weights of the animals which were given 5 cc. daily of each of the preparations. The animal which received the acid preparation aerated for one hour was chloroformed after two months and although some mild signs of scurvy were established it was nevertheless evident that the guinea-pig could have lived for some time longer. The animal which received the same dose of neutral preparation aerated for one hour commenced declining slowly after 15 days and died after 40 days from acute scurvy. Doubtful protection was achieved by 5 cc. of acid preparation aerated for two hours. These experiments show that the acidity undoubtedly had a protective action on the inactivation of the antiscorbutic factor by aeration at 100° .

Decitrated lemon juice as prepared above contains 0.8 %–1 % of solids, the best part of which consists of sugars. It is, therefore, reasonable to assume that the increase in the hydrogen-ion concentration when the alkaline juice is exposed to the air is due to the production of acids from the sugar, which according

to the extensive researches of Nef is oxidised in alkaline solution to various organic acids. Although there is a certain parallelism between the formation of acid and the inactivation of the antiscorbutic factor in the experiments described, in so far that both take place in alkaline solution in the presence but not in the absence of air, the parallelism is not complete. It is seen that about 80 % of the factor is destroyed by the treatment in about half an hour. Preliminary investigations show that the formation of acids proceeds at a much slower rate. After half an hour the hydrogen-ion concentration is only

Fig. 3.

just perceptibly increased. Whether the inactivation of the antiscorbutic factor is due to an intermediate change of the sugar before the formation of the acid or whether it is a change in which the sugar is not concerned cannot be answered from the experimental evidence so far available. The writer hopes that investigations on the subject which are now in progress may conduce to the elucidation of this problem.

SUMMARY.

Decitrated lemon juice made approximately $N/20$ alkaline ($p_H = 12.5$) and kept exposed to the air at room temperature loses about 80 % of its antiscorbutic potency in half an hour. After three hours a daily dose of 5 cc. fails to prevent or delay the onset of scurvy in guinea-pigs.

Decitrated lemon juice of the same reaction kept for 24 hours at room temperature in the absence of air shows no deterioration in its antiscorbutic potency.

There is no change in the hydrogen-ion concentration of the alkaline decitrated juice kept in the absence of air after 24 hours; when, however, it is exposed to the air there is an increase in the hydrogen-ion concentration.

When air is aspirated through a boiling solution of decitrated lemon juice for one hour the destruction of the antiscorbutic factor is delayed when the hydrogen-ion concentration is raised from p_H 6.6 to 2.2.

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