XVIII. THE ANTISCORBUTIC POTENCY OF APPLES. II.

BY MARY FORREST BRACEWELL, FRANKLIN KIDD,
CYRIL WEST AND SYLVESTER SOLOMON ZILVA¹.

*From the Biochemical Department, Lister Institute, London,
and the Low Temperature Research Station, Cambridge.*

(Received December 31st, 1930.)

SOME recent publications [Bracewell, Hoyle and Zilva, 1930, 1, 2] have dealt with an exploratory investigation instituted with the object of ascertaining whether a functional relationship exists between the antiscorbutic activity and the physiological condition of the apple fruit. With this end in view the potency of a number of English and imported varieties of known environment was assessed and the influence of empirical methods of storage and of heat on the vitamin content of apples was studied. The following salient facts emerged.

(1) The Bramley's Seedling is markedly more potent than the other varieties tested, which definitely vary in activity among themselves but to a smaller extent.

(2) There is no indication, so far, that "age of tree," soil or season has any bearing on the antiscorbutic activity of the apple.

(3) There is little deterioration of the vitamin when apples are stored either at 1° in the air (cold store) or at 10° in an atmosphere of approximately 10 % oxygen, 10 % carbon dioxide and 80 % nitrogen ("gas" store); the loss is, however, greater in the case of the "gas" stored apples.

(4) Heating Bramley's Seedling apples in their skins at about 115° for approximately 50 minutes has little effect upon their antiscorbutic activity.

While these results offer no indication so far whether vitamin C has any physiological function in the plant, they nevertheless point to the fact that the following up of this enquiry is advisable, not only from the vitamin aspect but also from the botanical point of view. Without drawing any broad lines of demarcation, the investigation is now being continued in collaboration with groups of workers interested in different fields of pomological research. In view of the lengthy nature of the investigation and of the seasonal character of the experimental material, it is proposed, for the sake of convenience, to record the results at suitable intervals even if they do not yield information from which definite conclusions can be drawn at the time.

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

The present communication deals with:

- (1) The antiscorbatic activity of further apple varieties.
- (2) The effect of low temperatures and of maturity on the antiscorbatic activity of the apple.
- (3) The effect of temperature of storage on the antiscorbatic activity of ageing apples.
- (4) The distribution of the vitamin in various parts of the apple.

The technique utilised in these tests was precisely the same as that already described [Bracewell, Hoyle and Zilva, 1930, 2].

The antiscorbatic activity of further apple varieties.

The varieties tested were Newton Wonder and Lane's Prince Albert.

The Newton Wonder apples were taken from fifteen year-old trees grown on a flinty loam over chalk, near Eynsford, Kent.

The Lane's Prince Albert apples came off 15-20 year-old trees grown on a chalk soil at Exning, near Newmarket.

The apples after being picked were stored at a constant temperature of 1° at the Low Temperature Research Station, Cambridge, and weekly consignments were delivered to London for testing purposes.

The test of the Newton Wonder apples began on 15th October, 1929, and that of the Lane's Prince Albert on 24th October, 1929.

Table I.

Dose g. daily	Lane's Prince Albert 1929	Newton Wonder 1929	Normal Bramley's Seedling 1929 Stored 3° 1st batch	King Edward 1929 Bristol High nitrogen	Worcester Pearmain 1928 Burwell	Normal Cox's Orange Pippin 1928 Burwell	Woodbine 1928 Bristol	Dabinett 1928 Bristol
3	+	+	++++	No protection	No protection	+	No protection	No protection
5	+++	++	++++	+	No protection	+	?	+++
10	++++	++++	++++	++	+	++	+	++++
20	++++	Vitiated test	Test not finished, but fully protected for 60 days	++++	+++	++++	++++	++++

It will be seen from Table I, in which other varieties are incorporated for comparison, that the Newton Wonder showed an activity of the order of Cox's Orange Pippin whilst the Lane's Prince Albert, like the Dabinett, falls between the Bramley's Seedling and the Cox's Orange Pippin in vitamin activity. It will be of interest to ascertain whether further tests will confirm this result.

The effect of low temperature (-20°) and of maturity on the antiscorbutic activity of the Bramley's Seedling.

In the first investigation of the series it was found that the early gathering of Bramley's Seedling made no appreciable difference to its vitamin C content. It was next of interest to ascertain whether in the still earlier stages of the life cycle of the apple the concentration of the vitamin is of the same order as when the fruit is ready for gathering. If that were so, it would indicate that the vitamin is either synthesised by the apple or that it is supplied to it as it grows on the tree. Alternatively, it might have been supposed that even at this early stage the fruit contains the same amount of vitamin as when it is fully grown. In this event the antiscorbutic potency of the immature apple tissue would have been proportionately higher than that of the mature apple.

Bramley's Seedlings, about 3.2 cm. in diameter and 23 g. in weight, gathered at the end of July, and fully grown apples of similar origin gathered in the middle of October were tested for comparison. The fruit was picked from 20 year-old trees grown on flinty loam over chalk near Eynsford, Kent.

The mature and immature apples were placed immediately after gathering in a constant-temperature room kept at -20° at the Low Temperature Research Station at Cambridge, and weekly supplies were removed from the room and despatched to London in a well-insulated box. On arrival the fruit was transferred immediately to a refrigerator in which a temperature of approximately -12° was maintained. Quantities necessary for the test were removed daily, thawed and brought up to a temperature suitable for feeding to the experimental animals.

The test on the immature Bramley's Seedlings began on 8th August, 1929, that on the normal¹ apples on 21st October, 1929 (1st batch). The normal fruit was also kept in the hard frozen condition until 20th February, 1930 (2nd batch), when it was again tested for its antiscorbutic potency.

Table II.

Dose g.	Frozen immature Bramley's Seedling 1929	Frozen normal Bramley's Seedling 1929, 1st batch	Frozen normal Bramley's Seedling 1929, 2nd batch	Normal Bramley's Seedling 1929 Stored 3° 1st batch
$\frac{1}{2}$	No protection	Not tested	Not tested	Not tested
1	+	"	"	"
3	+++	++	++	+++
5	++++	++++	++++	++++
7	++++	Not tested	Not tested	Not tested
10	++++	++++	++++	++++
20	Not tested	++++	++++	Test not finished, but fully protected for 60 days

The results are summarised in Table II. When compared with similar apples kept at 3° the frozen apples show a slightly but definitely lower vitamin content. There are indications, however, that this small loss was not incurred

¹ The term "normal" is used throughout this paper to denote that the fruit was gathered at the normal time.

in the process of freezing but during the period which elapsed between the thawing of the fruit and its consumption by the experimental animals. Precisely the same titre was obtained with these apples after 4 month's storage.

The immature Bramley's Seedlings seem slightly more active than the normal apples. Had they, however, possessed the same total amount of vitamin as the fully grown apples, a daily dose of 0.5 g., or even less, of tissue would have been sufficient to protect a guinea-pig from scurvy. The amount of vitamin in the apple therefore increases as it grows on the tree.

The effect of temperature of storage on the antiscorbutic activity of ageing apples.

In view of the observations made that the loss in the antiscorbutic activity of apples is somewhat greater when the fruit is stored in an atmosphere of nitrogen, carbon dioxide and oxygen at 10° (*i.e.* "gas" storage) than when it ages in the air at 1°, it was thought desirable to ascertain whether this higher loss was brought about by the difference in the temperature or in the composition of the atmosphere of the two methods of storage. Bramley's Seedling apples gathered at the normal time, of the same origin as those which were employed in the preceding experiments, were therefore allowed to age in the air at 3° and at 10°. Unfortunately circumstances did not permit a comparison to be made with apples "gas"-stored at 10°. In the case of the Bramley's Seedling variety a displacement of the metabolic balance of the tissues occurs during storage at 1°, eventually leading to low-temperature breakdown, whereas at 3°, the metabolic activities of the fruit proceed normally; hence the choice of the latter temperature in this experiment.

The tests on the first batch of apples stored at 3° began on 21st October, 1929, that of the second on 6th January, 1930, and that of the third on 22nd April, 1930. The apples stored at 10° were tested on 2nd February, 1930.

Table III.

Dose g. daily	Normal Bramley's Seedling 1929 Stored 3° 1st batch	Normal Bramley's Seedling 1929 Stored 3° 2nd batch	Normal Bramley's Seedling 1929 Stored 3° 3rd batch	Normal Bramley's Seedling 1929 Stored 10°
3	+++	+++	+++	+++
5	+++	+++	+++	+++
10	+++	+++	+++	+++
20	Test not finished but fully protected for 60 days	+++	+++	+++

The results obtained in this experiment (Table III) yielded no definite answer as to whether the greater deterioration in the antiscorbutic activity of apples in "gas" storage was due to the temperature or to the composition of the atmosphere, since the slightly lower activity of the sample of apples stored at 10° for about 3 months as compared with that of the samples stored at 3° was of an order which falls within the limits of the experimental error of the

method employed in the assessment of vitamin C. It is of interest to note, however, that no loss of activity was recorded in Bramley's Seedling apples stored for 6 months at 3°.

The distribution of vitamin C in various parts of the apple.

In all the experiments of the preliminary and of this investigation the cortex of the peeled apples was used for the determination of the relative activity of the fruit. Experiments are here described in which the distribution of the vitamin in the fruit, carried out on some of the 1929 apples discussed in the preceding sections, is studied.

Three sections of the apples were tested, the peel, the outer half of the cortex and the inner half of the cortex of the peeled apples. The results are presented in Table IV. As it was not possible to use the usual number of 6 guinea-pigs, the number of animals employed in each test is noted in the table.

Table IV.

Normal Bramley's Seedling 1929. Stored 3°, 1st batch								
Dose g.	Peel	No. of guinea-pigs	Outer cortex	No. of guinea-pigs	Inner cortex	No. of guinea-pigs	Total cortex from peeled apples	No. of guinea-pigs
$\frac{1}{2}$	++ +	4	Not tested	—	Not tested	—	Not tested	—
1	++++	4	"	—	"	—	"	—
$1\frac{1}{2}$	Not tested	—	"	3	"	—	"	—
3	++++	4	++++	2	+ +	3	++++	6
5	Not tested	—	++++	3	Vitiated test	—	++++	6
10	"	—	Not tested	—	"	—	++++	6
20	"	—	"	—	Not tested	—	Test not finished but fully protected for 60 days	6

Normal Bramley's Seedling 1929. High nitrogen sample						
Dose g.	Outer cortex	No. of guinea-pigs	Inner cortex	No. of guinea-pigs	Total cortex from peeled apples	No. of guinea-pigs
$\frac{1}{2}$	Not tested	—	Not tested	—	Not tested	—
1	"	—	"	—	"	—
$1\frac{1}{2}$	++	3	"	—	"	6
3	++++	3	++	3	++++	6
5	++++	3	++++	3	++++	6
10	Not tested	—	++++	3	++++	6
20	"	—	Not tested	—	Test not finished but fully protected for 55-60 days	6

Frozen immature Bramley's Seedling 1929				Newton Wonder 1929				
Dose g.	Peel	No. of guinea-pigs	Total cortex from peeled apples	No. of guinea-pigs	Peel	No. of guinea-pigs	Total cortex from peeled apples	No. of guinea-pigs
$\frac{1}{2}$	+ +	6	No protection	6	No protection	4	Not tested	—
1	++++	6	+	6	+	4	"	—
$1\frac{1}{2}$	Not tested	—	Not tested	—	Not tested	—	"	—
3	"	—	++++	6	++++	4	+	6
5	"	—	++++	6	++++	4	+ +	6
10	"	—	++++	6	Not tested	—	++++	6
20	"	—	Not tested	—	"	—	Not tested	—

The tests began at the following times.

Normal Bramley's Seedling apples, 1929, at 3°: peel October 2nd, 1929, outer cortex January 6th, 1930, inner cortex November 22nd, 1929.

Normal Bramley's Seedling apples, 1929. High nitrogen: outer cortex January 14th, 1930, inner cortex November 22nd, 1929.

Peel of frozen, immature Bramley's Seedling apples, 1929, on August 8th, 1929.

Peel of Newton Wonder apples, 1929, on 3 g. and 5 g. dose, October 15th, 1929, and on 0.5 g. and 1 g., November 13th, 1929.

It will be seen (Table IV) that in the case of the normal Bramley's Seedling apple in which the potency was determined in the peel, in the outer cortex and in the inner cortex, the peel is more than 3-4 times as active as the outer cortex, and over 6 times as active as the inner cortex and that the outer cortex, as might be expected, is somewhat more active than the total cortex of the apple. In the case of the immature Bramley's Seedling and of the Newton Wonder, the relationship of the activity of the peel to that of the total cortex of the apples is similar to that of the normal Bramley's Seedling.

In this connection it should be mentioned that Givens, McCluggage and Van Horne [1922] have found dried apple peel more active than dried apple. Scheunert [1930] also states that the vitamin activity of apples depends on the method of peeling.

It is impossible to say at this stage of the enquiry whether the disparity in activity between the different parts of the fruit has any physiological significance, but in view of the similar distribution of certain enzymes in the apple such a possibility must be borne in mind.

SUMMARY.

1. Newton Wonder apples possess a vitamin C content of the order of that possessed by Cox's Orange Pippin, whilst Lane's Prince Albert occupies an intermediate position between the latter and the much more potent Bramley's Seedling.

2. Bramley's Seedling apples can be frozen at - 20° and stored at this temperature for 4 months without losing appreciably in antiscorbutic activity.

3. Bramley's Seedling apples gathered at the end of July are not more active per g. than the normal apple from the same plantation gathered in October.

4. Bramley's Seedling apples stored at 3° in air for 5 months do not lose any of their antiscorbutic activity.

5. The concentration of vitamin C in the tissue of the apple increases as the skin is approached from the core and is more than 6 times as great in the peel as in the flesh near the core.

REFERENCES.

- Bracewell, Hoyle and Zilva (1930, 1). *Biochem. J.* **24**, 82.
 ——— (1930, 2). *Med. Res. Council, Special Report Series*, No. 146.
 Givens, McCluggage and Van Horne (1922). *Amer. J. Dis. Child.* **23**, 210.
 Scheunert (1930). *Der Vitamingehalt der Deutschen Nahrungsmittel.* (Zweite Ergänzte Auflage. Julius Springer.)