CLXXV. CHANGES IN THE ASCORBIC ACID AND GLUTATHIONE CONTENTS OF STORED AND SPROUTING POTATOES.

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THE indication given by Guthrie [1932] that the glutathione content of potatoes increases on sprouting has been investigated. Ascorbic acid has been included in the study and the exact time relations of this change, as well as the effects of storing the potatoes at different temperatures are here reported.

The ascorbic acid and glutathione contents of potatoes seem to be at a maximum just at maturity and to decline gradually on storing even when the potatoes are refrigerated, tending to come to a certain low plateau value. Forced or natural sprouting at any point in this decline, or plateau, resulted in a marked rise to rather high values, followed by a rapid drop to the low level again. Remarkable similarity between the changes in glutathione and in ascorbic acid has been observed.

The observed instability of ascorbic acid (vitamin C) on storage is of the greatest importance from the viewpoint of the nutritive value of potatoes.

EXPERIMENTAL.

Storage. Newly lifted potatoes were scrubbed, dried and stored at 15°, 10° or 5°. The author is grateful to the Director of the Low Temperature Research Station, Cambridge, for the opportunity of using their facilities and to Dr J. Barker, of the Station, for his advice and interest. Early potatoes were Duke of York variety, the late were at first Great Scot, later King Edward.

Sprouting. Moist sand in flat pans at 15–16° was used. The potatoes were usually cut in two. Late potatoes, which have a non-sprouting period of about 6 weeks after digging, were forced to sprout by treatment with ethylene chlorohydrin [v. Guthrie, 1932].

Occasional moisture determinations varied from 75·2 to 73·3% during the 5–8 days of sprouting. Results are not corrected for moisture except in the case of potatoes which had been stored 3 months.

Extraction. The potatoes, usually 4–6 in number, were scrubbed, cut into cubes of about 1 cm. dimensions, mixed and sampled by quartering until a quantity of about 50 g. remained. 25 g. were at once weighed into a beaker and just covered with 10% trichloroacetic acid, or 3% salicylsulphonic acid, (which has a slight stabilizing effect on the ascorbic acid). As soon as convenient the cubes were put in a mortar, smashed and ground to a pulp with sand. The pulp was quantitatively transferred to a graduated cylinder, rinsing with the acid from the beaker and the whole made up to a definite volume, usually 40 ml. Frequent vigorous shaking for an hour, followed by suction filtration yielded a slightly opalescent filtrate of about 30 ml. This filtrate contained 96–98% of the glutathione, and 85–92% of the ascorbic acid and, in general, was used directly

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or the present work. A second extraction of the residue in 10–15 ml. trichloroacetic acid yielded most of the remaining ascorbic acid. The procedure of Guthrie & Wilcoxon [1932] of dropping cubes into boiling water was found to give seriously lower values for both GSH and ascorbic acid, indicating destruction. The increased ascorbic acid values found by McHenry & Graham [1935] were possibly due to difficult end-points with a cloudy solution and adsorption of dye on starch.

Estimation of ascorbic acid. An aliquot of the extract after reduction and adjustment to ca. *p*H 2, was titrated with 2:6-dichlorophenolindophenol of about 0·004 % strength (the Tillmans titration). The dye was made up in *M*/500 phosphate buffer at *p*H 7·0, and stored at 0°. After the original standardization with pure ascorbic acid it was tested daily with a solution of *M*/10 ferrous ammonium sulphate, dissolved in *M*/500 H_2SO_4, covered with paraffin oil and set up permanently connected to the burette. The dye solution remained fairly constant for 3 weeks or more.

The dye was usually used in the burette. With small amounts of ascorbic acid in the test solution it is convenient to make a standard solution of pure ascorbic acid, add to the extract a given volume (an excess) of dye, and immediately back-titrate with standard ascorbic acid. By this procedure only quick-reacting substances are determined, and this makes the method more specific, since ascorbic acid reduces the dye much faster than other substances liable to be present, such as cysteine and glutathione [Hopkins *et al.*, 1935]. The results are expressed as mg. ascorbic acid per 100 g. fresh tissue, recognizing the possibility that not ascorbic acid alone was determined. The procedure of preliminary precipitation with mercuric acetate, recommended by Van Eekelen & Emmerie [1936], invariably gave results higher than the above and not as uniform. Recently Wachholder & Podesta [1936] have suggested that the Tillmans titration should be discarded as too unspecific. Their criticism is based on comparison with colorimetric methods, which are themselves not of proved specificity and are considered better for the simple reason that they give lower values.

Estimation of glutathione. The extract, or a portion of it, was treated with powdered zinc, shaken a few minutes and filtered. An aliquot, usually 10 ml., received 2 drops of 1 % starch, 2 ml. of 5 % KI and sometimes 1 ml. of *N* H_2SO_4. It was titrated with KIO_3 of suitable strength, made by diluting (usually 1 : 10) a stock solution containing 0·214 g. per 100 ml. The latter was standardized against cysteine or glutathione.

The figure thus obtained was presumed to represent the combined ascorbic acid and glutathione contents and was corrected for ascorbic acid. The results, calculated as mg. GSH per 100 g. fresh tissue, thus represent the total iodine titration less a correction. They are called glutathione with the reservation that the method greatly lacks specificity.

Results.

1. *Preparation of glutathione from sprouted potatoes.*

The results of Guthrie [1932] in preparing crystalline glutathione from sprouted potatoes have been confirmed, using about the same procedure. As pointed out by Guthrie this is the only known case of actual isolation of glutathione from a plant tissue. The white crystalline material obtained, which gave a strong nitroprusside reaction, contained 10·0 % S, but was not subjected to further analysis.

2. *Effect of sprouting fresh stock potatoes.*

An increase in glutathione during germination has been mentioned chiefly by Guthrie [1932, 1933]. An extensive literature, however, is available relating to vitamin C and germination. No previous study of potatoes has been found.

Fig. 1 shows that a large and rapid rise occurs in the glutathione and ascorbic acid contents of sprouting potatoes, reaching a maximum at 2 days, followed by

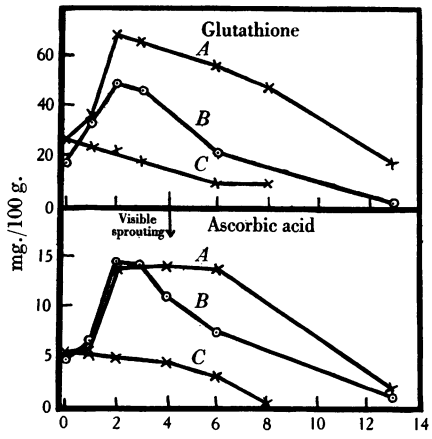

Fig. 1.

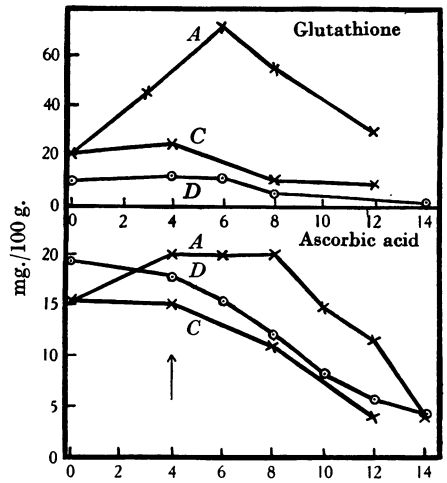

Fig. 2.

Fig. 1. Changes in the glutathione and ascorbic acid of potatoes during sprouting. *A*, Late (treated to sprout); *B*, early (sprouting); *C*, late (not sprouting).

Fig. 2. Changes in the glutathione and ascorbic acid of potatoes during sprouting. *A*, Late (treated to sprout); *C*, late (not sprouting); *D*, early (not sprouting).

a gradual decline to a very low value after 13 days. If no sprouting occurred no rise occurred. The same general effect has been observed many times but the exact time relations and the shapes of the curves may vary considerably. This is shown by Fig. 2.

3. *Effects of sprouting stock which had been stored.*

This stock had been stored 3 months at 5° and had dehydrated somewhat, containing 56 % moisture. The moisture content varied during sprouting and the results were calculated on the dry weight. The usual large increases in GSH and ascorbic acid were found even when calculated on fresh weights. The difference between cut and uncut stock was here more pronounced than usual, but in all previous cases some lag of the uncut had been observed. It is, of course, known that in cut potatoes respiration is greatly increased and possibly some metabolic processes are hastened with which the above substances are concerned.

4. *Effect of storing potatoes at 5°, 10° and 15°.*

The original plan of the investigation did not include a complete study of this aspect of the problem, and systematic results are not available. A more complete study, including animal tests, is planned for the near future.

The effect of storage on glutathione seems not to have been investigated before. On the other hand, the stability of ascorbic acid has been extensively studied in many tissues, though notably seldom in potatoes.

McKittrick & Thiessen [1932] reported some loss of vitamin C in potatoes after storing 6 months, but exact figures are not given. Yanovska [1933], to protect guinea-pigs from scurvy, found that 4 times more of stored potatoes were needed than of fresh. Murri *et al.* [1934] found that cranberries at 0° and 8° lost some of their vitamin C, apples at 10° lost all, whilst potatoes lost none. Recently Woods [1935] reported no loss of vitamin C after 3 months' storage. In this case the initial rapid drop may have been over at the start of the experiment.

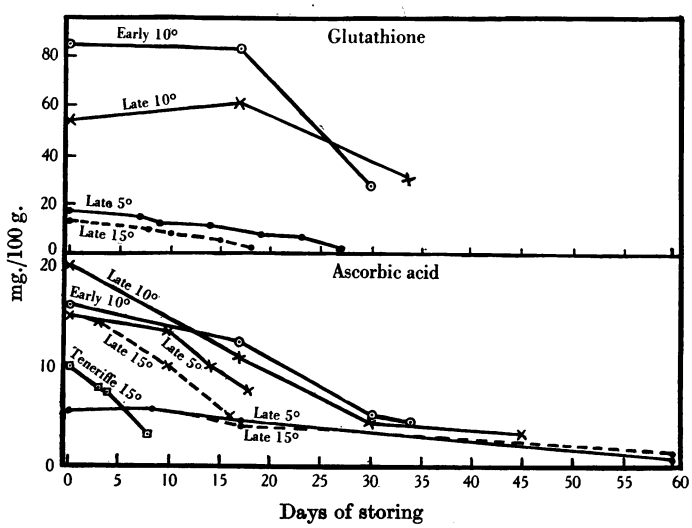

Fig. 3. Changes in the glutathione and ascorbic acid contents of different stocks of potatoes stored at 5°, 10° and 15°.

Fig. 3 shows the relation between time of storing at 5°, 10° and 15° and the contents of ascorbic acid and GSH, using different stocks of potatoes. The loss was rapid at all temperatures, though slightly more at 15°, the rate depending on the initial value. The ascorbic acid shows a tendency to come to a certain level, after which a much slower decrease occurs. In Fig. 3 the potatoes at 5° were stock purchased late in the season and had presumably reached the level obtained in 30 days by the other stock. The results also suggest a variation in different varieties of potatoes and possibly too in the same variety grown on different soils, but this has not been thoroughly studied. Such variations were suggested for vitamin C in potatoes by Richardson *et al.* [1931].

Discussion.

The results have a practical significance in their bearing on nutrition. The importance of vitamin C in diet needs no discussion, and the suggestion has been made that potatoes are the chief source of this vitamin for poor families. If present methods of storing threaten even this source, as suggested by these results, other methods should be explored. The results on sprouting potatoes indicate a possible solution of the problem, since a very short period of sprouting

(2–4 days), which was not sufficient to show any change in the "eyes" of the potatoes, restored a good part of the vitamin. On the other hand, once visible sprouting begins the ascorbic acid content falls rapidly to the vanishing point.

The results have also a theoretical importance in clearly demonstrating for the first time the great increase in glutathione during the sprouting of potatoes. A similar increase in germinating wheat seeds has since been observed and will be reported elsewhere. At the present time it is difficult to suggest specific reasons or functions associated with these changes. The increase is made before real sprouts appear and appears to be generalized throughout the tuber. The ascorbic acid content of the sprouts after about 2 weeks is higher than that of the tuber tissue, but the glutathione content is lower, this being the only point at which the usual parallelism between these two fails.

It is, indeed, of especial interest that the ascorbic acid and glutathione contents undergo the same changes, rising and falling together, in view of the intimate relationships found by Bersin *et al.* [1935] in animal tissue and by Hopkins (unpublished). It seems unlikely that a chemical reaction between the two is involved, but since the presence of GSH protects ascorbic acid from reversible oxidation (Hopkins), and since the total amounts of the two change simultaneously, it is possible that they are linked in action by being thermodynamically suitable to each other, with redox potentials in advantageous relation.

SUMMARY.

1. Sprouting of potatoes is associated with a large rise in the content of ascorbic acid (vitamin C) and of glutathione, followed by a decrease. Time curves for these changes are given. This occurs even after storing potatoes some months at 5°.

2. Potatoes stored at 15°, 10° or 5° rapidly lose ascorbic acid and glutathione, reaching a level at 20–30 days after which the loss is much slower. The importance of this loss from a nutritive standpoint is discussed.

3. Guthrie's isolation of crystalline glutathione from sprouted potatoes has been repeated.

The author is grateful to Sir F. G. Hopkins for his interest and advice.

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Note added 4 July 1936. Olliver [*J. Soc. chem. Ind.* 1936, **55**, 153], in an investigation of cooking and storing effects, found a rapid decrease in the ascorbic acid of stored potatoes, thus confirming the above results.