THE PHYSIOLOGY OF GROWTH IN APPLE FRUITS

I. CELL SIZE, CELL NUMBER, AND FRUIT DEVELOPMENT

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Summary

The problem of fruit size in the Australian apple variety Granny Smith was examined in relation to mean cell size and mean cell number. Cell size gradients in the fruit and changes in cell shape and packing during development were noted.

Observations of workers on other varieties that cell division ceased within four weeks of pollination were confirmed. Variation in size of fruits at maturity was shown to be due mostly to variation in cell number and only to a small extent to mean cell size. Cell enlargement was shown to continue throughout the life of the fruits on the tree.

I. INTRODUCTION

Growth of fruits is a problem, not only of considerable plant physiological interest but also of outstanding economic importance. In apples, for instance, fruit size has for a long time been regarded as an important factor in determining the keeping quality of apples in storage. Since keeping quality is related to the physiology of the fruit, it is of considerable interest to investigate the anatomical and histological causes of differences in fruit size and to relate these to physiological phenomena, both during the development of the fruit and during its senescent life after removal from the tree. The work described in this paper was undertaken to study the relationship between cell size and fruit size, and in a second paper this will be related to physiological and biochemical changes.

Smith (1940) has determined the cell size and cell number of several varieties of English apples. He has related size and number of cells in the flesh of mature apples to the sizes of the fruits at maturity, and correlated respiration rates and keeping quality with cell number. This work was based on mean cell size determinations made on tissue at a standard depth under the skin of the fruit. Smith suggested that if increase in size of the bigger apples were due entirely to more cells, then cell sizes of both large and small apples should be the same. If, on the other hand, increase in fruit weight were to be accounted for by cell enlargement alone, then cell size should have doubled for a doubling in apple weight. Since neither of these postulates was

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obeyed, he concluded that differences in sizes of mature apples were due both to the amount of cell division and to the degree of cell enlargement. Further, Smith has correlated the respiration rate of different varieties with cell number and has shown that there appears to be a correlation between respiration rate, expressed on a unit fresh weight basis, and number of cells per gram of tissue. It is also pointed out that those varieties with the greater numbers of cells and the higher respiration rates per unit weight are also those which have poorer keeping quality. In a more recent paper, Smith (1950) has extended these observations to evaluate the part played by cell multiplication and cell enlargement in the development of fruits of a number of varieties. In this paper, similar observations that have been made on the Australian variety Granny Smith, will be described.

Various authors have concerned themselves with the anatomy and histology of the apple fruit, but have not been primarily interested in the problem of size. Tetley (1930, 1931) studied the morphology and cytology of developing Bramley's Seedling apples and established the fact that cell division ceases a few weeks after fruit set. Thereafter, increase in size is mostly due to cell enlargement. MacDaniels (1940) was concerned with the morphology of pome fruits and MacArthur and Wetmore (1939, 1941) with developmental studies of the anatomy of McIntosh Red and Wagener apples. Apart from the work of Smith, the only investigation that bears directly on cell size is that of Tukey and Young (1942), who studied the varieties Lodi, Early Harvest, Twenty Ounce, McIntosh, and Rome, with histological observations on the McIntosh. Tukey and Young record that cell division in the pith seems to have ceased by three weeks after full blossom and, thereafter, increase in size is due to increase in the size of cells and intercellular spaces, some cells reaching 150 x 300 μ. In the cortical region also, cell division appears complete three weeks after full bloom and thereafter cell enlargement is responsible for increase in size, some cells attaining a size of 197 x 340 μ. Observations on the number of cells across the cortex indicate that there is very little increase in number after three weeks from full blossom.

The interpretation of the morphology of the apple fruit is uncertain. Two theories have been suggested. The receptacular theory considers the fruit to be composed of five drupe-like carpels contained in a fleshy receptacle, which is therefore regarded as a fleshy development of the stem, so that areas comparable with pith and cortex can be defined. The appendicular theory considers the carpels to be enclosed by fleshy tissue derived from the fused and enlarged bases of the floral appendages so that the apple flesh represents the floral tube formed from fused petals, sepals, and stamens. It is not necessary to discuss the relative merits of these two theories in this work; for convenience, the receptacular theory is followed and the terms pith and cortex are used for the regions respectively inside and outside the ring of ten vascular bundles.

This paper describes attempts to determine cell size, cell number, gradients in cell size within the fruit, and gross morphological changes during development, and to correlate fruit size, cell size, and cell number.

II. Material and Methods

The material used was the Granny Smith variety and was obtained from four trees. Tree 1 is at Orange, N.S.W., and trees 2, 3, and 4 are at the New South Wales Department of Agriculture Experiment Farm at Bathurst, N.S.W.

Two main experiments were carried out. For the first of these, *the experiment on size in mature fruits,* 43 fruits of weights ranging from 73.8 g. to 251 g. were taken from tree 1 at time of commercial picking in 1947, and the cell sizes determined. At commercial picking in 1948, another 24 fruits were taken, ranging in size from 101 g. to 210 g. and the cell sizes determined.

Table 1
DATES OF PICKING OF EARLY SAMPLES

Sample	Date	Days from Full Blossom
A	10.xi.47	21
B	17.xi.47	28
C	24.xi.47	35
D	9.xii.47	42
E	17.iii.48	149

For the second experiment, the *fruit growth experiment,* developing fruits were sampled at intervals from the Experiment Farm trees. Date of full blossom was taken as the approximate date of first petal fall, October 20, 1947. Between petal fall and December 10, 1947, samples were taken from tree 2 to examine the cell division stage. Fruit was not taken from the main experimental trees (3 and 4) over this period so as to avoid overthinning these trees. Fruit taken from tree 2 over this period consisted of four samples each of 24 fruits; sizes ranged from 1.4 to 20.95 g. Table 1 shows the dates of picking

Table 2
DATE OF PICKING AND DAYS FROM FULL BLOSSOM OF SUCCESSIVE SAMPLES FROM TWO TREES

Pick	No. of Fruit		Date	Days from Full Blossom
	Tree 3	Tree 4		
1	20	—	10.xii.47	51
2	20	—	31.xii.47	72
3	10	10	4.ii.48	107
4	10	10	18.ii.48	121
5	10	10	3.iii.48	135
6	10	10	31.iii.48	163
7	10	10	14.iv.48	177
8	10	10	28.iv.48	191
9	9	10	12.v.48	205

of these samples and of another sample taken from the same tree much later. From December 10, 1947 onwards, fruit was taken from the main experimental trees as shown in Table 2. Commercial picking date was April 28, 1948 — 191 days from full blossom.

(*a*) *Fruit Size.*—Each fruit was weighed with the stalk attached and was then cut in half transversely. Tracing paper was placed on the cut surface of the half fruit, and the regions of the tissues outlined; the areas of the various tissues were determined from these tracings with a planimeter.

(*b*) *Fixing Material.*—Anatomical studies of apple tissue are difficult because of the large intercellular spaces, which are filled with air; all cell examinations must be made with fixed material from which air has been excluded. Sections of the tissue to be examined were cut and fixed in acetic-alcohol (1 : 3), which removed the air from the intercellular spaces. After fixing, sections were stored in 70 per cent. alcohol until required. When the fruits were being examined for cell division the fixative used was formalin-alcohol (6 ml. formalin in 100 ml. 70 per cent. alcohol). The method of fixation made negligible difference to the mean cell diameters. This was tested in two ways:

(1) cylinders of tissue were measured when cut, and again after fixation; the largest change in dimensions was only about 3 per cent., and

(2) sections were examined in isotonic solutions and then transferred to fixative; no significant change in mean cell diameter was detected after fixation.

(*c*) *Staining.*—When the tissue of young fruits was being examined for cell divisions, safranin-haematoxylin was used. Water blue stained the nuclei and the cytoplasm of cells in mature tissues. It was unnecessary to use any stain in the measurement of cell size as the cell outlines in apple tissue are very distinct.

(*d*) *Estimation of Cross-Sectional Dimensions of Cells.*—A micro-projector was found to be very satisfactory to trace cell outlines. A calibrated micrometer was used to obtain the magnification (approximately ×100) of the projector.

Since the form of the cells was found to approximate to spheres or to oblate spheroids (elliptical on the major axis of rotation), the volume was calculated from the tracing of the cells by measuring the major and minor axes and using the formula $\frac{4}{3}\ \pi\ a\ b^2$ where a is half the length of the major axis and b is half the length of the minor axis.

(*e*) *Sampling.*—Sampling was done in different regions of the fruits to establish the gradients in cell size. Four regions of different cell size can be distinguished: the skin region of very small cells, the mid-cortical region in which the cells are much larger, the region of very small cells round the vascular tissue, and the pith region consisting of large cells, mostly elongated along the radius of the fruit. Although standard deviations were always high, indicating variability in cell size even within one region, it was found that measurement of 20 or 25 cells was sufficient for a sample of the mid cortex or pith, though in some experiments large numbers of cells were measured.

As the apple increases in size, the cells elongate along the radius of the fruit, so that cells that appear approximately circular in tangential longitudinal

section are oval in both radial longitudinal section and transverse section. Consequently, the measurements of the cells were taken in transverse section. In most determinations the sections were taken from the equatorial radii of the fruits.

III. Results

(*a*) *Size in Mature Fruits*

(i) *Mean Cell Size.*—Measurements of 25 cells in the mid region of the cortex were made and the mean volume of cells was calculated. Mean cell volume is plotted against fruit weight in Figure 1. The cell size of fruits of the 1948 pick appears to be slightly less than that of the 1947 pick, but in each case there is little increase in cell volume with increase in weight. Thus, taking a line of best fit (drawn by inspection) to the 1947 sample, the mean cell volume of a 70 g. mature fruit is 0.0030 cu. mm. and that of a 250 g. mature fruit is 0.0046 cu. mm., i.e. as the tissue volume measured by weight (assuming that the specific gravity of the cells does not change appreciably) increases 3.6 times, the mean volume of the cortical cells increases only 1.5 times. This

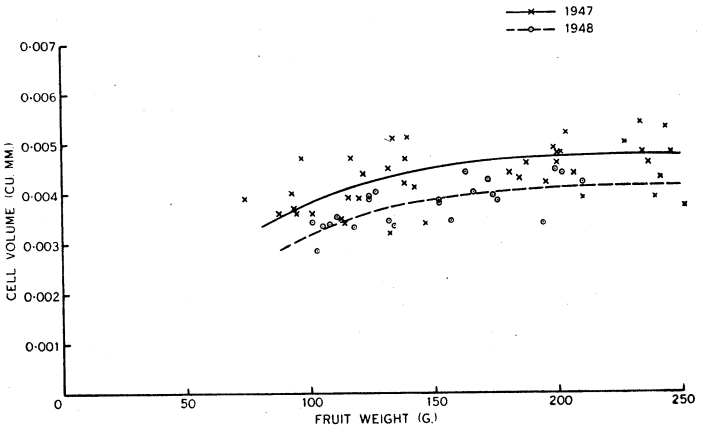

Fig. 1.—Relationship of mean volume of mid-cortical cells to fruit weight in apples from the same tree in two seasons.

is even more striking than the difference Smith (1940) obtained and interpreted as being due to a difference in cell number in the larger fruit. These results show that the difference in flesh volume with size of different mature fruits cannot be accounted for by the difference of the volumes of cells from the mid-cortical region. It could be accounted for by differences in cell number, provided that the mean cell size for the mid-cortical region can be taken as a reasonable value for the true mean of the fruit. There are, however, considerable variations in cell sizes in different regions of the fruit and it is necessary to examine the gradients in cell size to determine how far the cortical mean cell size represents the value for the fruit as a whole.

(ii) *Cell Size Gradients.*—No satisfactory investigations of the gradients in cell size have been reported, though the variations in different parts of the fruit have been referred to by earlier workers (Smith 1937; Tukey and Young 1942). Five apples from the same tree were taken and weighed; the weights ranged from 120.3 to 216.5 g. These five apples were cut transversely at the equator and the morphological details — cross-sectional area, position of vascular tissue, and size of carpellary cavity — were traced for subsequent measurement. Two transverse sections were then cut from each of four opposite radii in each apple; the first section on each radius extended from the skin to the region of the vascular bundles and the second section extended from the region of the vascular bundles to the carpel wall. From these two sections, it was possible to measure the cells in consecutive fields from the skin to the carpel wall across the fruit; 20 cells from each field were traced and the mean cell volumes were plotted against distance along the radii. Figure 2 shows the cell volume gradients for one of these fruits, which was typical. The cells immediately under the skin are very small but increase in size in the cortex, reaching a maximum size, which then changes little till the region of the vascular tissue is reached, when the cells diminish in size. They increase again in size in the centre of the pith. Cells on either side of the vascular tissue are small and tend to be elongated along a radius of the fruit, especially in the region between the sepal bundle and the dorsal carpellary bundle. Cells of the central pith area are also often considerably elongated along the radius of the fruit. The tendency of the cells of cortex and pith to reach approximately uniform size is apparent. The size of the fruit as a whole will be dependent on the total number of cells in the fruit, and the proportion of that number reaching the maximum size.

Since most of the fruit consists of regions with these cells of maximum size, the mean volume of cells from the cortex can be taken as the volume of cells contributing most to the fruit size. The mean cell volume is given by $3/a^3 \int_0^a v\, r^2\, dr$, where a is the radius of the apple (assumed spherical) and v is the cell volume at distance r from the centre. By inspection of Figure 2, it appears that the cell volume in the mid cortex will be very close to the mean value, and comparison with mean volumes calculated from the formula shows that this is true.

(iii) *Cell Number.*—The "cell number" is calculated by dividing the tissue volume* in the fruit by the mean cell volume estimated from the mid cortex. This is a fair estimate of the number of cells contributing most to the fruit size. This is the same method as used by Smith, except that in his work the cell volume was determined 4 mm. below the skin at the equator.

The "cell number" for the mature fruits of tree 1 in the 1947 and 1948 harvests is plotted against fruit weight in Figure 3. The range of cell numbers is from 17×10^6 in the smallest fruit to 62×10^6 in the largest, i.e. there are

* Tissue volume was determined from the tissue weight divided by the specific gravity of the cells, which was found to average 1.05.

about four times as many cells in the large fruits as in the small fruits. Two straight lines have been fitted to these two sets of data, by the simple linear regression technique, using the usual assumptions and taking fruit weight as the independent variate; the fit is not significantly improved by the inclusion of a quadratic term. The difference between the slopes of the two lines was not significant, but the difference in cell number for fruits of average weight in the two seasons was significant. Over the range of fruit weights investigated, the number of cells per gram was about 160,000 to 170,000. This compares closely with Smith's figure for cell number in Bramley's Seedling. Extrapolation of these lines of best fit is such that the origin is not included in the confidence interval at zero fruit weight. This would support the suggestion that, over the lower range of mature fruit weights, mean cell sizes are lower (cf. Fig. 1).

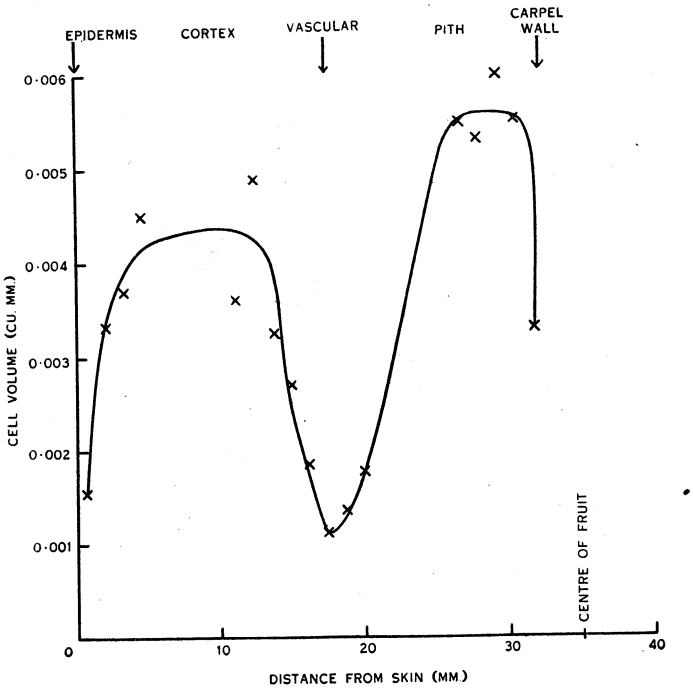

Fig. 2.—Gradient in cell volumes along an equatorial radius in a mature apple.

How far this "cell number" approximates to the true cell number laid down at the time cell division ceases will be discussed in the section on fruit development.

(iv) *Gross Size of Fruit; Cell Size and Air Spaces.*—The overall volume of the fruit is related not only to the number and volume of the cells but also to their packing within the fruit and the volumes occupied by intercellular spaces and carpel cavity. To examine the importance of the air spaces, 25 mature fruits were taken from the 1947 pick over the size range from 160 to 253 g.

and the volumes were determined by displacement. From the weights and volumes the specific gravities of the fruits were obtained; the latter decrease with increasing fruit weight. Using the specific gravity curve against fruit weight as a standard curve, it was possible to calculate the volumes of the fruits used in the main experiment; from this, volume of the tissue, i.e. weight divided by specific gravity of the cells (1.1), was subtracted and the volume of air space in each fruit was obtained. The percentage of air space plotted against fruit weight is shown in Figure 4. The percentage increases rapidly with size of fruit, but in the larger fruits approaches an asymptote at about 27 per cent. of the volume. To eliminate the possibility that this change in proportion of air space was due primarily to an increase in the carpel cavity in large fruits, a number of fruits were peeled and quartered and the carpel region removed. Determination of the specific gravity of the cortical region showed a decrease with increasing weight similar to that obtained with the whole fruit.

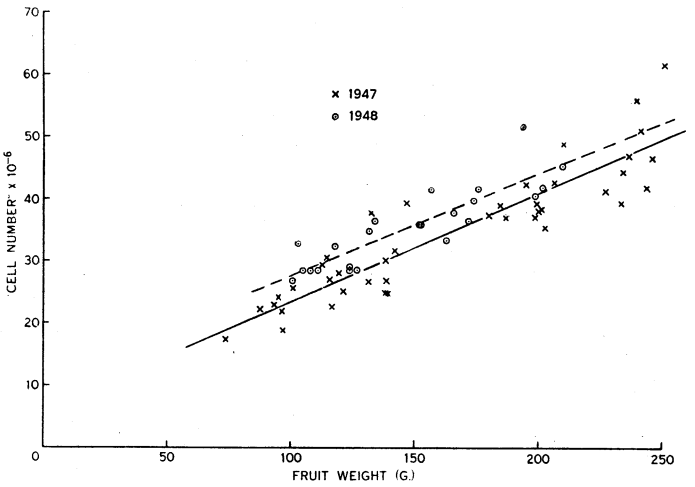

Fig. 3.—Relationship of calculated cell number to fruit weight; apples from the same tree in two seasons; regression equations were $y = 6.0 + 0.17\ x$ for 1947 and $y = 11.4 + 0.16\ x$ for 1948.

These changes in the relative proportions of cell space and air space indicate a difference in the packing of cells in larger fruit. It is noticeable that the change in percentage of air space is most marked in the smaller size ranges where the fruit size differences are due to cell size differences as well as cell number (cf. Fig. 1). This indicates that the larger the cells, the more loosely they are packed. In the larger size ranges where cell size does not increase with fruit size, the differences in percentage of air space with change in size of fruit are not so marked.

(*b*) *Fruit Growth Experiment*

(i) *Cell Number.*—Because of the uncertainty about the validity of the cell number determinations based on the cell sizes of mature fruits, it was thought desirable to estimate cell number on very young fruit to determine the

mean number and the range of numbers to be expected. For this purpose, fruit from tree 2 was used. Cell division was seen clearly in material collected on November 6, 1947. In the dividing cells, the cytoplasm collects in the centre of the cell where the nucleus divides and the two daughter nuclei can be seen clearly in juxtaposition across the new cell wall. The typical appearance of the cells at this stage is shown in Figure 5, which is a camera lucida drawing. By November 10, 1947, cell division appeared to have ceased, i.e. 21 days after full blossom. This agrees with the findings of other workers with other apple varieties. The volumes of the cells in fruit at this early stage were very uniform as the gradients had not yet been established and the cells can be treated as approximately spherical. Table 3 shows the results with these fruits (S.G. taken as 1.1).

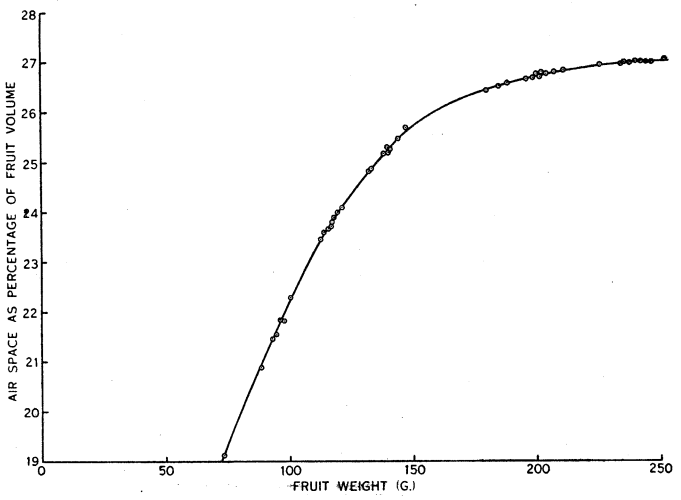

Fig. 4.—Relationship between percentage air space and fruit size.

These results confirm the conclusion that there is a wide range in cell number in these fruits and establish the order of magnitude of the cell numbers in Granny Smith apples. The range in cell number calculated in the above picks was from 21×10^6 to 74×10^6 and the mean cell number was 43×10^6. Later in the season, a sample of 19 fruits was taken from the same tree on March 17, 1948, i.e. 149 days from full blossom. The cell sizes for the mid-cortical region and the calculated "cell numbers" are shown in Table 4.

The mean calculated cell number at this stage is thus 45.0×10^6 and the range is from 30.6×10^6 to 65.7×10^6. This is not significantly different from the cell number of the small fruit and indicates that the method of obtaining "cell number" from mean cell volume gives a similar approximation.

(ii) *Gradients of Cell Size Within Fruit.*—Some fruits were examined for gradients in cell size across the equator from skin to carpel cavity, with particular reference to the cortical region. The method was as described for the

fruit size experiment. Figure 6 shows the change in cell diameter in different regions of the cortical tissue, of some of the fruit examined in this way. The gradients seen in the fruit of the fruit size experiment appear to become estab-

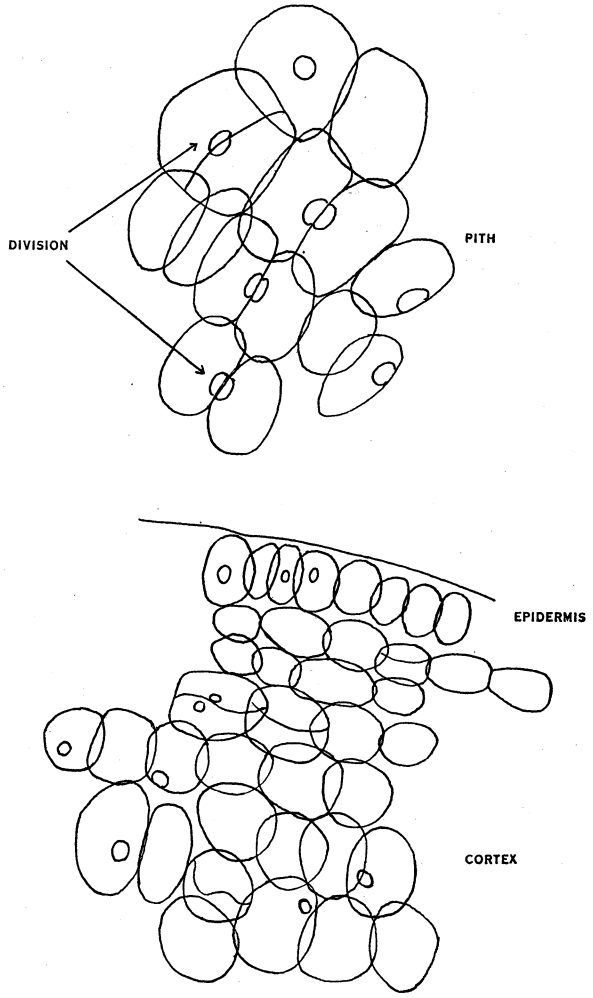

Fig. 5.—Typical appearance of the newly divided cells in young apples.

lished early in the life of the fruit and the cells of the cortex then seem to enlarge in such a way as to preserve the relative differences in size. Thus in fruit 4/10 from pick 9, 15 mm. of cortex contains 60 of the 85 cells (calculated number) with average cell diameter of 0.252 mm., which is significantly different from the mean diameter of the cells in the 4 mm. immediately under the epidermis. By use of these diagrams for gradient of cell diameter, the total number of cells across a given region can be estimated. In this fruit the estimated number is 85, which agrees reasonably with the counted number of 80.

The hypothesis that no cell division occurs after the early period can be tested further by comparing the cortical width, as determined from the area measurements of the whole fruit less that enclosed by the vasculars, with the

TABLE 3

MEAN CELL VOLUMES AND MEAN CELL NUMBERS OF FRUITS IN EARLY SAMPLES

Date of Pick	Days from Full Blossom	Weight of Fruit (g.)	Volume of Tissue (cc.)	Mean Cell* Volume (cu. mm. × 10^5)	Cell Number (× 10^{-6})
10.xi.47	21	1.40	1.27	2.13	59.5
		1.80	1.64	3.88	42.3
		2.31	2.10	5.10	41.2
		3.40	3.09	6.95	44.5
		4.45	4.05	13.20	30.7
17.xi.47	28	1.20	1.09	2.83	38.5
		1.60	1.45	4.59	31.6
		2.65	2.40	6.08	39.4
		3.15	2.86	4.90	58.5
		3.40	3.09	8.35	37.0
		3.05	2.78	6.01	46.1
		3.70	3.36	9.80	34.2
		3.28	2.98	13.9	21.4
		5.18	4.70	6.31	74.4
		5.75	5.23	9.40	55.7
		6.10	5.54	12.40	44.6
24.xi.47	35	7.35	6.7	15.1	44.3
		7.83	7.1	11.3	62.7
		8.12	7.4	19.6	37.8
		9.60	8.7	19.6	44.3
		8.70	7.9	23.0	34.3
		10.75	9.8	23.0	42.6
		9.07	8.3	19.6	42.3
		12.50	11.4	28.8	39.6
		12.70	11.5	26.8	42.9
		10.20	9.3	19.6	47.5
		11.42	10.4	40.8	25.4
		14.10	12.8	31.1	41.1
1.xii.47	42	12.45	11.3	28.0	40.4
		13.55	12.3	25.6	47.0
		14.15	12.7	32.6	38.7
		14.95	13.6	51.2	26.6
		17.35	15.7	55.6	28.2
		19.30	17.5	40.2	43.5
		15.45	14.0	31.6	44.3
		16.50	15.0	32.0	46.9
		20.95	19.1	39.8	48.0

* Except for the first pick, where 200 cells were measured, 100 cells were measured in each.

calculated cortical width based on the total assumed number of cells (78) and the mean major axis of the cells measured. The value of 78 cells in the cortex was the mean counted number in pick 1 when the cells were still countable. These results are given in Table 5.

The agreement between the observed and calculated trend in cortical thickness is in reasonable agreement with the hypothesis that cell enlargement alone is responsible for the increase in width of the cortex. Fruit of pick 9, which will be discussed later, departs anomalously. It should be pointed out

TABLE 4

MEAN CELL VOLUMES AND MEAN CELL NUMBERS OF FRUITS TAKEN LATE IN THE SEASON FROM THE SAME TREE AS THOSE SHOWN IN TABLE 3; DATE OF PICK MARCH 17, 1948, 149 DAYS FROM FULL BLOSSOM

Weight of Fruit (g.)	Volume of Tissue (cc.)	Mean Cell Volume (cu. mm. $\times 10^4$)	Cell Number ($\times 10^{-6}$)
142.02	129	36	35.9
161.51	147	48	30.6
161.41	146	38	38.5
163.10	148	39	38.0
167.46	152	39	39.0
172.31	156	37	42.2
174.15	158	39	40.5
177.50	162	36	45.0
180.20	164	47	34.9
185.14	168	34	49.5
188.20	171	40	42.7
189.50	173	36	48.0
200.10	182	39	46.7
201.07	183	45	40.7
202.85	185	32	57.9
205.72	187	43	43.5
222.10	202	34	59.5
239.16	217	33	65.7
249.91	297	40	56.5

that a small variation in the number of cells across the cortex, however, represents considerable increase in cell number. The average radius for fruit of pick 8 is 3.91 cm. Compare a spherical fruit (of average radius) with 80 cells

TABLE 5

RELATION BETWEEN WIDTH OF CORTEX AS MEASURED AND WIDTH OF CORTEX AS CALCULATED FROM MEAN MAJOR AXIS OF CELLS

Pick	Av. Weight (g.)	Av. Width of Cortex (cm.)	Mean Major Axis of Cells (mm.)	Calculated Width (cm.)
1	19.85	0.70	0.098	0.76
2	42.20	0.87	0.132	1.03
3	96.70	1.40	0.184	1.44
4	119.20	1.39	0.182	1.42
5	149.47	1.74	0.206	1.61
6	180.38	1.79	0.226	1.76
7	201.45	1.84	0.220	1.72
8	208.82	1.88	0.248	1.93
9	214.0	1.86	0.292	2.28

across the cortex with another spherical fruit having 90 cells across the cortex. The average radius for each cell for that pick is 0.011 cm. Then the radius of the larger fruit would be 4.13 cm. and the volumes of the two spheres would be 250 and 295 cc., the additional rows of cells contributing 45 cc. Such an addition of 10 rows of cells, i.e. an increase of 12½ per cent., would increase the total cell number of the fruit from 30×10^6 to 35.4×10^6, or an increase of 18 per cent.

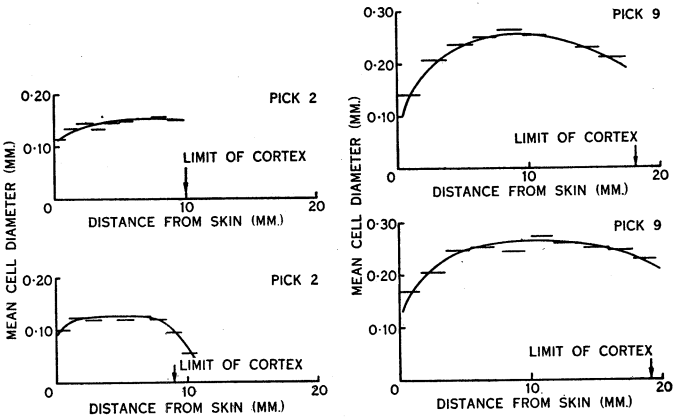

Fig. 6.—Gradient in cell size in the cortex of a young fruit (72 days from full blossom) compared with that of a mature fruit (205 days from full blossom).

So far considerations have been based on the increase in width at the equator of the fruit. Some measurements of gradients in cell size in other directions in the fruit were made in the development experiment. Observations showed that the gradients in the cortex, along radii other than those at the equator, did not differ significantly from those at the equator. It seems, therefore, that the measurements taken at the equator are likely to be satisfactory for obtaining mean cell volume.

(iii) *Gross Development and Cell Size.*—The development of the fruit, as measured by weight and plotted against number of days from full blossom, is given in Figure 7. This gives the usual form of growth curve. No direct measurements of volume were made on most of these picks. Simultaneously, the areas of the fruits across the equators were measured and these areas were used to determine a mean radius. The volumes of spheres of these radii were calculated and these are also plotted for comparative purposes in Figure 7 as the fruit "volume." The volumes of the fruits used in picks 9 and 10 were determined and are also shown on the graph; the calculated spherical volume underestimates the actual volume.

Within the fruit, the comparative areas of the different regions of tissue at the equator, as seen in transverse section, are given in Figure 8. Areas increase up to approximately 190 days from full blossom (mean weight 207 g.) and this increase is shown in both cortex and pith. The area of the carpel

cavities altered little with increasing fruit size, contributing little to the total area and therefore little to the total volume. The relative proportions of cortex and pith did not change very much; the cortex increased from 64 per cent. of the area in 20 g. fruit to 73 per cent. of the area in 214 g. fruit; meantime the pith decreased from 27.6 per cent. to 24.3 per cent. The relative proportions of the different tissues were constant after 135 days from full blossom (mean weight 150 g.).

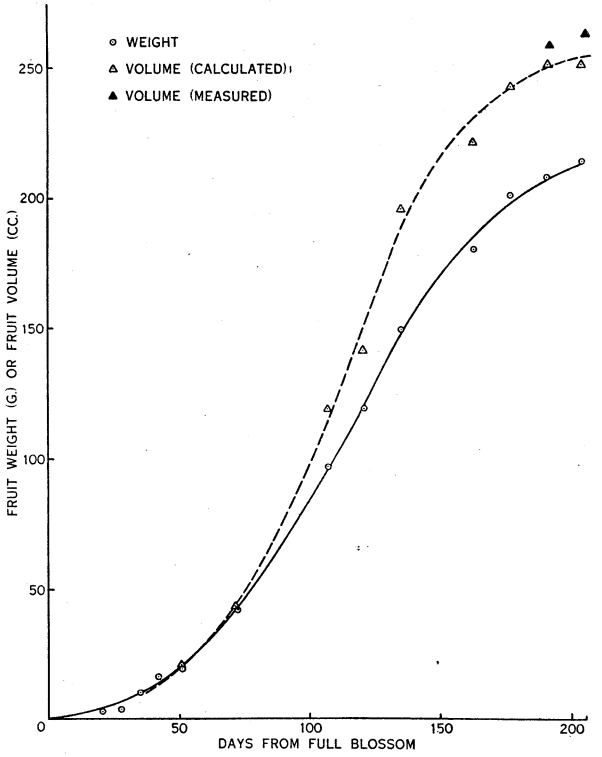

Fig. 7.—Increase in mean weights of fruits with time; calculated fruit "volumes" are included for comparison with the measured volumes of two samples.

Since all the evidence supports the view that, after the first few weeks, fruit growth is a matter of cell enlargement, provided that the specific gravity of the cells does not change during development, the applicability of the cell size determinations to the three-dimensional development of the fruit can be obtained from calculation of cell volumes and comparison with fruit weight. This comparison is better than that with fruit volume, which may be complicated by changes in the intercellular spaces as well as changes in cell volume. The specific gravity of the cells and their contents, which was shown to be about 1.05, does not alter markedly during the developing period. Thus volumes of cells can be compared with the weight of the fruit without introducing an error of any great magnitude. When the mean cell volumes of each

pick are plotted against mean fruit weights, a linear relationship between calculated cell volume and fruit weight is obtained over most of the range of sizes, though there is a suggestion that pick 9 departs anomalously (Fig. 9). The results are given in Table 6.

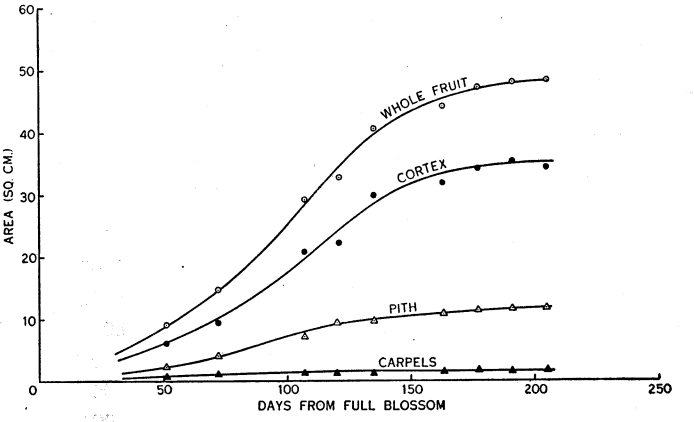

Fig. 8.—Changes in area of different tissues with time.

From the preceding discussion, it would be expected that, if the sample of 10 fruits from each tree were large enough to represent truly the cell sizes and cell numbers of the fruit on the tree at the time of picking, the relationship would be perfectly linear. The fruits of pick 9 give an indication of what

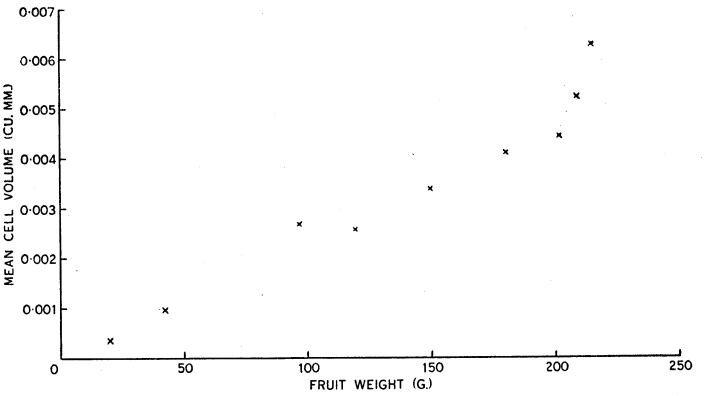

Fig. 9.—Mean volumes of mid-cortical cells of successive samples plotted against fruit weight.

is probably the principal cause of this departure. It can be seen from Figure 10 that this sample contains a wider range of size classes than either of the two preceding samples. Despite this, the cell volumes of the smaller fruits in this pick are as great as those of the larger fruits, therein resembling the cell size range in the fruit size experiment. If the cell size is used to calculate cell

number for pick 9, the same wide range in number that has been noted earlier is found. Thus the cell number ranges from 21.3 × 10^6 to 46.1 × 10^6 with a mean at 32.6, which is significantly different from the mean of pick 8. This seems to indicate that the sampling of 10 fruits per tree is not satisfactory to

TABLE 6

RELATIONS BETWEEN MEAN CELL VOLUME AND MEAN CELL NUMBER IN FRUITS OF SUCCESSIVE PICKS

Pick	Days from Full Blossom	Av. Weight (g.)	Tissue Volume (cc.)	Mean Cell Volume (cu. mm.)	Mean Cell Number (× 10^{-6})
1	51	19.9	18.9	0.00037	51.0
2	72	42.2	40.2	0.00096	41.8
3	107	96.7	92.0	0.00267	34.5
4	121	119.2	113.8	0.00255	44.6
5	135	149.5	142.2	0.00337	42.3
6	163	180.4	172.0	0.00409	42.1
7	177	201.5	191.8	0.00442	43.4
8	191	208.8	199.0	0.00521	38.2
9	205	214.6	204.5	0.00628	32.6

give a true picture of the relation of cell enlargement by volume to the total weight of the fruit, because the relationship may be obscured by the wide range in cell number.

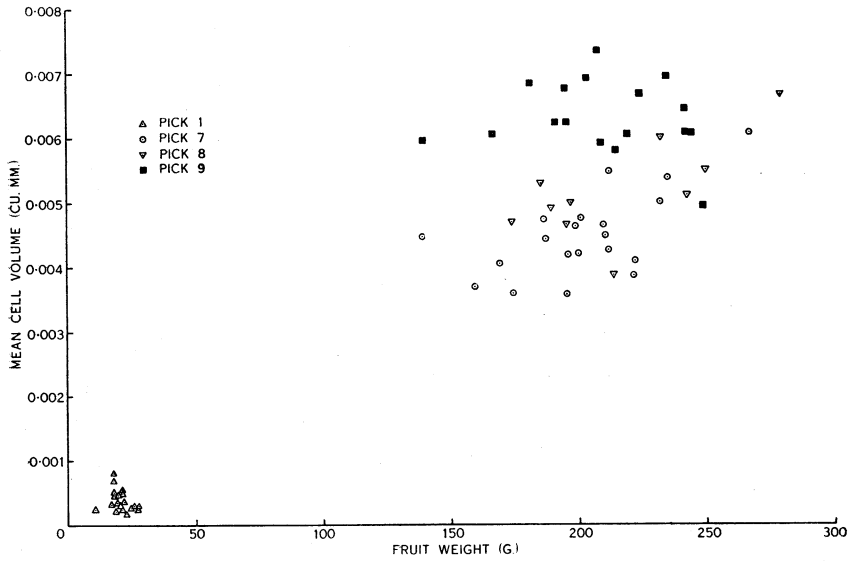

Fig. 10.—Mean values of mid-cortical cells of individual fruits in different samples plotted against fruit weight.

The results show that, as long as the fruit stays on the tree, cell enlargement continues, and at the end of this experiment the cell size was still increasing. The change in mean cell size with time from full blossom is plotted in Figure 11.

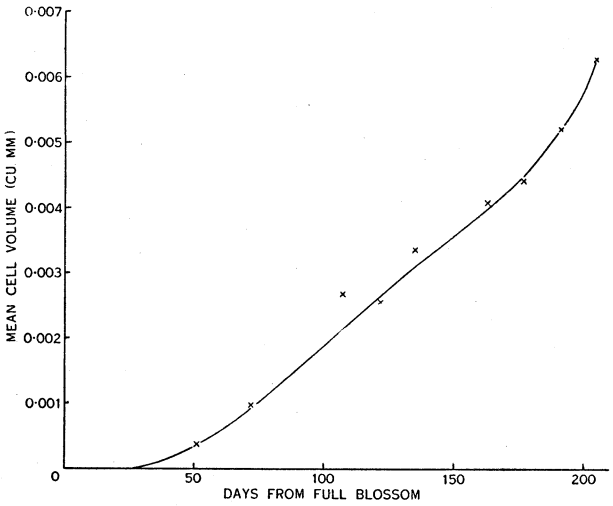

Fig. 11.—Mean volumes of mid-cortical cells of successive samples plotted against time from full blossom.

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