155. THE DISTRIBUTION AND CONCENTRATION OF ASCORBIC ACID IN THE POTATO (*SOLANUM TUBEROSUM*)

BY ALEXANDER MARTIN SMITH AND JOHN GILLIES

From the Chemistry Department, Edinburgh and East of Scotland College of Agriculture

(Received 29 July 1940)

SINCE the development of simple methods of estimating ascorbic acid, most fruits and vegetables have been assayed; a summary of the results has recently been prepared by Fixsen & Roscoe [1940]. Giroud [1938] reports a positive correlation in plant tissues between the ascorbic acid and the carotenoids and chlorophylls; Hausen [1935] states that the growth of plants is increased by the addition of ascorbic acid to the culture solution, whilst Virtanen [1936] finds that the concentration of the vitamin reaches a maximum during the early stages of growth. McHenry & Graham [1935] have found that ascorbic acid may be present in the reversibly oxidized form.

The values for potato tubers given by various investigators vary between about 5 and 40 mg. per 100 g. of tissue. These rather wide limits, as suggested in a previous paper by Smith & Paterson [1937], are partly accounted for by such factors as variety, health and storage conditions, so that the results obtained by different workers are not directly comparable. Normally stocks of potatoes to be sold for seed purposes must reach a certain standard of purity and freedom from certain virus diseases and for this purpose growing crops are graded by inspection. This means that during the dormant season the presence of virus in the tuber can be shown only by grafting experiments which take a long time to complete. The object of the present investigation was to make a comprehensive examination of the ascorbic acid content of the potato to see whether the incidence of virus infection were related to ascorbic acid content as had been indicated in earlier studies.

Methods

The indophenol method. Many methods for the determination of ascorbic acid have been described in recent years and, while most of them are based upon the reduction of 2:6-dichlorophenolindophenol, some workers make use of ascorbic acid oxidase known to be present in most plants. For the present investigation the indophenol method was adopted on account of its suitability for rapid routine determinations. It may be mentioned that indicators bearing the same name from two sources have been found to have different constitutions; the sodium salts had molecular weights of 290 and 340 so that the corresponding concentrations of 0.0005 *M* solutions were 0.0145 and 0.017 % respectively. The indicator was standardized by the method introduced by Menaker & Guerrant [1938]. This method compared well with the original method of Harris & Ray [1933] and had the advantage of being more easily carried out.

Tuber tissue. The methods of extraction depended upon the purpose of the experiment and the material examined. For example, to obtain representative

samples of tuber tissue, cylinders were cut in the direction of the major axis with a cork borer and portions of them were cut off with parallel knives, each portion weighing about 2 g. For the purpose of obtaining an idea of the concentration of ascorbic acid these pieces, which were always of the same dimensions, were simply shaken up with 10 ml. of 2% trichloroacetic acid for 1 min. and the supernatant liquid was titrated with standard indophenol. The results so obtained are referred to as "indophenol values". For complete extraction, the cores were immediately ground to a pulp with about 25 ml. of trichloroacetic acid and about 8 g. of pure sand. The extraction was repeated three times, the extract being filtered through glass wool into a standard flask and made up to a definite volume. It was sometimes necessary to centrifuge the samples and titrate an aliquot portion of the clear liquid.

Sprouts and leaves. The method used for sprouts and leaves was the same as for tuber cores but the amounts of material were much smaller and a micro-burette was used for the titrations. Many of these extracts were deeply coloured and had to be specially dealt with. The method used was based upon that introduced by McHenry & Graham [1935] which depends upon the fact that the plant pigments are insoluble in CHCl_3 . The technique was essentially that of Olliver [1938], the ascorbic acid solution being placed in a narrow tube with 2 ml. of CHCl_3 . Stirring was carried out by a stream of N_2 and the indicator was added until the CHCl_3 layer developed a definite pink colour. It was found that thorough stirring of the extract and the indicator was advisable before mixing the CHCl_3 and aqueous layers and sometimes it was necessary to centrifuge in order to break the emulsion.

Dehydroascorbic acid. A certain amount of dehydroascorbic acid was almost always found in potato tubers, although the amount varied considerably during the season. The total ascorbic acid was determined by passing H_2S for 15 min. into the extract (frothing could be reduced by the addition of 2 ml. of isopropyl alcohol) and allowing it to stand overnight in a stoppered flask. The excess of H_2S was then driven off with a stream of oxygen-free N_2 before carrying out the indophenol titration. The dehydroascorbic acid was calculated by subtracting from this total value the amount of ascorbic acid obtained by the usual method. There seems to be no reason to suspect that other reducing substances are formed in tuber extracts by this treatment; little or no dehydroascorbic acid could be found, for example, in tubers examined immediately after harvesting.

Freezing. The above processes are rather tedious for the examination of large numbers of samples and an attempt was made to save time by freezing the samples. Solid CO_2 was first used, the cores being packed for a few minutes into holes cut in a solid block of CO_2 ; this offered no advantage over the usual method because the frozen cores had subsequently to be ground up. Liquid air was also tried, the cores being placed in a small muslin bag or test tube, immersed in the liquid and then ground. A serious loss of ascorbic acid, however, always resulted from the freezing, the loss not being entirely accounted for by the formation of dehydroascorbic acid. For example, the average amount of ascorbic acid for 4 varieties was 7.5 mg. per 100 g. of unfrozen tissue and only 1.8 mg. per 100 g. of material after freezing; the amount of reversibly oxidized ascorbic acid accounted for only about half of the difference. Mills [1932] found similar results when liver was frozen. It is perhaps worth noting that a pure solution of ascorbic acid in 2% trichloroacetic acid did not show this loss of ascorbic acid on freezing except in presence of tuber tissue.

Results

Diurnal fluctuations in leaves. Preliminary tests showed that the hour of sampling had an important effect on the ascorbic acid content of the leaves and stem. A few plants of each variety were marked and leaflets were taken at intervals and immediately placed in trichloroacetic acid. The determination of the ascorbic acid was carried out as already described. The two varieties used were Kerr's Pink and Epicure and samples were taken in the various experiments at intervals of up to 48 hr. The tests were conducted about the middle of June when the tubers were beginning to form and the plants were about 18 in. high. Figs. 1 and 2 illustrate the nature of the results obtained and show that the

Fig. 1. Diurnal fluctuation of concentration of ascorbic acid in leaves of Kerr's Pink.

Fig. 2. Variation in concentration of ascorbic acid in leaves and stem of Kerr's Pink and Epicure with time.

fluctuations in the concentration of ascorbic acid are not unlike those for the nitrogen [Barton-Wright & McBain, 1933] and carbohydrate [Cockerham, 1939] in the plant. The concentration reaches a maximum early in the forenoon; the top of the plant has almost always a higher concentration than the lower part, the variations at the base of the stem being comparatively small.

Seasonal variations. Samples were always taken at 10 a.m. starting about the beginning of July and continuing until the end of the growing season. At one centre, both healthy tubers and tubers infected with severe mosaic and leaf roll belonging to 3 varieties (Arran Pilot, Arran Consul and Dunbar Cavalier) were planted and the leaflets nearest the growing tips of numbered plants were sampled along with tubers from the same plants. The figures showed that, on an average, the concentration of ascorbic acid in the leaves was twice as great as that in the tubers. The differences between healthy and diseased plants were rather irregular. In the second locality, 7 varieties were planted and the results are given in Table 1. It is obvious that the concentration of ascorbic acid in the young tuber varies with the stage of development, the maximum being reached some time in August, depending upon whether the variety is early, second early or late. There follows a sharp fall in concentration as the haulms ripen and die down. At all stages in the growth, the leaves have a much higher concentration than the tubers.

Table 1. *Ascorbic acid (mg./100 g.) in leaves (L) and tubers (T) at different times during the growing season*

Date ...	4 July		26 July		31 Aug.		14 Sept.	
Variety	L	T	L	T	L	T	L	T
Arran Pilot	120	27	101	14	59	30	—	17
Great Scot	84	17	76	25	54	26	—	18
Kerr's Pink	—	—	75	25	51	31	—	14
Arran Banner	—	—	90	20	60	34	—	22
Redskin	—	—	46	21	60	24	—	14
Majestic	—	—	49	21	52	30	—	14
Dunbar Cavalier	—	—	62	21	66	24	—	14

Effect of manuring. Ten plots were laid down in duplicate. The variety of potato chosen was Redskin of stock seed standard¹. In addition to a basal dressing of farmyard manure, varying amounts of N, K and P were added in the form of ammonium sulphate, potassium sulphate or nitrate and superphosphate. The tubers were harvested at the end of September, stored at a temperature of 8–10° for a period of 3 weeks and the concentration of ascorbic acid was determined as previously described. The results varied between 14.4 and 16.7 mg. per 100 g. of tissue, but it was not possible to correlate the differences with manurial treatment. This agrees with the results previously found by Smith & Paterson [1937]. Wachholder & Nehring [1938] also reported that farmyard and artificial manures made no substantial differences to the ascorbic acid content of a number of varieties of potatoes, whilst Virtanen [1936] found that, although unbalanced manuring lowered the vitamin C content of potato tubers, any adequate treatment gave almost constant values.

The ascorbic acid in mature tubers

The effect of storage. It was impossible to examine all the material immediately after harvesting and consequently it was necessary to discover how the results might be affected by storage. From previous work there was reason to suspect that the ascorbic acid decreased considerably during storage and that the amount of dehydroascorbic acid was very small at the time of lifting. The latter conclusion was confirmed by determining the amount of dehydroascorbic acid in a series of tubers of each of 6 varieties immediately after lifting in September. It is

¹ This is the highest standard of purity and health as recognized by field inspection.

unnecessary to give all the figures for they were remarkably uniform for the different varieties; the average values for ascorbic and dehydroascorbic acids were respectively 16.5 and 0.3 mg. per 100 g. of tissue. Further determinations, however, showed that the amount of dehydroascorbic acid increased fairly rapidly during storage. The results are given in Table 2 which shows the ascorbic and dehydroascorbic acid contents after different periods of storage at temperatures varying between 2° and 8°. In every case there was a substantial decrease in the amount of ascorbic acid during storage, the values after storing for 6 months being only about one-third of those after 1 month's storage. Similar results have recently been reported by Zilva & Barker [1939] in connexion with one variety which they studied. The proportion of dehydroascorbic acid increased during storage and amounted, on the average, after 6 months to more than a third of the ascorbic acid. This increase in dehydroascorbic acid does not account for the observed loss of ascorbic acid. In fact the amount of dehydroascorbic acid tends to remain constant, which would seem to indicate that it is formed at the expense of ascorbic acid and then further oxidized to an irreversibly oxidized form.

Table 2. *Ascorbic (A) and dehydroascorbic acid (D) in mg. per 100 g. tuber tissue after different periods of storage*

Storage time ... Variety	4 weeks		2 months		4 months		6 months	
	A	D	A	D	A	D	A	D
Arran Consul	16.4	2.9	8.7	1.6	4.9	3.9	5.2	2.7
Arran Chief	—	—	10.8	1.2	5.7	2.3	6.4	2.1
Di-Vernon	—	—	8.8	1.6	6.2	1.3	7.5	3.8
President	—	—	—	—	6.7	—	5.6	2.7
Dunbar Cavalier	13.9	3.3	11.3	0.7	7.1	0.8	6.3	3.4
Majestic	15.4	2.9	8.6	1.4	7.4	1.5	5.5	1.7
Doon Star	—	—	12.7	4.1	7.6	0.9	5.3	4.2
Epicure	—	—	—	—	7.7	1.2	5.2	1.8
Dunbar Standard	—	—	—	—	7.7	1.2	7.5	2.8
King Edward	23.4	1.1	18.8	2.4	8.1	1.1	5.6	2.4
Duke of York	—	—	—	—	8.3	1.8	7.4	2.4
Up-to-Date	19.2	2.0	—	—	8.5	1.7	8.0	1.8
Arran Banner	—	—	8.6	3.9	8.7	1.1	7.8	2.2
Gladstone	—	—	—	—	9.3	1.4	6.4	1.2
Redskin	26.7	1.6	12.2	1.6	9.7	2.9	5.1	4.4
Golden Wonder	19.4	0.6	10.9	1.8	9.8	1.7	6.6	2.6
Great Scot	20.1	4.3	10.4	1.6	—	—	7.2	1.8
Kerr's Pink	22.4	2.0	12.9	3.0	—	—	9.5	1.5
Average	19.7	2.3	11.2	2.1	7.7	1.5	6.6	2.5

The effect of variety. After any particular period of storage there are differences associated with the varieties which in many cases are outside the range of experimental error. It would be dangerous to emphasize these differences too much on account of the very large part played by storage. It will be noticed, for example, that the order of varieties according to ascorbic acid concentration is not constant at the various periods of storage. The results given in Table 2 are average figures obtained from several tubers from one source so that the influence of locality is not shown. Tubers from 4 other sources were also examined but the analyses unfortunately extended over a period of about 6 weeks and the data are consequently not strictly comparable. There was, however, a marked tendency for the results from one source to be higher than those from the others, but it is not known whether this was due to stage of maturity when lifted or to conditions of storage. It is unlikely that the difference was due, to climate because "stock

seed" is generally produced in the northern counties and, as already indicated, differences in normal manuring practice would not affect the results.

The effect of disease. One of the objects of the investigation was to examine the correlation, suggested by earlier work, between the amount of ascorbic acid in the tuber and the presence or absence of virus disease. The greatest difficulty in this type of work is the obtaining of tubers which can be guaranteed to be free from infection or to be infected with known viruses. For the most part we had to accept as healthy material tubers which had been placed in "stock seed" categories by field inspection of crops, whilst the infected material was taken from plants which were observed in the field to be suffering from severe mosaic or leaf roll. It has been shown [Scott, 1938] that "stock seed" tubers are not necessarily free from virus, and it is well known that mosaic may be due to a combination of viruses. The results obtained from the examination of many varieties, healthy and infected with severe mosaic or leaf roll, are summarized in Table 3. It will be noticed that after 1 month's storage 13 out of 17 varieties examined show a greater concentration of ascorbic acid in the infected than in the healthy tubers. After 4 months' storage, the results are not so striking and those for ascorbic and dehydroascorbic acid together are not more conclusive. Indophenol values were also obtained by the quick method of extraction previously described, and here it will be seen that most varieties again show a higher value for infected than for healthy material. The results for leaf roll material are not so complete but once again there is a tendency for the concentration of ascorbic acid to be greater in the diseased than in the healthy tubers.

Table 3. *Ascorbic (A) and dehydroascorbic acid (D) in mg. per 100 g. tissue and "indophenol value" (I) for healthy and diseased tubers after 1 and 4 months' storage*

Variety	Storage ...		1 month				4 months				1 month		
			A		D		A		D		Indophenol value		
			H	M	H	M	H	M	H	M	H	M	L.R.
Arran Consul			16.4	16.8	2.9	4.2	4.9	5.3	3.9	1.8	0.46	0.54	—
Arran Chief			15.4	16.1	—	—	—	—	—	—	0.45	0.52	—
Di-Vernon			—	—	—	—	—	—	—	—	0.56	0.87	—
Dunbar Cavalier			13.9	26.4	3.3	0.1	7.1	7.5	0.8	0.5	0.65	0.61	—
Majestic			15.4	18.4	2.9	3.1	7.4	7.2	1.5	0.7	0.44	0.41	0.37
Doon Star			19.0	21.0	—	—	7.6	6.9	0.9	4.3	0.46	0.51	0.49
King Edward			23.4	28.3	1.1	0.0	8.1	9.3	1.1	5.4	0.96	1.21	1.62
Duke of York			24.0	22.0	—	—	8.3	11.7	1.8	1.2	—	—	—
Up-to-Date			19.2	20.6	2.0	4.2	8.5	9.9	1.7	0.5	0.90	1.04	0.91
Arran Banner			22.3	25.0	—	—	8.7	7.7	1.1	4.0	0.89	0.94	1.04
Gladstone			22.4	19.3	—	—	—	—	—	—	0.93	—	1.02
Redskin			26.7	24.7	1.6	1.2	9.7	10.1	2.9	2.0	0.63	0.91	0.80
Golden Wonder			19.4	17.5	0.6	0.0	9.8	5.7	1.7	4.1	0.60	0.60	0.67
Great Scot			20.1	21.3	4.3	1.1	8.5	8.9	—	—	0.55	0.55	—
Kerr's Pink			22.4	29.8	2.0	2.0	11.8	9.3	—	—	1.07	1.27	0.99
Arran Pilot			18.8	23.3	13.8	5.5	9.8	9.5	—	—	0.72	0.70	—
Catrina			24.9	31.6	—	—	—	—	—	—	1.32	1.27	—
Sharpe's Express			24.1	25.2	—	—	—	—	—	—	1.14	1.37	—
Eclipse			—	—	—	—	—	—	—	—	0.55	1.05	0.53

H = healthy; M = severe mosaic; L.R. = leaf roll.

Several factors have to be borne in mind when considering these results. Some doubt must always exist with respect to the freedom of the healthy material from infection. Further, there is the question of the time of storage,

for, although storage in the laboratory could be controlled, it was not possible to obtain precise information from various growers as to the date of lifting and the conditions of storage between lifting and dispatch to the laboratory. These results should not, therefore, be stressed unduly; nevertheless, the recurrence of the difference between healthy and diseased material would seem to be more than a mere accident. In order to make a proper study of this question it would be necessary to carry out a long term investigation with material specially selected and tested and grown under those conditions which are necessary for carrying out virus research work.

Distribution of ascorbic acid in the potato tuber

Various sections of the outer layer and of the internal tissue of the tuber were examined. The concentration of ascorbic acid on the surface varied rather indefinitely but it was greater at the rose than at the heel end; in the inner tissue it was also greater at the rose than at the heel end, and, in fact, showed a consistent gradation. Typical results for a core running from the heel to the rose end and divided into 6 sections are given in Table 4.

Table 4. *Distribution of ascorbic acid within tuber tissue*

Section ...	Heel		Centre		Rose	
	1	2	3	4	5	6
Ascorbic acid (mg./100 g.)	4.3	5.5	6.8	7.1	7.6	8.1

These results were obtained after sprouting had begun and do not necessarily represent the normal distribution in the tuber throughout the dormant season.

Ascorbic acid in tubers and sprouts grown in the light and the dark. For the purpose of this investigation 12 tubers of each of 4 varieties were placed on trays exposed to the light and comparable lots of 12 tubers were kept on trays in the dark. The varieties were Golden Wonder, King Edward, Great Scot and Arran Pilot. After sprouting was sufficiently advanced, 6 sections were cut from each sprouted tuber. In every case when the tuber had been exposed to the light there was an increase in the concentration of ascorbic acid from the heel end of the tuber to the middle or tip of the sprout; when the tuber had sprouted in the dark the concentration gradient was not so steep and not quite so regular and the highest concentration was, with one exception, found in the middle of the sprout. The concentration of ascorbic acid in all cases was greater in the middle and tip of those sprouts exposed to the light than in the corresponding positions of those sprouts which had developed in the dark. Table 5 contains the average values for the 4 varieties and gives a very good idea of the nature of the gradient in tubers growing in the light and in the dark.

Table 5. *Distribution of ascorbic acid (mg./100 g.) between tuber and sprout*

Position ...	Tuber			Sprout		
	Heel	Middle	Rose	Base	Middle	Tip
Light	6.2	6.7	8.8	10.0	14.5	14.3
Dark	7.7	7.1	10.1	11.1	12.5	8.8

Source of ascorbic acid in the growing plant. Several tubers of each of 4 varieties were weighed and placed in trays exposed to the light and a corresponding set was stored in the dark. The varieties were Great Scot, King Edward, Golden Wonder and Redskin. At the start of the experiment the average concentration

of ascorbic acid in the tubers of each of the 4 varieties was determined so that the total amount of ascorbic acid in each tuber set aside to sprout was known. In due course the sprouts of each tuber were cut off, the sprouts and tubers weighed separately and the ascorbic acid contents determined. From these data it was possible to calculate how much ascorbic acid had been lost from the tuber since the start of the experiment and to say whether or not ascorbic acid had been synthesized. The results for 4 tubers of each variety are given in Table 6. They include figures for dehydroascorbic acid in the case of the variety Redskin.

Table 6. *Transfer of ascorbic acid from tuber to sprout*

Storage	Variety	Date ...	Tubers						Loss of A from tuber	Sprouts			Net loss of A
			14. ii. 40			6. v. 40				6. v. 40			
			W	A	D	W	A	D		W	A	D	
Light	Great Scot	500	53.0	—	421	41.6	—	11.4	12.8	2.8	—	8.6	
	King Edward	403	29.8	—	335	22.1	—	7.7	6.2	1.8	—	5.9	
	Golden Wonder	372	36.5	—	282	25.8	—	10.7	6.7	1.4	—	9.3	
	Redskin	469	51.6	13.1	376	40.5	6.7	11.1	8.7	1.8	1.2	9.3	
Dark	Great Scot	554	58.7	—	386	37.0	—	21.7	68.0	4.6	—	17.1	
	King Edward	366	27.1	—	303	23.9	—	3.2	12.5	1.8	—	1.4	
	Golden Wonder	385	37.8	—	281	23.5	—	14.3	25.1	2.2	—	12.1	
	Redskin	432	47.5	12.1	280	27.3	1.4	20.2	37.7	3.0	2.0	17.2	

W = weight of tubers or sprouts in g.

A = weight of ascorbic acid in mg.

D = weight of dehydroascorbic acid in mg.

These results confirm the conclusion drawn from the previous section, that there is a greater concentration of ascorbic acid in the sprouts grown in the light than in those grown in the dark. The actual weight of ascorbic acid present in the sprouts grown in the dark is greater, however, due to the much greater weight of sprout tissue. Over the 3 months during which sprouting had been going on there was an average net loss of ascorbic acid of 1.9 and 2.8 mg. per 100 g. original tuber tissue when sprouted in the light and dark respectively. This indicated that ascorbic acid had not been synthesized to any extent at this stage in the life cycle, but was simply passing from the tuber to the sprout. There was also a net loss of dehydroascorbic acid which was greater in the case of storage in the dark. It is possible that the loss is connected with the increase in respiration due to sprout formation and this would account for the greater loss in the case of the long etiolated sprouts formed in the dark.

SUMMARY

The concentration of ascorbic acid in the leaves fluctuates considerably during the day but shows a maximum value in the early forenoon; it is on the average much greater in the leaves than in the tubers. In the tubers it reaches a maximum in August and falls quickly as the plant ripens off. During storage the value decreases until, after 6 months, it is only about one-third of the value at the time of harvesting. Variations in normal manuring practice do not produce significant effects. There is usually a greater concentration in tubers from plants affected with severe mosaic or leaf roll than in tubers presumed to be healthy. The concentration of ascorbic acid increases from the heel to the middle or tip of the sprout; the gradient is more pronounced when sprouting has taken place in the light than when it has occurred in the dark. There is apparently no synthesis of ascorbic acid during the early stages of sprouting but rather a net loss which is greater when sprouting has taken place in the dark.

Part of this investigation was carried out with the aid of a grant from the Agricultural Research Council and the Department of Agriculture for Scotland for which the authors are grateful. They would also like to acknowledge their indebtedness to Dr Cockerham, Dr Robertson and Mr Scott for advice and assistance in securing special material and to many merchants and farmers for supplies of tubers for the investigation.

REFERENCES

- Barton-Wright & McBain (1933). *Ann. appl. Biol.* **20**, 549.
Cockerham (1939). *Ann. appl. Biol.* **26**, 417.
Fixsen & Roscoe (1940). *Nutr. Abst. Rev.* **9**, 795.
Giroud (1938). *Protoplasma Monographien*, Bd. **16**. Berlin.
Harris & Ray (1933). *Biochem. J.* **27**, 303.
Hausen (1935). *Nature, Lond.*, **136**, 516.
McHenry & Graham (1935). *Biochem. J.* **29**, 2013.
Menaker & Guerrant (1938). *Industr. Engng Chem. (Anal.)* **10**, 25.
Mills (1932). *Biochem. J.* **26**, 704.
Olliver (1938). *Analyst*, **63**, 2.
Scott (1938). *Scot. J. Agric.* **21**, 121.
Smith & Paterson (1937). *Biochem. J.* **31**, 1992.
Virtanen (1936). *Nature, Lond.*, **137**, 779.
Wachholder & Nehring (1938). *Bodenk. Pflanzenernähr.*, **9-10**, 708.
Zilva & Barker (1939). *Rep. Food Invest. Bd., Lond.*, 1938, D.S.I.R. p. 199.