XIII. THE ANTISCORBUTIC POTENCY OF APPLES.

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Introduction.

It is not unreasonable to suppose that the vitamin C content of a fruit is controlled by a number of physiological factors and, consequently, that the minimum protective dose for a particular fruit should vary in accordance with some of its characteristics. That being so the establishment of a definite relationship between the antiscorbutic activity and the physiological condition of a plant would in no small measure contribute to our knowledge of the character of a vitamin and might even reveal its identity. It has been already pointed out [Zilva, 1927, 1928] that further progress by chemical purification must be slow, since, presumably, the removal of "impurities" beyond a certain stage brings about a degree of instability in the vitamin which makes it almost impossible to ascertain its presence by biological tests. An indirect approach on the above lines to the subject seems, therefore, to be justified at this stage of the study of the antiscorbutic factor.

This communication is devoted to exploratory experiments designed to detect the relations, if any, between the antiscorbutic activity of apples and the variety, soil, age and conditions of storage. The effect of heating on antiscorbutic activity is also discussed. It may be considered to be only preliminary in character, since, if a connection between the antiscorbutic activity of the apple and one of its physiological functions is to be definitely established, it must be done by a process of elimination. This procedure is, however, complicated by the inter-relation of a number of factors and, further, by technical difficulties arising from the seasonal character of the experimental material and the long duration and laboriousness of the tests.

It was essential in an investigation of this character to attain the highest degree of accuracy in order to establish reliable quantitative criteria. Standardised conditions, which necessitated the use of a great number of animals, were therefore observed. Only general results are given in this paper. Full details of these experiments will be communicated in due course in a special report to the Medical Research Council.

Historical.

Holst and Frölich [1912] found that out of four guinea-pigs kept on a diet of rye bread baked with yeast, supplemented with 30 g. of raw apple daily, three succumbed on the 39th, 51st and 52nd days respectively—the last two showed symptoms of scurvy at the *post-mortem* examination. The fourth animal was still alive after 87 days' subsistence on this diet. They conclude that the apple is not a good source of the antiscorbutic factor.

Robison [1919] investigated the antiscorbutic activity of concentrated apple juice, prepared by the "Kestner evaporator." In this process the duration of heating is less than one minute and the maximum temperature attained is about 102° F. The jelly which was tested contained about 83 % of total solids and a daily dose of 4 g. of this concentrated juice (equivalent to 24 g. of pressed juice) protected guinea-pigs weighing 400 g. from scurvy; a daily dose of 1 g. of this concentrated juice was barely sufficient to afford complete protection, whilst one of 0·5 g. delayed the onset of scurvy for only a short time.

The British Mission sent to Vienna soon after the war [Chick, 1920] observed that 50 g. of raw apple juice administered daily as an antiscorbutic failed to prevent the onset of early symptoms of scurvy in children.

Givens, McClugage and Van Horne [1922] protected guinea-pigs from scurvy with daily doses of 10 g. of raw apple juice. When the raw apple was subjected to any considerable heating, such as is ordinarily employed in preservation by desiccation or canning, the vitamin in the apple was considerably inactivated. Dried apple peelings were found by these authors to be active in daily doses of 2 g. (22·26 % of original weight).

Kohman, Eddy and Carlsson [1924] administered 5, 6 and 7·5 g. daily of raw, cold-stored, apple to guinea-pigs. The animals on all the doses were losing weight on the 20th day of the experiment. The stored apples were, therefore, replaced at this stage by fresh, early summer apples, purchased in the open market. This produced considerable improvement in the animals, although the above doses of the fresh apple were inadequate to protect guinea-pigs from scurvy. The authors consider this observation as indicating that raw apples in storage gradually lose their antiscorbutic potency. These workers have further found that apples kept in 2 % salt solution for about 18 hours before processing retained their original antiscorbutic activity; on the other hand, some other methods of canning had a deleterious effect on the antiscorbutic activity of the fruit.

The antiscorbutic activity of different varieties of apples.

The following English varieties, from different seasons and grown on different soils, were investigated: Bramley's Seedling (cooking variety), Worcester Pearmain (dessert variety), Cox's Orange Pippin (dessert variety), Woodbine (cider variety), Dabinett (cider variety) and King Edward (cooking

variety). Various imported varieties were also investigated and will be discussed later.

The results are summarised in Table I. Bramley's Seedling stands out as markedly the most active of the varieties tested. The high antiscorbutic content was established in these apples coming from different parts of England and picked at different seasons, and, therefore, it is unlikely to be accidental. The other cooking variety, King Edward, which resembles the Bramley's Seedling in many respects, is very much less active. The next in order of activity is the Dabinett, but when compared with Bristol Bramley's grown in an adjacent orchard on a more or less similar soil, it is between three or four times less active. Of the eating varieties, Cox's Orange Pippin is about twice as active as the Worcester Pearmain, but they both fall far below Bramley's Seedling in activity.

Table I. *Relative antiscorbutic potency of English varieties.*

Dose g.	Normal Bramley 1927, Canterbury	Normal Bramley 1928, Burwell	Normal Bramley 1928, Bristol	Worcester Pearmain 1928, Burwell	Normal Cox's Orange Pippin 1928, Burwell	Woodbine 1928, Bristol	Dabinett 1928, Bristol	King Edward 1928, Burwell
3	+ + +	+ + + +	+ + + +	No protection	±	No protection	No protection	No protection
5	+ + + +	+ + + +	+ + + +	„	+	?	+ + ±	„
10	+ + + +	+ + + +	+ + + +	±	+ + ±	+	+ + + +	±
20	+ + + +	Not tested	+ + + +	+ + +	+ + + ±	+ + + +	+ + + +	+ +

It has been found that there was no relationship between the age of the tree from which the apples were picked and the antiscorbutic activity. The Bristol Bramley's Seedlings were picked from trees eight years old and the Canterbury and Burwell Bramley's Seedlings from trees about twice this age, yet the difference in the antiscorbutic activity of these apples is not significant. On the other hand, all the Burwell apples (Cox's Orange Pippin, Worcester Pearmain and Bramley's Seedling), differing so much in activity, came from trees of approximately the same age. Nor can there be a direct relationship between the soil on which the apples are grown and their vitamin content, since the Bramley's Seedlings represent three different types of soil, whilst the Burwell and Long Ashton apples which showed a varying vitamin content came from similar soils.

The antiscorbutic activity of early gathered apples.

These experiments were not instituted with the intention of making a detailed study of the relationship between the varying stages of the age cycle on the antiscorbutic activity of the apple. Such an inquiry is in progress. They were intended to be more of an exploratory character in order to ascertain whether a very marked difference in activity is obtained by picking apples earlier. The experiments were carried out on the Canterbury Bramley's Seedling and the Burwell Cox's Orange Pippin. Apples of these varieties

were picked from the same trees 14 days before the normal apples were gathered. It will be seen from Table II that there is no marked difference between the activity of these apples and that of the normal picking from the same trees a fortnight later. Nor is there to be observed a marked difference in the activity in the case of the Cox's Orange Pippin,

Table II. *Relative antiscorbutic potency of early and normal apples.*

Dose g.	Early Bramley 1927, Canterbury	Normal Bramley 1927, Canterbury	Early Cox's Orange Pippin 1928, Burwell	Normal Cox's Orange Pippin 1928, Burwell
3	+ + + ±	+ + +	±	±
5	+ + + ±	+ + + +	+	±
10	+ + + +	+ + + +	+ ±	+ + ±
20	+ + + +	+ + + +	Not tested	+ + + ±

INFLUENCE OF STORAGE ON THE ANTISCORBUTIC POTENCY OF APPLES.

Preliminary observations were made on the vitamin C in apples which aged after gathering under two empirical methods of storage, namely, "cold storage" and "gas storage." In the former case the apples were kept at 1° (constant) in the air and in the latter in an atmosphere of 10 % carbon dioxide, 11 % oxygen and 79 % nitrogen at 10°. The storing was done at the Low Temperature Research Station, Cambridge.

Table III shows that little deterioration had taken place in the vitamin during storage and that the loss, such as it was, was more marked in the "gas-stored" apples. This disparity in the inactivation of the vitamin is particularly marked in the Cox's Orange Pippin. The question as to what extent it is characteristic of this variety cannot at present be answered, since only one storage experiment has so far been carried out with it. It is further seen that early picking and soil do not seem to influence the behaviour of the antiscorbutic factor in apples on storage.

Table III. *Relative antiscorbutic potency of stored apples.*

Dose g.	Early Bramley 1927, Canterbury	Early Bramley 1927, Canterbury. Cold-stored 1°, 98 days	Early Bramley 1927, Canterbury. Gas-stored 10°, 98 days	Normal Bramley 1927, Canterbury	Normal Bramley 1927, Canterbury. Cold-stored 1°, 98 days	Normal Bramley 1927, Canterbury. Gas-stored 10°, 98 days
3	+ + + ±	+ + +	+ ±	+ + +	+ + ±	+ ±
5	+ + + ±	+ + + ±	+ + +	+ + + +	+ + +	+ + + ±
10	+ + + +	+ + + +	+ + + ±	+ + + +	+ + + +	+ + + +
20	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +

Dose g.	Normal Bramley 1928, Burwell	Normal Bramley 1928, Burwell. Cold-stored 1°, 105 days	Normal Bramley 1928, Burwell. Gas-stored 10°, 140 days	Cox's Orange Pippin 1928, Burwell	Cox's Orange Pippin 1928, Burwell. Cold-stored 1°, 79 days	Cox's Orange Pippin 1928, Burwell. Gas-stored 10°, 83 days
3	+ + + +	+ + + ±	+ + +	±	No protection	No protection
5	+ + + +	Test not finished	+ + + ±	+	±	,,
10	+ + + +	,,	+ + + +	+ + ±	+ + +	,,
20	Not tested	Not tested	+ + + +	+ + + ±	+ + + +	±

THE ANTISCORBUTIC ACTIVITY OF IMPORTED APPLES.

The following imported varieties have been tested: *Cleopatra* Louden, Western Australia (May, 1927) and Karragullen, Western Australia (April, 1928), *Jonathan* (May, 1927), *Strawberry Pearmain*, Louden, Western Australia (May, 1927), *Cleopatra*, Baghdad, Tasmania (May, 1927) and New Zealand (May, 1927), *Kings*, Ontario (November, 1927) and Burlington, Ontario (January, 1929), *Kings*, Nova Scotia (November, 1927), *Jonathan*, British Columbia (November, 1927), Penticton, British Columbia (October, 1928) and *Cox's Orange Pippin*, Penticton, British Columbia (October, 1928).

Circumstances did not favour the collection of as many details about the imported experimental material as was possible in the case of the English apples but it will be seen from the above that the general aim was to get (1) a number of varieties, (2) one variety from different places and (3) one variety from as nearly as possible the same place two consecutive seasons.

Table IV. *Relative antiscorbutic potency of imported dessert apples.*

Dose g.	Cleopatra 1927, Australia	Cleopatra 1928, Australia	Jonathan 1927, Australia	Strawberry Pearmain 1927, Australia	Cleopatra 1927, Tasmania	Cleopatra 1927, New Zealand
3	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested
5	No protection	No protection	No protection	?	No protection	No protection
10	?	,,	?	±	± ?	± ?
20	± ?	+ +	+	+ ?	+ +	+

Dose g.	Kings 1927, Ontario	Kings 1928, Ontario	Kings 1927, Nova Scotia	Jonathan 1927, British Columbia	Jonathan 1928, British Columbia	Cox's Orange Pippin 1928, British Columbia
3	No protection	Not tested	No protection	No protection	No protection	No protection
5	±	No protection	,,	±	±	± ?
10	+ + +	+ ?	+	+ +	+	± ?
20	+ + + +	Not tested	+ + + ±	+ + + +	+ + +	+ ±

It is seen from Table IV that the Australian and the New Zealand apples were not as potent as the Canadian apples, which were almost as potent as the English dessert varieties. On the other hand, there is comparatively little difference in activity among the different varieties coming from various places in the same country and belonging to two seasons, which is consistent with the observations made on the English apples. How can one explain the difference in the activity of the Australian, Canadian and English apples? In view of the results obtained in the storage experiments it would seem that this disparity is most probably due to the different lengths of time that elapse between the picking and the testing of the apples. It is to be noted that the Ontario King apples (January, 1929), which could only be tested in 5 g. and 10 g. doses, about two months later than the majority of the Canadian apples showed, as far as one can judge, from this incomplete test, a vitamin content roughly of the order of the Australian apples and were decidedly lower in

potency than the same variety of the previous year tested earlier (November, 1927). Another point of interest is that British Columbia Cox's Orange Pippin, although tested early in October, shows a lower potency than the other varieties of the same origin. Unfortunately, the test, to some extent, was marred by the fact that most of the test animals on the highest dose did not consume their entire 20 g. of apple. The result, however, indicates the possibility that the antiscorbutic activity of Canadian Cox's Orange Pippin as in the case of this English variety, has a tendency to deteriorate more quickly than that of other varieties.

The effect of heat on the antiscorbutic activity of Bramley's Seedling.

As Bramley's Seedling is a cooking variety, it was of interest to ascertain the influence of heat on its antiscorbutic activity. The potency of heated apples before storage, after cold storage and after gas storage was, therefore, investigated. It was advisable to study the effect of heat on stored apples since evidently the stability of vitamin C is controlled by other factors than the vitamin itself [Zilva, 1927, 1928] and consequently there was the possibility that observations made on unstored apples might not hold true of apples which had undergone changes on storage.

The apples were placed, in their skins, in a drying oven previously brought up to 160°. On the introduction of the apples the temperature fell to about 115°, at which temperature the heating continued for about 50 minutes; by this time the flesh of the apples was quite soft. On several occasions observation was made on the temperature of the apple by introducing a thermometer into the tissue to the depth of the core. The temperature rose from about 25° after 5 minutes to about 95° at the end of the heating. After cooling the soft pulp was scooped out and administered to the guinea-pigs in equivalent doses of the raw apple (there was usually a loss of about 10 % of the original weight in the process of heating). The Burwell Bramley's Seedling (October, 1928) were used in these tests. Results of the experiments (Table V) show that very little destruction of the vitamin took place during the heating, whether freshly picked, cold-stored or gas-stored apples were used.

Table V. *Relative antiscorbutic potency of heated apples.*

Dose g.	Normal Bramley 1928, Burwell	Heated normal Bramley 1928, Burwell	Normal Bramley 1928, Burwell. Cold-stored 1°	Heated normal Bramley 1928, Burwell. Cold-stored 1°	Normal Bramley 1928, Burwell. Gas-stored 10°	Heated normal Bramley 1928, Burwell. Gas-stored 10°
3	+ + + +	+ + + +	+ + + +	+ + + + ?	+ + +	+ + +
5	+ + + +	+ + + +	Test not finished	+ + + +	+ + + +	+ + +
10	+ + + +	+ + + +	,,	+ + + +	+ + + +	+ + + +
20	Not tested	+ + + +	Not tested	+ + + +	+ + + +	+ + + +

The chemical composition of the apple varieties tested.

The biological test for vitamin C yields, of course, the composite effect of the batch as a whole. It gives no information concerning the individual variation of the apples in antiscorbutic potency. Under these circumstances, in order to obtain a fair comparison between certain chemical data and the vitamin content of apples, the material to be tested should be in a stable and uniform condition which would represent both chemically and biologically the average of the batch. An elaboration of such a method is in progress. In order, however, to obtain some information about the chemical composition of the fruits tested, a few representative apples of each batch were analysed. Although the number of estimations of each sample of apples was too few for the formation of any definite conclusions, nevertheless, the data offered some information which at this stage of the inquiry may be considered of an indicative nature.

Figures more or less similar to those previously obtained by other workers for these varieties have been recorded and afford no information which can be correlated with the biological activity. Particularly striking was the similarity in the general chemical composition of the Bramley's Seedling and the King Edward, which differ so much in their vitamin content. There was one chemical dissimilarity, however, namely that of the nitrogen content. Unfortunately, owing to an oversight, the nitrogen of only two batches was determined, namely, those of the gas-stored Burwell Bramley's Seedling (1928) and of the stored King Edward (1928), which were carried out on three representative apples of each batch in March, 1929. The percentages of N were as follows: King Edward, 0·061, 0·070, 0·072; Bramley's Seedling, 0·027, 0·035, 0·038. In a work of this nature these figures are too few for drawing categorical conclusions but they are, nevertheless, significant enough to be considered in future research.

Conclusions.

One of the main objects of this investigation was to ascertain whether a functional relationship existed between the antiscorbutic principle and any other factor or factors in the apple. The experimental evidence so far obtained does not supply the answer. Considering the number of variables involved, it would indeed have been a very fortunate coincidence if the first set of preliminary experiments were to have supplied definite information. We have, nevertheless, made a number of observations, some of which may be considered as more or less definite, whilst others can serve at this stage only as indications. If some of these observations are not capable of full interpretation at present, their significance will, in all probability, be disclosed as the investigation develops.

It is no surprising feature to find a variation in the antiscorbutic activity of apples, but the persistent high vitamin content of the Bramley's Seedlings

attracts special attention. Judging from the results so far obtained one may exclude age of tree, soil and season as being directly responsible for this high activity, since, as we have seen, the Cox's Orange Pippins, Worcester Pearmains and Dabinetts were grown under similar conditions and yet were less active. The contrast is even more marked when the Bramley's Seedlings are compared with the King Edwards, a variety resembling them so much. Apart from the difference in the vitamin C content the only disparity which was observed in these two varieties was in the nitrogen content, but, as already stated, the number of estimates was few. Whether this property of the Bramley's Seedling is "racial" and if so, whether it is associated with any other outstanding characteristic, has still to be established. It would be further of interest to ascertain whether the smaller degree of difference in the antiscorbutic activity observed among the dessert and cider varieties is definitely characteristic of these apples.

That high antiscorbutic activity is the property of the average Bramley's Seedling, whether freshly picked or stored and that it persists after heating, is a point of some interest to the dietetician. Yet one can hardly consider the lesser antiscorbutic activity of the dessert varieties, English or imported, as inimical to their nutritive value, since in the consumption of these varieties under normal conditions the vitamin C requirements are usually amply covered by the general diet; much rather is the dessert apple valued for its palatability, flavour and other characteristic properties. This point is stressed owing to the fact that there is a tendency at present to give wrong values to experimental facts obtained in vitamin research when applied to practical hygiene and commerce.

It is also quite difficult at this stage to interpret the significance of the disparity in activity of the apples which were stored at 10° in an atmosphere of oxygen, carbon dioxide and nitrogen and of those which were allowed to age in the ordinary atmosphere at 1°. What bearing, if any, vitamin C has on the physiological changes of the apple in its progress through youth, maturity and senescence is a problem which still awaits solution and which we hope will be elucidated by the experiments which are now in progress.

Summary.

1. Of a number of apple varieties tested for their antiscorbutic potency, Bramley's Seedling was found to be markedly more active than all the other varieties, which differed among themselves comparatively very much less in their vitamin C content. There were no indications that the character of the soil, the age of the tree or season had any bearing on the antiscorbutic activity of the apple.

2. Bramley's Seedlings picked from the same tree 14 days before the normal crop were approximately of the same antiscorbutic activity.

3. There was little loss in the vitamin C content of apples stored at 1° in the air or at 10° in a mixture of carbon dioxide, nitrogen and oxygen for about

3 months. The gas-stored apples showed, however, a definitely greater deterioration in the vitamin.

4. Tests carried out on a number of imported dessert apples showed that the activity was higher in those cases in which the time elapsing between the picking of the fruit and the testing was the shortest. There was also no indication in the case of these apples of any very marked difference in activity which could be correlated with the difference in variety.

5. Heating of Bramley's Seedlings in their skins hardly affected their antiscorbutic activity.

It is with great pleasure that we take this opportunity of acknowledging our indebtedness to Drs. West and Kidd of the Low Temperature Research Station, Cambridge, and Professor Barker of the Agricultural and Horticultural Research Station, Long Ashton, for much valuable help and advice. It is evident from the nature of the inquiry that without their assistance the pomological aspect of this investigation would have been considerably restricted.

Our thanks are also due to Mr P. G. H. Barter of the Empire Marketing Board for his assistance in collecting the imported varieties of apples.

Having had the privilege of consulting Sir William Hardy at various stages of this inquiry we cannot conclude without expressing our high appreciation of his stimulating encouragement.

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