CLXXXVII. THE ANTISCORBUTIC FRACTION OF LEMON JUICE. IX.

By SYLVESTER SOLOMON ZILVA¹.

From the Biochemical Department, Lister Institute, London, S.W. 1.

(Received October 9th, 1930.)

THIS communication deals in the main with two aspects of the antiscorbutic factor, namely, its fractionation and the mechanism controlling its spontaneous inactivation.

The method of fractionation introduced some years ago by the author was based on the precipitation of the active principle by lead acetate at p_H 7-7.2 after removing the acids from lemon juice and clearing the resulting solution with alcohol or neutral lead acetate or with both in succession. In the early stages [1924] basic lead acetate was employed as the precipitating agent, but as the work progressed it became apparent that very unequal results were obtained, which were then ascribed to the alkalinity of the basic lead acetate, and consequently a modification, which consisted in the precipitation of the vitamin at p_H 7-7.2, was introduced [1927]. Further experiments have, however, shown that the slightly alkaline reaction of the medium was not alone responsible for the low activity of some of the fractions obtained [1928, 1929] and that some uncontrolled factor, possibly varying with the character of the juice employed, was involved. It was therefore felt that evidence sought along this path might not only yield information conducive to the preparation of fractions of more uniform activity, but might afford knowledge with regard to the general complexity of the problem. Results obtained so far seem to favour the view that the variable activity obtained by precipitation with lead acetate in the neutral zone is controlled by the character of the juice. Thus a quantity of lead acetate which precipitates the greater part of the active principle in one juice fails to bring down much of the vitamin in another case, but, by adjusting the quantity of the precipitating agent, the activity of the precipitate obtained from the juice can be greatly enhanced.

What the factor in the lemon juice which controls the precipitation of the antiscorbutic factor is, has not so far been ascertained, but the following results will make it plain that the fractionation of the principle is a complicated subject requiring critical judgment in its manipulation and in the interpretation of the results.

Since the publication of the above method of fractionation, experiments

¹ Member of the Scientific Staff, Medical Research Council, working with grants for assistance and expenses.

dealing with its extension and its application to other sources of the antiscorbutic factor have been published by several workers. Their results are, however, quite contrary not only to what the author's experience would lead him to expect, but to his actual observations; which suggests that it is necessary to exercise caution in order to interpret correctly the available evidence on the subject. To emphasise this necessity, the experiments on the fractionation of the vitamin are here communicated.

The experiments described in the section of this communication dealing with the spontaneous inactivation of the antiscorbutic factor are an extension of the work recorded recently [1929]. In that communication it was shown that the addition of autoclaved decitrated lemon juice or of quinol to unheated decitrated juice accelerated the destruction of both the reducing principle¹ and the antiscorbutic factor of the mixture at neutral reaction. It was further found that the iodine-reducing capacity of the autoclaved solution increased² and that the activity of the peroxidase present in lemon juice persisted after the heating. A plausible explanation of the accelerated inactivation of the vitamin after autoclaving presented itself. A substance, probably of a phenolic character, was formed in the autoclaving which on oxidation in the air acquired the property of acting in conjunction with the thermostable peroxidase as an oxidiser of the reducing substance, the disappearance of which, as has been shown [1927, 1928], precedes the loss of the antiscorbutic activity. The experiments described in this communication support this view. The addition of benzoquinone, quinhydrone or ethereal extracts from autoclaved decitrated juice accelerated the inactivation of the vitamin in unheated decitrated juice to much the same extent as if the juice itself had been autoclaved.

Williams and Corran [1930] have recently shown that in the case of lemon juices adjusted with hydrochloric acid to various hydrogen ion concentrations ranging from p_H 0.6 to 2.2 and stored for 7–8 months, the antiscorbutic activity greatly deteriorated when the p_H was less than 1.8. Experiments are described here to show that there is no appreciable destruction of the reducing principle or of the antiscorbutic factor in decitrated lemon juice kept at p_H 1 or less for one week, so that the destruction of the vitamin observed by these workers, if it be directly due to the acidity and not to any other factor inherent in the conditions under which they were working, must be comparatively slow.

¹ Szent-Györgyi [1928] suggests that the reducing principle is probably identical with the hexuronic acid which was isolated by him from the adrenal cortex and from fruit and vegetable juices. No direct evidence has yet been produced to substantiate this suggestion. Until such evidence is available the author proposes to use the expression "reducing principle."

² Experiments performed since the publication of this observation have shown that there is not so much an increase in the decolorising capacity as increase in the speed at which the decoloration of the iodine takes place. There is no very significant difference between the amounts of iodine that are decolorised by autoclaved and unheated juice if an excess of the reagent is added and the residual iodine is titrated back after 24–48 hours. It must, however, be noted that some destruction of the reducing principle, which also decolorises iodine, takes place in the autoclaving.

EXPERIMENTAL.

The variable activity of antiscorbutic fractions from lemon juice.

In the concentration and purification of the antiscorbutic factor the author has always adopted the procedure of assessing the activity of a preparation by reference to its equivalent of the original lemon juice. In the early experiments it was found that doses equivalent to 1.5 cc. of the juice were capable of keeping young guinea-pigs alive for periods of about 60 days; these animals were, however, not always free from scurvy at that time. Although the same dose of lemon juice or of decitrated juice is capable of preventing the development of any macroscopic scorbutic lesions and even inducing good growth during this period, it is assumed, considering the risk of loss in activity involved in the preparation, that the best part of the vitamin of the juice in such cases is obtained in the final fraction. As already stated, experience has shown that preparations of such high activity could not always be obtained in spite of the fact that, as far as it was possible to judge, uniform conditions were observed in the manipulation. It would require too much space to describe in detail all the fractionations performed during the last few years, but an analysis of these will afford a fair view of the variations in activity. Before discussing the results it is, however, necessary to give a detailed description of the method of preparation, though mention of it has already been made in previous publications of this series, since it is found that small deviations from the procedure often give negative results in the case of juices capable of yielding active fractions. The details given were applied to small batches of juice varying from 50 to 200 cc. These were always prepared and dosed daily except during the week-end. Less frequent preparation and storage of large batches led mostly to considerable loss in activity.

The acids are removed from the lemon juice by the addition of an excess of precipitated chalk and the mixture is allowed to stand at room temperature for about 1 hour. The liquid is then filtered through a Büchner funnel and to the filtrate (about p_H 6.4) an equal volume of neutral lead acetate is added. After immediate filtration through a Büchner funnel this filtrate (p_H 5.0–5.4) is brought up to p_H 7.2 by a solution of 6 % ammonia. The precipitate formed is quickly centrifuged and taken up in 10 % acetic acid, yielding usually a clear or slightly opalescent solution. The lead is then removed from the solution by the addition of just sufficient saturated magnesium sulphate (3–5 cc. for 50 cc. of decitrated juice) and subsequently of 2 volumes of 96 % alcohol and allowing the mixture to stand for 10–15 mins. The filtrate after being evaporated under reduced pressure at 40°–50° to a volume of 2–5 cc., when the excess of acetic acid has been distilled off, is diluted with distilled water to a convenient volume. Enough 20–25 % ammonia is then cautiously added to reduce the acidity to p_H 5.0–6.0 and the solution is brought up to its original volume with distilled water. The preparations must be administered to the animals immediately, since the inactivation of purified active solutions proceeds much

more quickly than does that of original juices of similar activity [Zilva, 1928]. The decomposition of the lead precipitate with H_2S instead of magnesium sulphate yields less satisfactory results. The above manipulation should not take more than 3–4 hours.

The 11 tests reviewed here in which over 95 animals are involved cover a period of several years and were performed at various seasons during the years 1925–30. All the animals referred to, unless otherwise stated, were chloroformed after 55–60 days and the degree of scurvy assessed at the *post mortem* examination.

Of the animals receiving a dose equivalent to 1.5 cc. of lemon juice, in six out of eleven sets, comprising 20 animals, hardly any delay in the onset of, or fatal termination due to, scurvy had been noticed. In the remaining five sets, comprising 17 animals, all the guinea-pigs survived the experimental period. In 6 of these animals hardly any scurvy could be detected, the others all showed marked scurvy at the *post mortem* examination.

Of the animals receiving a dose equivalent to 3 cc. in four sets out of nine, comprising 13 guinea-pigs, hardly any delay in the onset of and fatal termination due to scurvy could be observed. The remaining five sets comprising 13 animals, of which only 1 guinea-pig showed marked scurvy at the *post mortem* examination, survived the experimental period.

Of all the animals receiving a dose equivalent to 5 cc., in two sets out of eleven, comprising 6 animals, hardly any delay in the onset of and fatal termination due to scurvy could be observed. The remaining nine sets, comprising 22 animals, of which only 1 guinea-pig showed definite scurvy at the *post mortem* examination, survived the experimental period.

Out of one set of 4 guinea-pigs receiving a dose equivalent to 10 cc. of lemon juice, 1 animal died of an intercurrent disease without showing any signs of scurvy after 64 days. The remaining animals were chloroformed after 92–97 days and were found almost entirely protected from scurvy.

No correlation was found to exist between the activity of the various fractions and the season when they were prepared. As very little time usually elapsed between the arrival of the lemons in this country and their utilisation in the experiment, the date of the test can be used more or less as an indication of the time of their picking. Unfortunately it was difficult to obtain detailed information about cultural conditions owing to the fact that the fruit was bought in the open market. The following experiments, however, throw some light on one of the causes responsible for the low activity of some of the preparations.

The influence of various quantities of neutral lead acetate on the fractionation of the antiscorbutic factor.

It will be noticed that an equal volume of saturated neutral lead acetate was added to the decitrated lemon juice in the preparation of the antiscorbutic fractions in the experiments described above and that in the early experiments preparations were obtained which showed considerable potency in

doses equivalent to 1.5 cc. of the original lemon juice. In the search for the cause of the unequal activity in some of the later preparations, it was observed that when an equal volume of neutral lead acetate was employed a fraction was sometimes obtained possessing very little reducing capacity (1–3 cc. of 0.02 % phenolindophenol per 5 cc. of the solution) and, as anticipated, such fractions were found almost inactive antiscorbutically. On the other hand, it was possible to adjust the quantity of neutral lead acetate so that the reducing capacity of the fraction was increased with a simultaneous increase in antiscorbutic activity. The experiment, which is represented graphically in Fig. 1,

Fig. 1. +, died; C, chloroformed; S, scurvy; SS, slight scurvy; VSS, very slight scurvy; N, normal; ↓, onset of scorbutic symptoms; |, beginning of the daily administration of the dose.

demonstrates this point. It is seen that when an equal volume of neutral lead acetate was added, the juice did not show any activity even in doses equivalent to 5 cc. of the original juice, a quantity which decolorised on the average only 2.5 cc. of 0.02 % phenolindophenol. On the other hand, when the amount of neutral lead acetate was reduced to one-fifth of the volume of the juice, all other conditions remaining the same, the antiscorbutic potency was greatly enhanced. An equivalent of 5 cc. of this preparation reduced on the average about 7.5 cc. of 0.02 % phenolindophenol. The reducing capacity for phenolindophenol of the final fraction may, therefore, be utilised in this case as an index for the determination of the optimum amount of neutral lead acetate necessary to employ in the manipulation in order to achieve good antiscorbutic activity. So far it has been found that amounts of reagent varying from 18 % to 25 % of the volume of the juice are required to achieve the best results, although, as is evident from the earlier experiments, good potency can sometimes be obtained with equal volumes of lead acetate. When a uniform supply of lemons is used, the same amount of precipitating reagent can be used during the 2 months' duration of the test; the reducing capacity of the

fraction as shown by its reduction of phenolindophenol varies in such cases within certain limits, but is always above the minimum which experience has shown is necessary to ensure potent fractions, namely, above about 6 cc. of 0.02 % of the indicator for an equivalent of 5 cc. of juice. When the batch of lemons is not uniform in character, as sometimes happens, the quantity of neutral lead acetate used must be adjusted according to the daily variation in the titrations of the reducing capacity of the fractions.

It must here be emphasised that an increase in the reducing capacity in preparations obtained as described above, as measured by the decoloration of phenolindophenol, above the minimum necessary to insure antiscorbutic activity, in no way indicates a proportionate increase in the antiscorbutic activity. Beyond this limit this reducing capacity is more an index of the stability of the antiscorbutic potency.

Attempts at further purification of the active factor.

The first obvious procedure to attempt in order to effect further purification was the reprecipitation of the active fraction, but this had to be given up since no activity could be obtained in the second precipitate. Two experiments are cited here to illustrate the difficulties.

In the first experiment after taking up the active precipitate in 10 % acetic acid, 25 % ammonia was added cautiously until the neutral zone was almost reached and then the neutralisation was completed with 6 % ammonia. No precipitate was formed at p_H 7.0, as it was in the case of the first precipitation. When the p_H was raised to about 8 a precipitate began to form, the maximum precipitation being obtained between p_H 8 and 9. This precipitate was decomposed and tested in the usual way and, as will be seen from Fig. 2, it

was found to be inactive in a dose equivalent to 5 cc. of the original juice, whilst the first precipitate from the same juice was active in this dose. An equivalent of 5 cc. of the first precipitate reduced 7.5 cc. of 0.02 % phenolindophenol. A similar quantity of the second precipitation reduced 2.5 cc.

In the second experiment the first lead precipitate was decomposed with H_2S , and the H_2S removed by concentration of the resulting solution at $40^\circ\text{--}50^\circ$ under reduced pressure to a small volume. After diluting to its original volume, it was brought up to p_{H} 7-7.2 with 6 % ammonia. In this case the maximum precipitation was obtained at this reaction. The precipitate was decomposed in the usual way and, as will be seen from Fig. 2, there was a marked diminution in the activity in this second precipitate. The activity of the first precipitate in this experiment was not very high but this is not unusual when the lead precipitate is decomposed with H_2S instead of magnesium sulphate.

The above experiments, characteristic of several performed on these lines, are quoted here in view of the fact that Grettie and King [1929] and Sipple and King [1930] claim to have obtained very little loss in activity in the second precipitation, although the fractions were not even prepared every day but at least once a week and stored under anaerobic conditions before being tested.

Bezssonoff also claims to have obtained an active fraction from cabbage juice after several precipitations. This writer [1922, 1923] asserted that by clearing cabbage juice with neutral lead acetate, decomposing the precipitate with H_2S and concentrating to dryness under reduced pressure and low temperature, an active powder was obtained. Later [1925], he applied the author's principle but, instead of using basic lead acetate, precipitated the vitamin from the juice previously cleared with lead acetate and acetic acid by adjusting the reaction of the solution with sodium hydroxide to p_{H} 8.2. Further purification was carried out by taking up the precipitate in acetic acid, precipitating it twice more, decomposing it with H_2S and extracting with alcohol and with acetone. This procedure it is claimed yielded active crystals.

Considering the disappointment experienced by the author in attempting to obtain any antiscorbutic activity in the second precipitate, and the difficulty of manipulating active fractions in general without impairing their activity, the results obtained by Bezssonoff seemed rather surprising. This feeling was further strengthened by many observations that cabbage juice was the least stable source of the antiscorbutic factor so far handled. The experiments described below show that, in fact, in the author's hands, extremely disappointing results were obtained in the fractionation of this juice.

The outer green leaves without the stalk of the cabbage were thoroughly minced and pressed out through chain cloth by means of a hand press. The juice was then centrifuged to remove suspended matter and the untreated supernatant liquid (preparation A) was tested as a control. To another portion of this centrifuged juice 35 % of its volume of a saturated solution of neutral lead acetate was added, a quantity of reagent with which was usually obtained the highest reducing capacity in the resulting supernatant solution with this material, and the heavy precipitate centrifuged and rejected. The supernatant liquid was then decomposed with H_2S and the filtrate concentrated to a small volume under reduced pressure at a temperature of $40\text{--}50^\circ$ in order to remove the H_2S . After diluting the residue to its original volume with distilled water

and filtering off the little suspended matter, this preparation (preparation B) was ready for dosing. To another portion of the supernatant fluid, from the neutral lead precipitate, which was not decomposed with H_2S , normal sodium hydroxide was added until the reaction was brought up to p_{H} 8.2. The precipitate formed was suspended in water, decomposed with H_2S , concentrated as before to a few cc. and made up to its original volume with distilled water (preparation C) and dosed. To a portion of this solution 35 % of its volume of neutral lead acetate was added, and the reaction was again adjusted with sodium hydroxide to p_{H} 8.2. The precipitate thus formed was decomposed and treated as before and the resulting preparation (preparation E) tested. These preparations were made up daily, except during the week-end when double doses were given, and were administered to guinea-pigs as soon as they were ready. Doses equivalent to 1.5 cc., 3 cc., 5 cc. and 7 cc. were tested in each case. Preparation A was fully protective for 58 days even in equivalents of 1.5 cc. and promoted growth. Preparation B was protective in equivalents of 7 cc. doses only, very slight protection was afforded by the equivalent dose of 5 cc. of juice; in preparation C only one animal receiving an equivalent dose of 7 cc. lingered on for 55 days and finally died of scurvy, all the other animals in this test succumbed to the disease in 30–40 days. Preparation E showed no signs of activity even in doses equivalent to 7 cc. of the juice.

It is thus seen that although small batches were worked up daily under conditions which made it possible to take the greatest precautions against the spontaneous inactivation of the vitamin, no significant activity could be demonstrated even in the first precipitate. This result was not unexpected since this cabbage juice was found to possess, after the removal of the chloroplasts and proteins, a reducing capacity which varied somewhat with the amount of juice yielded by the leaves often from day to day within very wide limits (4–23 cc. of phenolindophenol per 5 cc. of solution) and which was usually low as compared with lemon juice and swede juice.

Even batches of cabbage juice which showed the highest reducing titres after being cleared with neutral lead acetate very rarely yielded a second precipitate with a reducing capacity characteristic of active fractions. It is conceivable that higher activity could have been obtained in preparation B and possibly some activity in the first precipitate (preparation C) if a juice of uniform high reducing capacity were obtained after clearing with neutral lead acetate. The above experiments, however, demonstrate the difficulty of preparing active fractions beyond the first precipitation in the neutral or slightly alkaline zone, even in highly reducing juices.

The spontaneous inactivation of the antiscorbutic factor in autoclaved decitrated lemon juice.

The series of experiments about to be described were instituted with the object of testing the hypothesis that the accelerated inactivation of the antiscorbutic factor in neutral solution produced by the previous autoclaving of

the decitrated lemon juice is due to a chain of reactions in which the thermostable peroxidase and a substance formed during the heating are concerned. Previous attempts [Zilva, 1929] have shown that the addition of autoclaved decitrated lemon juice or of quinol to unheated juice had a similar inactivating influence. In these experiments the effects of the addition of quinhydrone, benzoquinone or an ethereal extract from autoclaved juice were studied. In Exp. 1, quinhydrone (30 mg. per 50 cc. of juice) was added and the solution kept at p_H 7 for 24 hours in the cold room before dosing. In Exp. 2, benzoquinone (10 mg. per 50 cc. of juice) was added and the solution stored as in Exp. 1. In Exp. 3 the ethereal extract from decitrated lemon juice which had been heated at 143° for one hour in an autoclave was added to unheated decitrated lemon juice and stored in the cold room for 7 days at p_H 7. The preparation and addition of this extract were carried out as follows.

50 cc. of autoclaved decitrated lemon juice were shaken in an atmosphere of nitrogen with 75 cc. of ether for about 45 min. The separated ethereal layer was then added to 50 cc. of decitrated juice, adjusted to p_H 6, and the ether removed by means of a water-pump at room temperature. The resulting brown solution contained a trace of matter in suspension which was not filtered off. After adjusting the reaction to p_H 7, the preparation was stored at this reaction for 7 days before dosing. The ethereal extract did not reduce phenolindophenol but decolorised iodine.

The addition of the quinhydrone or the benzoquinone had a very deleterious action on the reducing principle, since hardly any phenolindophenol was decolorised after 24 hours' storage at p_H 7. The oxidising effect of the ethereal extract from the autoclaved juice was slower but the reducing principle was usually absent after 7 days' storage.

In Exps. 1 and 2, 1.5 cc., 3 cc. and 5 cc. doses were tested, using three guinea-pigs on each dose. In Exp. 3, the 3 cc. and 5 cc. doses only were tested on the same number of animals. All the guinea-pigs in the above experiments succumbed to scurvy within one month. A control set of animals showed that the decitrated lemon juice was active immediately after the addition of the ethereal extract.

The effect of hydrochloric acid on the stability of the antiscorbutic factor.

Experience has shown that the manipulation of the antiscorbutic factor in a medium of high hydrogen ion concentration has, presumably, no deleterious effect on the vitamin within short periods of time. In view, however, of Williams and Corran's [1930] observation that lemon juice, to which hydrochloric acid was added to lower the p_H to less than 1.8, when stored for a few months lost a great part of its antiscorbutic potency, it was thought necessary to devise an experiment with the direct object of ascertaining whether any appreciable loss takes place under these conditions of acidity in the space of a week.

The reaction of the daily doses of the decitrated lemon juice was adjusted to approximately p_H 1 by the addition of 4*N* hydrochloric acid. The limits during the entire testing periods were p_H 0.96 and p_H 1.12. The solution was then stored in the cold room for 7 days under aerobic conditions. At the end of this time the hydrogen ion concentration was once more determined and it was usually found that when a change in reaction did take place it was of the order of no more than 0.1 p_H . During the storage the reducing capacity of the juice fell from about 25 cc. to about 16 cc. of phenolindophenol per 5 cc. of juice. The biological test (1.5 cc., 3 cc. and 5 cc. with three animals on each dose) revealed that the guinea-pigs were protected from macroscopic lesions of scurvy for 60 days and that all the animals grew during the test period. The loss in the antiscorbutic potency during a week was, therefore, hardly appreciable. The phenolindophenol titration figures seem to suggest further that the loss of the reducing principle was even somewhat smaller under the above conditions of storage than when decitrated lemon juice is stored at p_H 7. An experiment in which the juice was stored for a week at p_H 0.6–0.8 yielded similar results. Thanks are due to Miss M. F. Bracewell for carrying out the electrometric determinations in this experiment.

DISCUSSION.

The experiments described in this communication indicate to some extent the difficulties associated with the fractionation of the antiscorbutic factor. Whilst these difficulties may impede the further concentration of the active principle they are at the same time instrumental in the unfolding of an aspect of the subject which promises to reveal some very interesting facts concerning the character and mode of action of the vitamin. The results recorded by the author during the last few years leave little doubt that the activity of the vitamin is controlled by the presence of the reducing principle which in itself is not antiscorbutically active. Preliminary attempts to replace the reducing principle after mild oxidation have so far failed to activate antiscorbutic fractions which have lost their potency through the destruction of the former. Although this failure, considering the complexity of the subject, may not be final, it is nevertheless not unreasonable to assume at this stage of the inquiry that the reducing substance acts irreversibly as a protective agent against the oxidation of the vitamin. In addition, further evidence is accumulating which suggests that, at least in a neutral medium, the reducing substance may be oxidised by means of a peroxidase and a substance acting like an organic peroxide, the latter being formed from a compound present in the autoclaved juice by exposure to air. This compound is possibly of a phenolic character and formed in the process of autoclaving, since the addition not only of the ethereal extract of autoclaved juice, but of quinol or its immediate oxidation products, quinhydrone or benzoquinone, accelerates the destruction of the reducing principle and of the vitamin. The chemistry of this and of the reducing principle is being studied further.

If it be assumed that the destruction of the antiscorbutic factor takes place in the series of reactions described above, then the stability of an active solution can be roughly gauged by the amount of phenolindophenol it reduces. Iodine, on the other hand, is decolorised both by the reducing principle and by the substance or substances formed on autoclaving, and consequently the amount reduced does not necessarily indicate the stability of an antiscorbutic fraction; it may under certain circumstances even serve as an index of its impending speedy inactivation. It is, in fact, possible that the relation of the decolorising capacity of iodine to that of phenolindophenol may be found to be indicative of the stability of an antiscorbutic solution even before heating.

So far this hypothesis of the inactivation of the antiscorbutic factor has been discussed here mainly in relation to the concentration of the active principle with the ultimate purpose of isolating and identifying it. If a chain of reactions similar to that outlined above does actually take place in the expressed juice, may it not be, as has already been suggested [Zilva, 1927], an indication that a cycle of similar reactions, in which the vitamin forms a link, takes place in the living cell of the plant? Further, may not the onset of scurvy in the animal be prevented not by the ingestion of a single principle, which the organism cannot synthesise, but by a number of links active in the cycle of reactions of the plant cell? It is hoped that information obtained in the inquiries which have been pursued by the writer and his colleagues on the function of the vitamin in the plant and in the animal may answer this.

SUMMARY.

1. Several years' work on the fractionation of the antiscorbutic factor is reviewed and it is shown that variable activity is obtained under presumably the same conditions of fractionation.

2. Batches of lemon juice sometimes yield fractions of low or no antiscorbutic activity when equal volumes of a saturated neutral lead acetate solution are used in the manipulation. It is, however, possible by adjusting the quantity of the precipitating reagent to obtain from the same juice preparations of high activity. The conditions must therefore be adjusted in each case in order to obtain uniform high activity.

3. Fractions of which the phenolindophenol decolorising capacity falls below a certain limit are found antiscorbutically inactive. This reducing property, however, is not an index of antiscorbutic activity itself but serves rather as an indication that the vitamin has been protected from inactivation.

4. The reprecipitation of an antiscorbutic fraction from lemon juice or from cabbage juice with lead acetate in the neutral or slightly alkaline zone (p_H 8-9) yields an inactive preparation.

5. The addition of an ethereal extract from autoclaved lemon juice, which decolorises iodine but not phenolindophenol, of quinhydrone or of benzoquinone to unheated lemon juice accelerates markedly the destruction of the reducing principle and of the antiscorbutic factor in a neutral medium in the presence of

air. It is possible that a substance of phenolic character, oxidisable in the air, is formed in the process of autoclaving. This compound in its oxidised form destroys, in conjunction with the peroxidase present in the juice, the reducing principle that acts as an agent for the protection of the antiscorbutic factor.

6. Decitrated lemon juice kept aerobically at p_H 1 or at p_H 0.6–0.8 for 7 days does not lose its reducing capacity for phenolindophenol to a greater extent than when kept at p_H 7 and shows little loss of its antiscorbutic activity.

REFERENCES.

- Bezssonoff (1922). *Compt. Rend. Acad. Sci.* **175**, 846.
— (1923). *Bull. Soc. Hyg. Aliment.* **11**, 14.
— (1925). *Compt. Rend. Acad. Sci.* **180**, 970.
Grettie and King (1929). *J. Biol. Chem.* **84**, 771.
Sipple and King (1930). *J. Amer. Chem. Soc.* **52**, 420.
Szent-Györgyi (1928). *Biochem. J.* **22**, 1387.
Williams and Corran (1930). *Biochem. J.* **24**, 37.
Zilva (1924). *Biochem. J.* **18**, 632.
— (1927). *Biochem. J.* **21**, 689.
— (1928). *Biochem. J.* **22**, 779.
— (1929). *Biochem. J.* **23**, 1199.