THE PHYSIOLOGY OF GROWTH IN APPLE FRUITS

IV. SEASONAL VARIATION IN CELL SIZE, NITROGEN METABOLISM, AND RESPIRATION IN DEVELOPING GRANNY SMITH APPLE FRUITS

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Summary

Results of investigation of cell size, fruit size, nitrogen metabolism, and respiration rate over three successive seasons confirm the main conclusions of earlier papers in this series. Fruit left on the trees for a period beyond normal commercial maturity showed a very large increase in soluble nitrogen with only slight increase in protein nitrogen. The relation of the nitrogen and organic acid metabolism to the climacteric rise in respiration is discussed.

I. INTRODUCTION

Earlier papers in this series recorded observations on the physiology of the Granny Smith apple fruit, with particular reference to cell enlargement (Bain and Robertson 1951; Robertson and Turner 1951). This paper reports the investigation of fruits developing on the same trees in three later consecutive seasons. Further, by an improved sampling technique and by continuing the sampling over a longer period, a more detailed study of several aspects was possible.

In two complete seasons, October to June of 1949-50 and 1950-51, and one part season, February to June of 1949, the anatomical and chemical changes occurring during the development of the fruits were investigated. Analysis included determination of cell size, cell number, respiration rate, total and protein nitrogen, total organic acids, starch, and cell wall material. Ninety-two fruits from 10 samples in the 1949-50 season were examined individually for each of these factors.

An attempt has been made to relate respiration rate and changes in chemical composition to cell size changes, and to discuss the metabolic processes associated with the climacteric rise in respiration.

II. MATERIAL AND METHODS

(*a*) *Sampling*

In the three seasons samples of Granny Smith apples were taken from two trees at Bathurst used in the earlier work. Most of the results described were from one tree in the two complete seasons; results from the other tree are also discussed where there is any appreciable difference. Samples were taken every 3 weeks from two trees in 1949-50 and monthly from one tree only in 1950-51.

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In 1949-50 one tree had approximately 500 fruits at 40 days from full blossom and the other had approximately 560; in 1950-51, at 54 days from full blossom, the numbers were 250 and 180 respectively. Picking dates and times from full blossom are shown in Table 1.

TABLE 1

DATE OF SAMPLING SEASON

Days from Full Blossom	1948-49		1949-50		1950-51	
	Pick	Date	Pick	Date	Pick	Date
0	—	—	1	6.x.49	—	—
40	—	—	2	15.xi.49	—	—
54	—	—	—	—	1	26.xi.50
59	—	—	3	4.xii.49	—	—
74	—	—	4	19.xii.49	—	—
76	—	—	—	—	2	18.xii.50
102	—	—	5	16.i. 50	—	—
104	—	—	—	—	3	15.i.51
123	—	—	6	6.ii.50	—	—
129	1	9.ii.49	—	—	—	—
132	—	—	—	—	4	12.ii.51
144	—	—	7	27.ii.50	—	—
155	2	7.iii.49	—	—	—	—
160	—	—	—	—	5	12.iii.51
165	—	—	8	20.iii.50	—	—
169	3	22.iii.49	—	—	—	—
183	4	5.iv.49	—	—	—	—
188	—	—	—	—	6	9.iv.51
193	—	—	9	17.iv.50	—	—
198	5	20.iv.49	—	—	—	—
207	—	—	10	1.v.50	—	—
211	6	3.v.49	—	—	—	—
216	—	—	—	—	7	7.v.51
225	7	19.v.49	—	—	—	—
228	—	—	11	22.v.50	—	—
239	8	30.v.49	—	—	—	—
242	—	—	12	5.vi.50	—	—
244	—	—	—	—	8	4.vi.51
263	—	—	13	26.vi.50	—	—
277	—	—	14	10.vii.50	—	—

In 1948-49 each sample consisted of 15 fruits. To improve the sampling in subsequent seasons, each tree was divided into 20 sections, with roughly equal numbers of fruits at the beginning of the season. Each pick contained one fruit, taken at random, from each section. The fruits of each sample were bulked for analysis. In the 1949-50 season five fruits from picks 5-14 were kept as individuals for all the analyses and the remaining 15 were treated in bulk.

(*b*) *Respiration Rate*

Respiration rate was measured by the Pettenkofer method at 25°C., as described by Turner (1949). The results are given as equivalent rates at 20°C. assuming a Q_{10} of 2. Individual respiration rates were measured by enclosing each fruit in a glass shell; the bulk samples were enclosed in large vacuum desiccators.

(*c*) *Cutting and Drying*

After the respiration measurements the fruits were cut transversely and four thin transverse sections were taken from one half on two diameters at right angles. The sections were kept in formalin : alcohol (60 ml. : 1000 ml. 70 per cent.) for anatomical study.

After the sections for the measurement of cell size had been removed, the fruits were peeled and the cortical tissue was sliced thinly. For moisture estimation one-quarter of each fruit was grated, and an aliquot of the carefully mixed material dried at 70°C. in a weighed aluminium can, first in an air-draught oven for several hours and then in a vacuum oven for 6 hr. Material for analysis was dried in the same way, cooled, ground finely, and stored in air-tight jars.

(*d*) *Analytical*

The analytical methods used were those described by Turner (1949) except for the estimation of total organic acids. In the method used, 0.5-1.0 g. dried apple powder was taken up in about 200 ml. distilled water. This was titrated with 0.1N sodium hydroxide, using phenolphthalein as indicator, to determine free acids. A further 0.5-1.0 g. dried apple powder was weighed in a porcelain basin and ashed at a low temperature to avoid loss of potassium. It was heated on a gauze mat while the fumes were given off and later on a triangle for about 10 min. until the ash was a light grey colour. After cooling, 5 ml. 0.1N hydrochloric acid were added to the ash and allowed to stand for 5 min. This was diluted to approximately 200 ml. and boiled to expel carbon dioxide. It was cooled immediately and back-titrated with 0.1N sodium hydroxide to determine the equivalents of combined acid. The free and combined acids together made up the total acids. The results of some analyses by this method were compared with the 48-hr. ether-extraction method of Pucher, Wakeman, and Vickery (1941). This gave a slightly higher result but the difference did not justify the extra work involved in using the ether method.

III. Results

(*a*) *Anatomical*

(i) *Cell Size.*—As in the earlier work (Bain and Robertson 1951), 25 cells from two transverse sections of the cortex across the equator of the fruit were traced and measured to calculate the average cell volume. The results for tree 2 in three seasons are set out in Table 2.

Changes in cell volume in both 1949-50 and 1950-51 seasons followed closely the changes in fruit weight with time (Figs. 1 and 2). Both fruit weight and

cell volume in the 1950-51 season were very much lower than in the 1949-50 season. From an examination of the individual fruits in the 1949-50 samples, cells from the small and large fruits within a pick had much the same cell

TABLE 2

CHANGES IN MEAN CELL VOLUME AND MEAN FRUIT WEIGHT WITH TIME FROM FULL BLOSSOM

Days from Full Blossom	1948-49		1949-50		1950-51	
	Fruit Weight (g.)	Cell Volume (cu. mm.)	Fruit Weight (g.)	Cell Volume (cu. mm.)	Fruit Weight (g.)	Cell Volume (cu. mm.)
0	—	—	0·106	—	—	—
40	—	—	7·75	0·0003	—	—
54	—	—	—	—	13·95	0·0004
59	—	—	24·73	0·0007	—	—
74	—	—	42·97	0·0015	—	—
76	—	—	—	—	27·79	0·0007
102	—	—	81·85	0·0024	—	—
104	—	—	—	—	66·05	0·0020
123	—	—	123·84	0·0024	—	—
129	116·29	0·0038	—	—	—	—
132	—	—	—	—	98·97	0·0030
144	—	—	149·99	0·0038	—	—
155	153·67	0·0051	—	—	—	—
160	—	—	—	—	132·12	0·0035
165	—	—	192·84	0·0047	—	—
169	179·13	0·0056	—	—	—	—
183	197·13	0·0059	—	—	—	—
188	—	—	—	—	170·28	0·0039
193	—	—	250·01	0·0056	—	—
198	195·75	0·0059	—	—	—	—
207	—	—	246·38	0·0055	—	—
211	202·91	0·0064	—	—	—	—
216	—	—	—	—	193·01	0·0042
225	217·01	0·0062	—	—	—	—
228	—	—	245·88	0·0058	—	—
239	222·08	0·0061	—	—	—	—
242	—	—	296·51	0·0058	—	—
244	—	—	—	—	182·78	0·0046
263	—	—	267·25	0·0063	—	—
277	—	—	257·60	0·0060	—	—

volume, so that cells in both were growing at the same rate. Cell volume increased throughout the season but at a lower rate after about 190 days from full blossom when the fruits were commercially mature.

In the 1948-49 season the fruit development was different. Cell volume was larger than in either of the other seasons but the fruit weight was less than in the 1949-50 season.

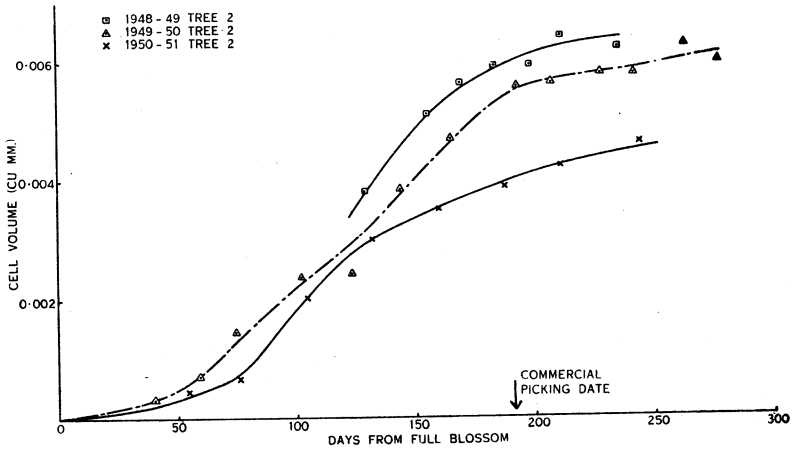

Fig. 1.—Changes in cell volume with time.

(ii) *Cell Number.*—Cell number was determined by the method of Bain and Robertson (1951), and the results for the three seasons are given in Table 3. Cell volume varied little within a pick but cell number ranged from about 12,000,000 to 83,000,000 with an average in the later picks of about 40,000,000. This accounted for the difference in fruit weight in fruit of the same age and agrees with earlier work.

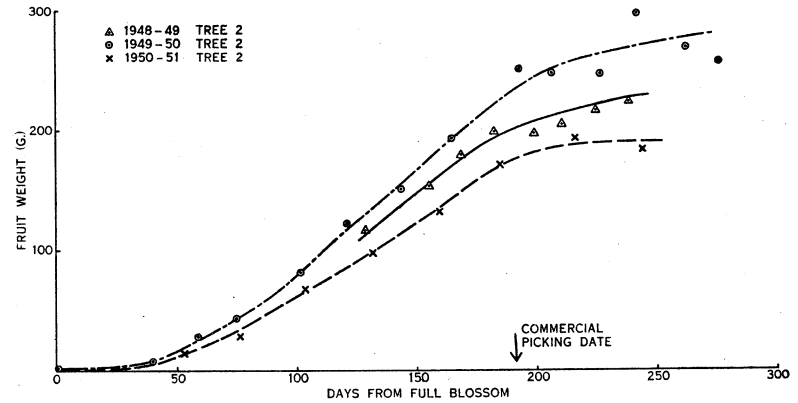

Fig. 2.—Changes in fruit weight with time.

In the 1950-51 season, additional samples were taken at full blossom, at 3 weeks, and at 6 weeks from full blossom. The results are shown in Table 4. The change in cell volume during the first 3 weeks was very small but the cell number increased about 10 times. By 6 weeks, the cell number was approximately equal to the mean cell number for the season, showing that cell division had almost ceased; cell volume had increased considerably in the second 3 weeks.

Mean cell number for the three seasons is plotted against time in Figure 3. In analysing these data it was found that the distribution of the number of cells within a pick was noticeably skew but the logarithm of the cell number was approximately normally distributed. Using the logarithmic transformation, the differences between the means of the picks were significant in relation to the variation between fruits within the picks. The cause of these differences between picks is not known. The cell number was significantly lower in 1948-49 than in the other two seasons.

TABLE 3

MEAN CELL NUMBER IN RELATION TO TIME

Days from Full Blossom	1948-49 Cell Number $\times 10^{-6}$	1949-50 Cell Number $\times 10^{-6}$	1950-51 Cell Number $\times 10^{-6}$
0	—	2·69	—
40	—	22·0	—
54	—	—	28·8
59	—	31·7	—
74	—	26·9	—
76	—	—	41·0
102	—	31·4	—
104	—	—	29·6
123	—	46·9	—
129	29·9	—	—
132	—	—	29·6
144	—	35·5	—
155	22·2	—	—
160	—	—	34·7
165	—	37·3	—
169	30·8	—	—
183	31·2	—	—
188	—	—	40·0
193	—	41·0	—
198	33·4	—	—
207	—	40·1	—
211	32·6	—	—
216	—	—	41·5
225	30·5	—	—
228	—	38·8	—
239	32·0	—	—
242	—	46·8	—
244	—	—	36·0
263	—	38·9	—
277	—	39·3	—

The earlier work of Tetley (1930, 1931) and of Bain and Robertson (1951) showed that cell division was completed in about 3 weeks from full blossom. The points in Figure 3 indicate a slight increase in cell number from 50 days to the end of the season. We do not believe that this necessarily contradicts the

conclusions of the earlier work, in which direct observation failed to show cell division after about 3 weeks. The apparent increase in cell numbers shown in Figure 3 may arise from the method of calculating cell number from measure-

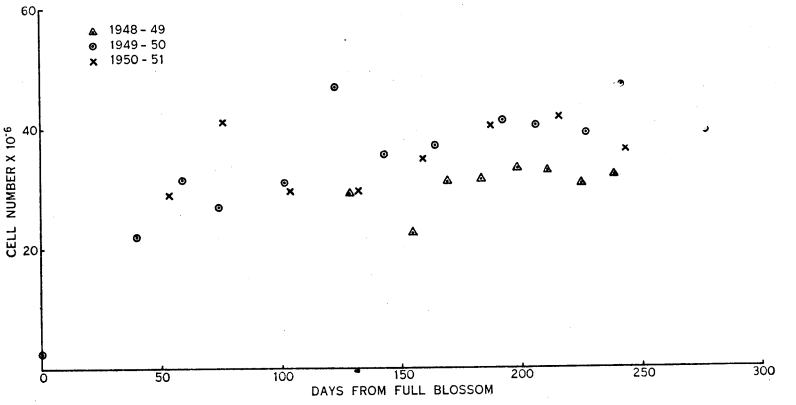

Fig. 3.—Changes in cell number with time.

ments of cells in the mid cortex; this method may not be equally satisfactory over the whole season for fruits of different sizes. We use the calculated mean cell numbers for expressing analytical results; any errors introduced by doing this will not affect the conclusions reached in this paper.

TABLE 4

CHANGES IN CELL VOLUME AND CELL NUMBER DURING THE FIRST 7 WEEKS OF FRUIT GROWTH

Pick	Date	Tree 2		Tree 3	
		Mean Cell Volume (cu. mm.)	Mean Cell Number $\times 10^{-6}$	Mean Cell Volume (cu. mm.)	Mean Cell Number $\times 10^{-6}$
1	3.x.50	0·000036	1·6	0·000027	2·4
2	25.x.50	0·000038	20·5	0·000044	18·5
3	15.xi.50	0·000249	33·8	0·000259	35·5

(iii) *Fruit Size.*—The results of the three seasons confirm the observation given in the previous paper that variation in fruit size is determined early in development by the amount of cell division. Within one season the cell volume in individual fruits of the same age but of different size was fairly uniform so that the variation in fruit size was determined by cell number and, to some extent, by the amount of air space. Between seasons, however, the size of cells may be the overriding factor in determining fruit size. This is shown by com-

parison of the 1949-50 and the 1950-51 seasons when the fruits had similar cell numbers, but in the later season they had smaller cells and hence the fruit size was lower, possibly because of a dry period early in development.

(*b*) *Analytical*

(i) *Dry Weight.*—Dry weight as a percentage of fresh weight increased about 7-8 per cent. during the season. The dry weight per cell was proportional to cell volume, both in time and in the individual fruits of a pick. Increase in dry weight per cell with time in tree 2 is shown in Figure 4.

In 1949-50, fruits on tree 3 behaved differently from those on tree 2 for about a month after the date of commercial maturity and showed a loss of dry weight. This appeared to be a real check in the growth of the fruit and was reflected in a similar decrease in total and protein nitrogen, starch, and organic acids.

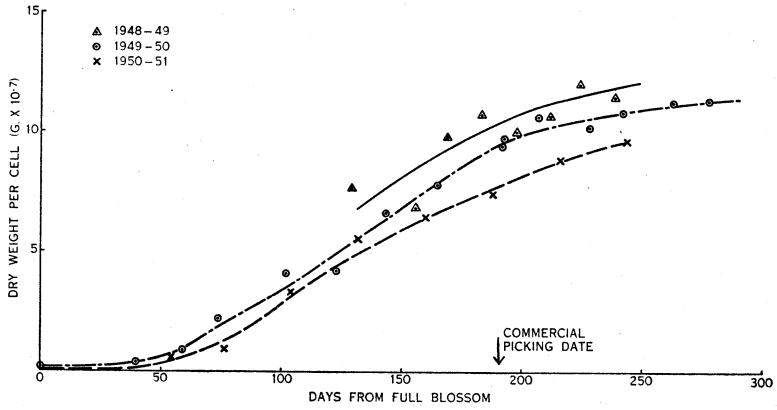

Fig. 4.—Changes in dry weight per cell with time.

(ii) *Starch.*—Starch was estimated on the dried material. A comparison with estimations on fresh material indicated that drying did not alter the results within the limits of experimental error. It is unlikely that any appreciable change in starch content occurred during the 72 hr. between picking and cutting. Kidd *et al.* (1950) found that synthesis of starch in Bramley's Seedling apples continued for 1-2 days after the fruits were removed from the tree; starch then began to decrease.

Starch was detected in the cells in measurable quantities at 60 days from full blossom in 1949-50 and at 76 days in 1950-51. Starch determinations on the individual fruits of each pick in 1949-50 showed the mean starch content per cell to be very variable between fruits. Starch per cell rose from about 0.2×10^{-8} g. at 60 days to 11.9×10^{-8} g. at 165 days from full blossom. It decreased again rapidly after this to 0.3×10^{-8} g. at 263 days. In 1950-51 starch per cell increased from 0.2×10^{-8} g. at 76 days to 5.9×10^{-8} g. at 160 days and dropped again to 0.3×10^{-8} g. at 244 days from full blossom (Table 5, Fig. 5).

The peak in starch synthesis occurred at the same time in all three seasons but in the latest season, when the cell volume and fruit weight were considerably lower, the amount of starch per cell was only half that formed in the earlier seasons.

TABLE 5

CHANGE IN STARCH AND CELL WALL MATERIAL PER CELL WITH TIME IN EACH SEASON

Days from Full Blossom	1948-49	1949-50		1950-51	
	Starch per Cell ($g. \times 10^{-8}$)	Starch per Cell ($g. \times 10^{-8}$)	Cell Wall Material per Cell ($g. \times 10^{-8}$)	Starch per Cell ($g. \times 10^{-8}$)	Cell Wall Material per Cell ($g. \times 10^{-8}$)
0	—	—	—	—	—
40	—	—	2·0	—	—
54	—	—	—	—	1·9
59	—	0·2	4·3	—	—
74	—	2·1	8·6	—	—
76	—	—	—	0·2	2·5
102	—	8·3	8·5	—	—
104	—	—	—	1·8	6·4
123	—	8·3	4·9	—	—
129	6·6	—	—	—	—
132	—	—	—	4·9	8·3
144	—	11·0	10·6	—	—
155	—	—	—	—	—
160	—	—	—	5·9	6·1
165	—	11·9	9·8	—	—
169	12·2	—	—	—	—
183	9·9	—	—	—	—
188	—	—	—	4·3	6·2
193	—	6·0	18·4	—	—
198	1·7	—	—	—	—
207	—	9·2	9·9	—	—
211	2·8	—	—	—	—
216	—	—	—	1·5	6·5
225	0·6	—	—	—	—
228	—	4·4	10·9	—	—
239	0·9	—	—	—	—
242	—	2·1	10·8	—	—
244	—	—	—	0·3	5·6
263	—	0·3	13·1	—	—

In individual fruits during the time that the amount of starch was decreasing, the correlation between starch (g. per cell) and respiration rate (mg. CO_2 per cell per hour) was significant when averaged over picks 8 and 9 ($+0.87$ on 5 degrees of freedom, $P =$ approx. 1 per cent.) but was not significant when averaged over picks 10, 11, and 12. However, as will be discussed later, the climacteric rise in respiration follows a few weeks after the beginning of starch

breakdown, which must be connected in some way with the characteristic metabolic change taking place in the cells at about 190 days from full blossom.

(iii) *Total Organic Acids.*—Total organic acids per cell, calculated as malic acid, increased from 0.3×10^{-8} g. at full blossom to 3.2×10^{-8} g. at 102 days from full blossom. These figures were very similar in the 1949-50 and 1950-51 seasons. After 102 days in tree 2 of the 1949-50 season the acids increased less rapidly to 4.4×10^{-8} g. at 190 days and remained at this level, with slight fluctuations till the end of the season (Table 6, Fig. 5). The decrease in

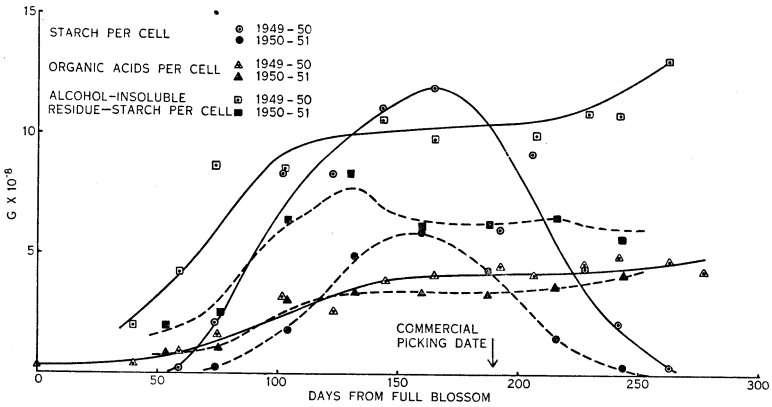

Fig. 5.—Changes in starch per cell, total organic acids per cell, and cell wall material with time.

dry weight at 210 days in tree 3 was due partly to decrease in acids; later in the season the concentration of acids increased again, which indicated a synthesis in the cells. The increase in acids in the 1950-51 season from 3.3×10^{-8} g. to 4.2×10^{-8} g. between 190 days and the end of the season (244 days) may be real but the data were not sufficient to test the significance properly. From

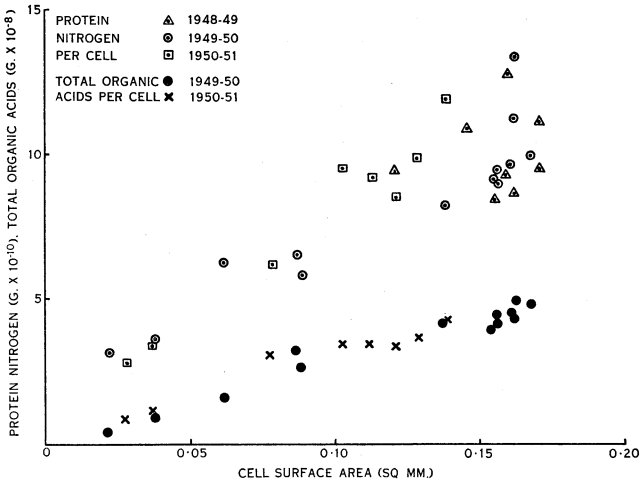

Fig. 6.—Relationship between protein nitrogen per cell, total organic acids per cell, and cell surface area.

comparison with both trees of the previous season it seems that until about the beginning of the climacteric rise in respiration the acids increase in the cells to a certain level, which is maintained during the climacteric and post-climacteric periods. When plotted against mean cell surface, the total acid per cell showed a linear relationship (Fig. 6).

TABLE 6

CHANGES IN TOTAL, PROTEIN, AND SOLUBLE NITROGEN AND TOTAL ORGANIC ACIDS PER CELL WITH TIME

Pick	Days from Full Blossom	Fruit Weight (g.)	Total Nitrogen per Cell ($g. \times 10^{-10}$)	Protein Nitrogen per Cell ($g. \times 10^{-10}$)	Soluble Nitrogen per Cell ($g. \times 10^{-10}$)	Total Organic Acids per Cell ($g. \times 10^{-8}$)
1948-49						
1	129	116·29	19·4	9·4	10·0	
2	155	153·67	23·0	10·8	12·2	
3	169	179·13	19·8	8·4	11·4	
4	183	197·13	—	8·6	—	
5	198	195·75	18·3	9·3	9·0	
6	211	202·91	18·7	9·5	9·2	
7	225	217·01	25·2	11·1	14·1	
8	239	228·08	36·3	12·8	23·5	
1949-50						
1	0	0·106	2·1	1·2	0·9	0·3
2	40	7·75	5·7	3·1	2·6	0·4
3	59	24·73	8·2	3·6	4·6	0·9
4	74	42·97	12·3	6·2	6·1	1·6
5	102	81·85	14·2	6·5	7·7	3·2
6	123	123·84	11·7	5·8	5·9	2·6
7	144	149·99	14·8	9·1	5·7	3·9
8	165	192·84	16·1	8·2	7·9	4·1
9	193	250·01	18·6	9·0	9·7	4·4
10	207	246·38	19·8	9·4	10·4	4·1
11	228	245·88	24·2	9·6	14·5	4·5
12	242	296·51	25·9	11·2	14·7	4·9
13	263	267·25	33·1	9·9	23·2	4·8
14	277	257·60	32·3	13·4	18·9	4·3
1950-51						
1	54	13·95	4·9	2·8	2·1	0·8
2	76	27·79	6·6	3·4	3·2	1·1
3	104	66·05	16·9	6·1	10·8	3·0
4	132	98·97	20·6	9·5	11·2	3·4
5	160	132·12	19·0	9·2	9·8	3·4
6	188	170·28	18·3	8·5	9·8	3·3
7	216	193·01	25·3	9·9	15·4	3·6
8	244	182·78	36·4	11·9	24·4	4·2

(iv) *Alcohol-insoluble Residue Minus Starch.*—This fraction represented an approximate measure of the cell wall material. It increased throughout the

season but less rapidly towards the end when the cells were not changing very much in volume (Table 5, Fig. 5). It showed an approximately linear relationship with cell surface, as in the earlier work, indicating that as the cell wall increased in area it remained approximately constant in thickness.

(v) *Total Nitrogen.*—The results of total nitrogen estimations (g. per cell) for the three successive seasons are plotted against time in Figure 7. The trends in each season were similar, showing a steady increase in nitrogen in the cells during the early growing period. Late in the season, another rapid increase in nitrogen made the final concentration at 277 days double that at 190 days from

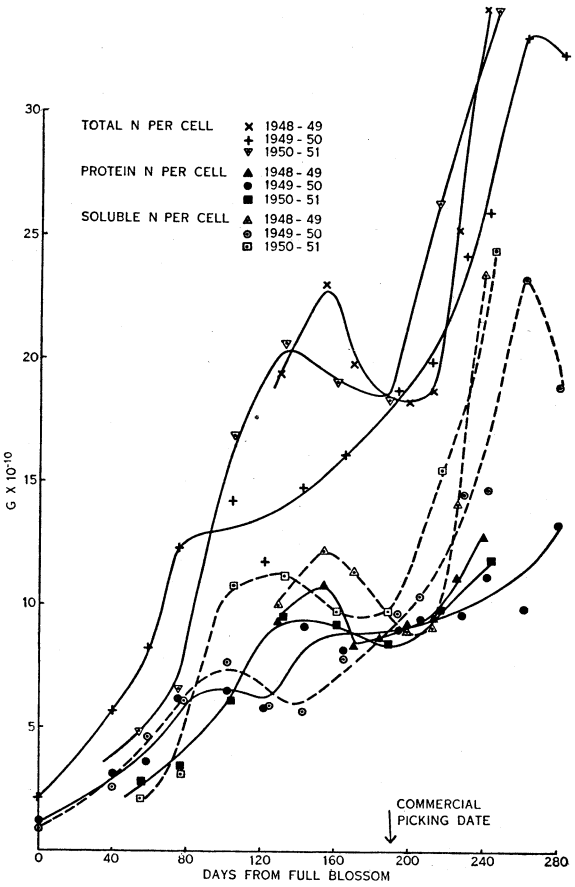

Fig. 7.—Changes in total nitrogen per cell, protein nitrogen per cell, and soluble nitrogen per cell with time.

full blossom. In two seasons there was an apparent decrease in total nitrogen for about 60 days prior to commercial picking. Tree 3 in 1949-50 behaved similarly because the intake of nitrogen stopped at 170 days and the amount per cell decreased for 6 weeks, after which there was another rapid intake; the decrease of total nitrogen corresponded with the loss of dry weight mentioned earlier.

(vi) *Protein Nitrogen.*—Protein nitrogen (g. per cell) made up approximately 50 per cent. of the total nitrogen per cell during enlargement (Table 6, Fig. 7). Amount of protein nitrogen per cell followed similar trends in all seasons. After about 120 days from full blossom, no further increase in protein content in the cells was observed for about 90 days. From 210 to 280 days from full blossom, further synthesis of protein took place. In tree 3 in 1949-50, the fall in total nitrogen content of the cells was followed by a reduction in protein nitrogen in the picks at 193 and 207 days from full blossom.

(vii) *Soluble Nitrogen.*—The soluble nitrogen content changed in much the same way as the protein nitrogen up to 190 days from full blossom (Fig. 7). For about 50 days before the climacteric rise in respiration, the amount of soluble nitrogen did not increase in the cells. However, after the climacteric rise began, there was a rapid import of soluble nitrogen by the cells and this was followed by further protein synthesis. The rapid increase in soluble nitrogen during the last 70 days was observed in both trees in all seasons.

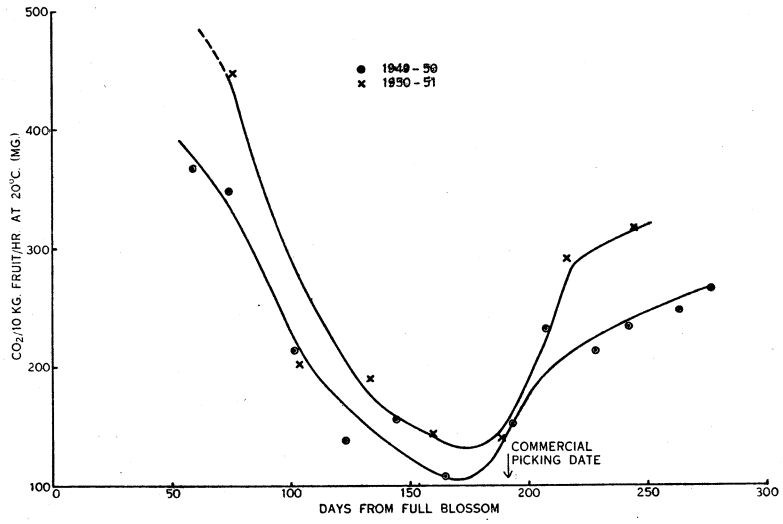

Fig. 8.—Changes in respiration rate per unit weight of fruit with time.

(viii) *Respiration Rate.*—Respiration rate was determined from the mean of seven individual fruits measured separately in the 1949-50 season and from two bulk samples, each of 10 fruits, in the 1950-51 season. The results were calculated per unit weight, per fruit, and per cell. From a comparison of the percentage difference in results from individual fruit of the same pick, the variation when the results were expressed per cell was very much smaller than either per fruit or per unit weight. For this reason, calculation of respiration rate per cell was found to be a very satisfactory means of comparing respiration rates between fruits of different sizes and between fruits of different picks.

In the 1949-50 season, respiration per unit weight of fruit, which is the usual way of expressing the rate, decreased rapidly from about 3300 mg.

CO_2/10 kg./hr. at full blossom to a minimum of 110 mg./10 kg./hr. at 160 days from full blossom. At about the date of commercial maturity of the fruit (190 days) a climacteric rise began and the respiration continued to rise slowly till the end of the season at 277 days (Fig. 8). In Figure 9 all measurements of respiration rate during the 70 hr. after each sampling are plotted to indicate the variation in rate during that time. The line joining each pick has been drawn through the mean. The curves would have been essentially the same in form if drawn through the highest or lowest values in each pick. The same type of drift over the 70 hr. was observed in individual fruits within a pick. The amount of variation in the readings from individual fruits seemed to increase with the age of the fruit and was most marked about the time of the climacteric rise.

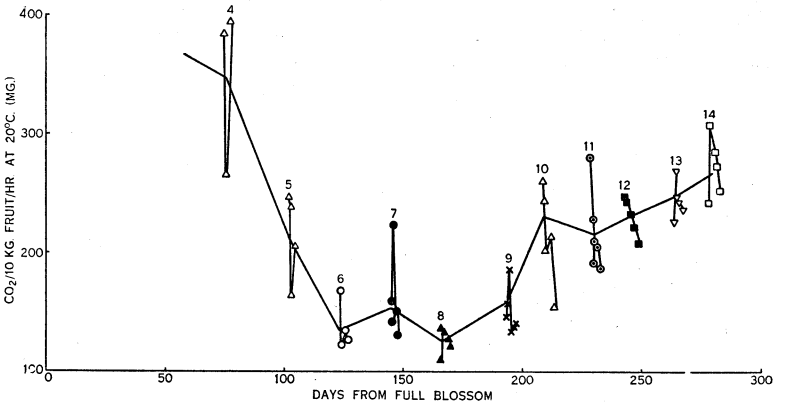

Fig. 9.—Changes in respiration rate per unit weight with time, showing the drift in the readings from which the mean value of each pick was obtained.

Within each pick respiration per cell was approximately proportional to cell volume. Fruits of different weights but similar cell volumes showed almost the same respiration rates per cell but more variable respiration rates per fruit or per unit weight. From Table 7 it is evident that calculation of respiration rate per fruit and per unit weight did not give comparable results between fruits of the same pick or between picks, and that respiration per cell gave the only really satisfactory means of expression.

The climacteric rise is seen clearly when expressed as respiration per cell. These results are given in Table 8 and plotted against time in Figure 10. In 1949-50 respiration rate per cell rose from 1.3×10^{-8} g. CO_2 per cell per hour at full blossom to 8.7×10^{-8} g. CO_2 per cell per hour at 193 days. Within the next 14 days it rose to 14.1×10^{-8} g., then increased slowly to 17.1×10^{-8} g. CO_2 per cell per hour at 277 days. In the later season respiration rate per cell followed the same pattern with the climacteric rise occurring at the same time as in both earlier seasons and at the same respiration rate per cell. It was not possible from the results to tell whether the increase in rate took place over a period of a few days or of 2-3 weeks, since no intermediate samples were taken.

IV. Discussion

(*a*) *Cell Size*

The observations on mean cell size over three successive seasons confirm the principal conclusions of Bain and Robertson (1951), but with fruit kept on the tree till the end of the season, the increase in cell volume is progressively diminished after about 190 days from full blossom. This was not observed by

Table 7

COMPARISON OF RESPIRATION EXPRESSED AS RATE PER CELL, RATE PER FRUIT, AND RATE PER UNIT WEIGHT

Pick No.	Fruit Weight (g.)	Cell Volume (cu.mm.)	CO_2/ Cell/Hr. at 20°C. (mg. $\times 10^{-7}$)	CO_2/ Fruit/ Hr. at 20°C. (mg.)	CO_2/ 10 Kg./ Hr. at 20°C. (mg.)	Variability Within Each Pair as a Percentage of the Mean when Expressed as:		
						Respiration per Cell	Respiration per Fruit	Respiration per Unit Weight
7	153·1	0·0041	0·81	2·50	147·86	1·23	4·50	17·60
	138·6	0·0040	0·82	2·39	176·40			
9	292·5	0·0058	1·00	4·26	150·14	1·95	45·11	2·22
	172·4	0·0054	1·02	2·69	153·51			
9	192·0	0·0062	1·09	2·87	159·67	0·92	18·25	5·78
	153·7	0·0058	1·08	2·40	150·70			
9	255·6	0·0067	1·06	3·43	134·12	0	14·59	2·17
	302·9	0·0067	1·06	3·97	131·24			
10	266·0	0·0058	1·53	5·92	222·44	0·65	19·46	9·66
	198·7	0·0052	1·54	4·87	245·03			
10	257·5	0·0065	1·65	5·39	195·15	0	27	7·02
	309·2	0·0059	1·65	7·06	209·34			
11	258·6	0·0062	1·49	5·17	200·03	0·67	11	8·26
	252·2	0·0068	1·48	4·64	184·16			
13	279·3	0·0060	1·63	6·49	232·29	0	6·85	10·35
	235·4	0·0055	1·63	6·06	257·64			
13	279·3	0·0060	1·63	6·49	232·29	3·0	7·85	7·50
	252·6	0·0059	1·68	6·00	250·38			

Bain and Robertson, whose observations ceased at 200 days. The fruits of one season (1950-51) were smaller and the cell volumes less than those of the other two seasons.

An increase in calculated cell number during the period of cell enlargement was observed in the three seasons discussed in this paper but not by Bain and Robertson (1951). We do not know whether this increase was real, and due to the persistence of a small amount of cell division, or was apparent, and due to the method of calculating cell numbers. The variation in cell number between picks was wide and erratic and will not be discussed further until more information has been obtained.

TABLE 8

CHANGE IN RESPIRATION RATE PER CELL WITH FRUIT WEIGHT AND TIME IN TREE 2

Days from Full Blossom	1948-49		1949-50		1950-51	
	Fruit Weight (g.)	CO_2/Cell/ Hr. at 20° C. (mg. $\times 10^{-8}$)	Fruit Weight (g.)	CO_2/Cell/ Hr. at 20° C. (mg. $\times 10^{-8}$)	Fruit Weight (g.)	CO_2/Cell/ Hr. at 20° C. (mg. $\times 10^{-8}$)
0	—	—	0·106	1·30	—	—
40	—	—	7·75	2·86	—	—
54	—	—	—	—	13·95	5·73
59	—	—	24·73	2·87	—	—
74	—	—	42·97	5·58	—	—
76	—	—	—	—	27·79	3·05
102	—	—	81·85	5·64	—	—
104	—	—	—	—	66·05	4·49
123	—	—	123·84	4·09	—	—
129	116·29	5·18	—	—	—	—
132	—	—	—	—	98·97	6·35
144	—	—	149·99	7·04	—	—
155	153·67	6·98	—	—	—	—
160	—	—	—	—	132·12	4·64
165	—	—	192·84	6·22	—	—
169	179·13	6·40	—	—	—	—
183	197·13	7·37	—	—	—	—
188	—	—	—	—	170·28	5·10
193	—	—	250·01	8·68	—	—
198	195·75	8·68	—	—	—	—
207	—	—	246·38	14·11	—	—
211	202·91	8·96	—	—	—	—
216	—	—	—	—	193·01	15·61
225	217·01	11·84	—	—	—	—
228	—	—	245·88	14·23	—	—
239	222·08	11·38	—	—	—	—
242	—	—	296·51	13·29	—	—
244	—	—	—	—	182·78	16·22
263	—	—	267·25	15·81	—	—
277	—	—	257·60	17·05	—	—

(*b*) *Nitrogen Compounds*

The changes in amount of the nitrogen compounds during the three seasons confirm the observations recorded by Robertson and Turner (1951). Total and

protein nitrogen per cell increase with increasing cell volume in the preclimacteric period. Within a pick the fruits with the larger cell volume had more protein nitrogen per cell; for example in pick 8, the fruit with the greatest cell volume, 0.0058 cu. mm., had the highest protein nitrogen, 0.101×10^{-8} g. per cell. In the same pick the fruit with the smallest cell volume, 0.0043 cu. mm., had the lowest protein nitrogen per cell, 0.071×10^{-8} g. per cell. Similarly the fruits with the lower cell numbers, which were mostly those with the larger cells, had the highest protein nitrogen per cell.

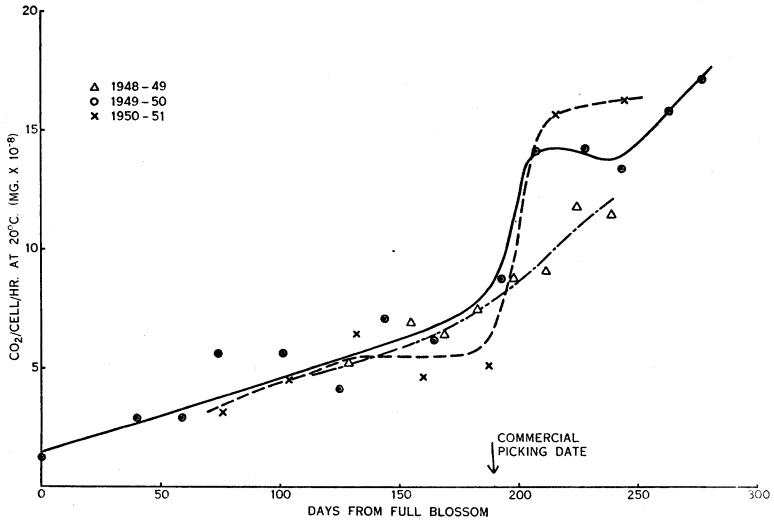

Fig. 10.—Changes in respiration rate per cell with time.

If protein nitrogen can be taken as a measure of cytoplasmic content, and if, as suggested by Robertson and Turner (1951), the cytoplasm increases with increase in cell surface but remains approximately constant in thickness, a linear relationship between protein nitrogen per cell and cell surface would be expected. This linear relation was obtained in both seasons, which confirms this interpretation. The results from tree 2 are shown in Figure 6. The results from later picks of tree 3 in 1949-50 did not show the linear relationship but this was explained by the marked drop in protein nitrogen at the same time as the decrease in dry weight; after this temporary setback, protein nitrogen increased. The last sample for both trees was high in protein nitrogen, suggesting increased rate of protein synthesis at the end of the life of the fruit on the tree. Protein synthesis was relatively more rapid than increase in dry weight in the last two samples. If, then, the protein nitrogen level is a true measure of cytoplasmic protein, the cytoplasm forms a layer of constant thickness inside the cell wall during cell expansion, but protein apparently may continue to increase later when increase in cell surface is only small.

Mean protein nitrogen and mean soluble nitrogen were correlated during growth up to 190 days with a correlation coefficient of + 0.844 (with 7 degrees of freedom). At commercial maturity, protein and soluble nitrogen contents were

correlated, thus being consistent with the results of Robertson and Turner (1951). After this time, however, the soluble nitrogen increased rapidly relative to the protein nitrogen. This was not recorded by Hulme (1951) whose observations ceased at 160 days, or by Robertson and Turner (1951) whose observations were not continued beyond 205 days. This increase in soluble nitrogen may be the result of protein degradation in the senescent leaves supplying the fruits. The composition of the soluble nitrogen fraction is now being studied.

In all three seasons, there is evidence that both protein and soluble nitrogen, which have been increasing up to 120 days, then remain stationary until 190 days when the soluble nitrogen again increases rapidly. After 190 days, the protein nitrogen also increases, but only slowly. The check in the increase of protein and soluble nitrogen after about 120 days varied in the different seasons. In 1950-51, the increase in protein appeared to lag behind the increase in soluble nitrogen. The lack of net protein synthesis at a time when soluble nitrogen content is high shows that some factor other than nitrogen supply controls protein synthesis, and possibly cell enlargement, at this stage.

(*c*) *Organic Acids*

The total organic acids per cell increased, as observed by Robertson and Turner, up to 190 days from full blossom, but did not increase markedly during the remainder of the season when the nitrogen compounds increased so rapidly and when the respiration rate increased in the climacteric. At this period, however, an increase in acids in the fruit of one tree showed a capacity for synthesis. Mean total organic acids per cell were correlated with mean protein nitrogen per cell over the whole period with a coefficient of + 0.93 (with 12 degrees of freedom). This confirms the high correlation found by Robertson and Turner in samples of fruit up to commercial maturity. In all living material investigated the acids form the connecting link between the respiration mechanism and protein synthesis. Turner (1949) found evidence of a tricarboxylic acid cycle in mature Granny Smith fruits. It is possible, as suggested by Robertson and Turner (1951), that the increase in acid content with increase in protein content in developing fruit can be explained by a steady-state relation between acids and nitrogen compounds. Perhaps, towards the end of the life on the tree when the respiration has increased, the steady-state organic acid content does not change, an increased rate of formation being offset by an increased rate of utilization, due both to protein synthesis and to use as additional substrates in respiration. Homogenates of apple tissue in the present season (1951-52) show that there is an increase in enzymes capable of oxidizing organic acids at the time of the onset of the climacteric. Kidd *et al.* (1951) found that the rate of metabolism of malic acid in Worcester, Pearmain, and Bramley's Seedling apples was unaffected by the onset of the climacteric rise, which agrees with our observation that the total organic acid content does not change at the time of the climacteric rise.

A linear relationship between cell surface and total organic acids was obtained in this work (Fig. 6) and agrees with the observations of both Kidd

et al. (1951) and Robertson and Turner (1951). The latter authors interpret the high correlation of protein nitrogen with total organic acids and of total acids with cell surface to indicate a steady-state relationship between protein nitrogen, organic acids, and respiration. Kidd *et al.*, however, suggest that the organic acids are not related to protein synthesis but are formed as a side reaction of the metabolism of cell wall formation. Evidence from our data is insufficient to decide between the two hypotheses but would favour the Robertson and Turner interpretation as it appears that the total acids continue to be formed in the late picks after cell wall formation has ceased.

(*d*) *Respiration and the Climacteric Rise*

Robertson and Turner (1951) found some evidence of a climacteric rise in respiration at the end of their experiment. In all three seasons discussed in this paper, a climacteric rise in respiration took place at about 190 days from full blossom, at the time of commercial maturity. A number of metabolic changes occur in the cells about the time of the climacteric rise and provide further information as to the possible cause.

Prior to the onset of the climacteric rise in respiration, protein nitrogen per cell was related to respiration rate per cell. In individual fruits those with larger cells had a high protein nitrogen per cell and a high respiration rate per cell. Hulme (1951) has shown that the ratio of respiration rate to protein nitrogen content is reasonably constant during development from a period soon after cell division until the onset of the climacteric. Our data over the two complete seasons agree approximately with this, but in 1949-50 season this ratio for the mean respiration rates and protein nitrogen contents in the different picks decreased from 0.9 to 0.76 (not significant at $P = 0.05$) and in the 1950-51 season from 0.9 to 0.6 (just significant at $P = 0.05$), before the onset of the climacteric rise. The 12 observations, over the 2 years, taken together, are homogeneous and the decrease is significant (at $P = 0.01$).

In contrast, at the climacteric, the increase in respiration rate was not accompanied by a corresponding increase in protein nitrogen. A number of workers (e.g. Gregory and Sen 1937) have found a relation between respiration rate and protein nitrogen during growth but the mechanism of control has not yet been worked out. The difference between preclimacteric and climacteric cells in our work suggests that the factors controlling respiration rate and protein synthesis at the two stages are different. The protein nitrogen level was the same in all seasons at the time of the climacteric rise and a small increase in protein took place after the rise. Since both soluble nitrogen and respiration rate are considerably increased after the climacteric rise, net protein formation must be limited by some other factor. Possibly the transfer of energy even from the increased respiration is sufficient only for the resynthesis of the unstable protein in the cells by that time; thus at higher nitrogen contents the turnover of nitrogen would be increased without the net protein content increasing in proportion.

Other observations on the climacteric rise confirm those of Robertson and Turner (1951). The increasing protein turnover in synthesis and degradation

may result in the increase in respiration rate, because, as Robertson and Turner suggest, the more rapid removal of phosphates from the phosphate carrier systems, thus freeing phosphate acceptors, would probably increase respiration rate. The increase in respiration rate is being studied further, both in cut tissue and in homogenates from apples of different maturities. Some evidence has been obtained which supports the hypothesis and will be published soon.

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