LXVIII. THE ACTION OF REDUCING AGENTS ON THE ANTISCORBUTIC FACTOR INACTIVATED BY AERATION.

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THE greatest difficulty in the chemical manipulation of the antiscorbutic factor is the readiness with which this principle is inactivated by oxidation. Not only does this impediment limit the scope of the investigation by vastly restricting the choice of methods but it adds greatly to the labours of the investigator, since doses have to be prepared daily during the testing period of six weeks. Storage of active fractions in bulk under conditions which prevent oxidation is not always suitable. It is therefore evident that if the activity of the antiscorbutic vitamin lost by oxidation in the process of fractionation could be regenerated, it would be of the utmost importance in facilitating research in this field of vitamin chemistry, apart from the valuable information it would contribute to this elusive problem.

Our scanty knowledge of the chemistry of the antiscorbutic factor does not enable us to follow up the chemical change brought about by oxidation in the active solution. On the assumption that the active molecule is directly oxidised, it may be supposed either that it is degraded or that it is oxidised without disintegration.

In the latter case there exists the possibility of reactivating the oxidised principle by suitable reducing methods. We have therefore studied the effect of a few such methods on the antiscorbutic factor inactivated by aeration. The choice of methods, as is usual in this work, was restricted by the fact that the final preparation had to be suitable for feeding tests on guinea-pigs. Attempts were made to reduce the inactivated solutions (*a*) by hydrogen in the presence of platinum black as a catalyst, (*b*) electrolytically and (*c*) by nascent hydrogen from acid and metal.

CATALYTIC REDUCTION BY MEANS OF PLATINUM BLACK AND HYDROGEN.

The catalyst was prepared by Zdrawkowitch's method [1876]: 10 cc. of a 5 % H_2PtCl_6 solution was added drop by drop to a boiling mixture of 4 cc. of 10 % potassium hydroxide and 6 cc. of glycerol. The precipitated platinum black was then centrifuged, washed on a glass filter with distilled water until the washings were free from chlorine and dried at 55°.

The activity of this catalyst was demonstrated in the following experiment. 15 cc. of benzaldehyde, 10 cc. of absolute alcohol and 0.1 g. of the dried platinum black were introduced into a stout bottle of about 150 cc. capacity fitted with a ground glass stopper and tap. This bottle was exhausted by a vacuum pump to 3 mm. and hydrogen introduced at a pressure of one atmosphere and shaken up. The washing out with hydrogen in this way was carried out three times, after which the bottle was shaken for two hours. When opened an absorption of gas was observed. The hydrogen was changed as before by exhausting and washing with the gas three times and the reacting mixture was then submitted to a further two hours' shaking. After this it was filtered and the filtrate treated with an excess of a saturated aqueous solution of sodium bisulphite and with ether. After thorough shaking, the ethereal layer was separated, shaken with sodium carbonate, washed with water and dried over sodium sulphate. On removing the ethyl alcohol and ether a yield of 7 g. of crude benzyl alcohol was obtained, which was free from benzaldehyde and which on oxidation with alkaline permanganate yielded benzoic acid.

The hydrogen used for the reduction was obtained from a cylinder and passed through a succession of tubes containing a 10 % solution of lead nitrate, silver nitrate solution and solid potassium hydroxide.

Lemon juice decitrated by the calcium carbonate-alcohol method [Zilva, 1924] was used as a source for the antiscorbutic factor. This material, although not the purest possible to obtain, is moderately free from most of the impurities of the original juice, and presumably contains nothing that could interfere with the process of reduction.

Utilising the observation that the antiscorbutic activity of a neutral solution can be destroyed by aspirating air through it at room temperature [Zilva, 1922] the decitrated juice was adjusted to approximately p_H 7.4 and air was aspirated through it for about 15 hours by means of a filter pump. The p_H gradually rose to about 8.0 in this process during a few hours and the reading then remained stationary.

The reduction was carried out as in the case of the benzaldehyde described above: 0.1 g. of freshly prepared catalyst was used per 100 cc. of juice. No perceptible absorption of hydrogen was observed in this case after shaking for four hours with an intermediate change of hydrogen. On removing the catalyst by filtration the juice was acidified with citric acid. A fresh batch was reduced daily and fed to the test guinea-pigs on the day of reduction, except during the week-end when a two days' supply was reduced, a part of which was stored in a vacuum.

Decitrated lemon juice not previously inactivated by aeration was also reduced as above in order to demonstrate that the process of reduction had no deleterious effect on the activity of the juice. Every batch of lemon juice was therefore tested daily (a) after inactivation by aeration; (b) after reduction following the inactivation; and (c) after reducing the active decitrated juice without aeration.

The aerated decitrated juice was tested in daily doses of 1 cc., 3 cc. and 5 cc., two animals being used on each dose. The reduced aerated juice was tested in the same doses, except that three animals were used on the 5 cc. dose. The reduced active juice was tested in the same way as the reduced aerated juice. All the animals in the first two groups succumbed within four weeks of being placed on the scorbutic diet and manifested signs of scurvy at the post mortem. All the animals in the third group lived for 57 days, after which period they were chloroformed. Only in one animal receiving a daily dose of 1.5 cc. slight signs of scurvy were observed. No regeneration of the antiscorbutic factor was therefore effected by this reduction.

ELECTROLYTIC REDUCTION.

The same source for the antiscorbutic factor and the same method of inactivation were employed in this experiment as in the previous one. Precautions were taken to prevent the antiscorbutic factor, in the event of regeneration, from diffusing from the cathode into the anode compartment and thus becoming oxidised. An ordinary porous cell would not have fulfilled this condition, and recourse was had to a collodion thimble soaked in 80 % alcohol for 24 hours [Brown, 1915; Zilva and Miura, 1921; Connell and Zilva, 1924]. Such a thimble does not permit the passage of the antiscorbutic factor [Zilva and Miura, 1921], whilst it does not prevent the flow of the electric current.

The inactivated decitrated lemon juice was acidified with citric acid to the extent of 1 % and placed in the collodion thimble. The thimble was then introduced into a liquid consisting of a 1 % aqueous citric acid solution in which the anode was placed. The cathode consisted of a piece of platinum foil of about 1.5 square inches coated with platinum black, and the anode of ordinary platinum foil. Two carbon electrodes immersed in tap water were introduced into the circuit as an adjustable resistance. These were arranged at a distance to yield a current of about 0.5–1.0 ampère from the main direct current of the lighting circuit of 200 volts. A glass cooling coil was placed inside the electrolytic cell just outside the collodion thimble to prevent any appreciable rise in temperature. The solutions were electrolysed for seven hours. In order to make sure that the collodion thimbles used in the experiment were impermeable to the antiscorbutic factor, active decitrated lemon juice was dialysed in such thimbles under similar conditions and tested for activity. In fact the same thimbles were used on alternate days for electrolysis and dialysis. The active decitrated lemon juice was also submitted to electrolysis for seven hours with the object of ascertaining whether the electrolytic reduction had any action on the activity of the antiscorbutic factor. Four preparations were tested out in this experiment, namely: aerated decitrated lemon juice; electrolysed aerated decitrated lemon juice; active decitrated lemon juice dialysed for seven hours in a collodion thimble soaked in 80 % alcohol for 24 hours; and electrolysed active decitrated lemon juice.

Three doses, *i.e.* 1.5 cc., 3 cc. and 5 cc. per day with three guinea-pigs for each dose were employed in these tests. In the case of the aerated and electrolysed aerated lemon juice these doses were found not to prevent or even delay the onset of scurvy. The other two preparations were found to be active, although the tests were somewhat marred by the death from unknown causes of some of the animals. The protocols of the animals, however, left no doubt as to the retention of the antiscorbutic activity by these preparations. Thus in the case of electrolysed, active decitrated lemon juice one animal receiving a daily dose of 1.5 cc., two receiving a dose of 3 cc. and one receiving a dose of 5 cc. survived the usual test period of two months and after being chloroformed showed no signs of scurvy at the post mortem. Similarly, of the animals fed on the dialysed juice, one animal on the 1.5 cc. dose survived for 53 days showing no scorbutic signs at the post mortem. Of the others, two animals on the 3 cc. dose and three animals on the 5 cc. dose were chloroformed after two months and were found not to be scorbutic. The animals which died during the test from unknown causes did not show any scorbutic lesions at the autopsy.

REDUCTION BY NASCENT HYDROGEN FROM ACID AND METAL.

In this experiment magnesium filings were introduced into the flask containing the juice, citric acid was then added gradually and the mixture shaken. At the end of the reaction the solution was treated with excess of absolute alcohol, filtered, evaporated in a vacuum and made up to the original volume of the juice. This preparation had a disturbing effect on the animals in spite of the removal of the bulk of magnesium citrate, and it was impossible to judge from the results whether the reduced solution was antiscorbutically active or not.

CONCLUSIONS AND SUMMARY.

It is seen from the above experiments that the inactivated solutions could not be regenerated by hydrogenation in the presence of platinum black as a catalyst and by electrolytic reduction. All reasonable risks of re-inactivation of the antiscorbutic factor in the case of regeneration were eliminated in the technique. It is true that in the hydrogenation experiment the medium was slightly on the alkaline side of neutrality, but the reduction took place under strictly anaerobic conditions for only four hours. It was previously found [Zilva, 1923] that active solutions made *N*/20 alkaline did not appreciably deteriorate when kept under anaerobic conditions even for 24 hours. It is, therefore, hardly probable that the reaction in this case could have had any effect on the antiscorbutic activity, were it regenerated. It is also to be pointed out that, in the electrolysis experiment, any dissolved oxygen which was generated at the anode and which diffused into the collodion thimble would have been at once removed by the hydrogen generated there at the cathode.

This investigation has by no means exhausted all the reducing methods. It is, however, evident that if it is at all possible to regenerate the inactivated antiscorbutic factor by reduction, the task is by no means straightforward. As already mentioned, the aim of the experiments was to find out whether it was possible to regenerate the antiscorbutic activity by ordinary reducing methods in order to facilitate the chemical study of the active principle. This is evidently not the case, and it is considered that any further efforts in this direction at the present stage would not be profitable.

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