that the osmotic effect appears in such a short time as shown here. In order to see how rapidly the cell wall extensibility is affected by auxin, longitudinal tension of 2·5 gm. was applied to stem pieces soaked for various periods in 200 mgm./litre solution of IAA. By this method[9,10], an increase in extensibility became observable in 15 min. of auxin action, but not in 10 min. Hence the osmotic change occurs earlier than the change in cell wall extensibility.

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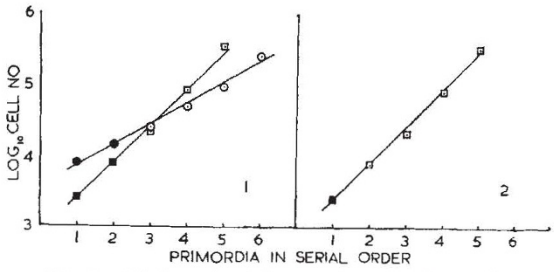
Determination of Cell Numbers in Leaf Primordia

Sunderland and Brown[1] have pointed out that there are two major problems involved in the determination of the number of cells in leaf primordia: (1) numerous macerating fluids[2] tend to disintegrate the relatively delicate cell wall system of leaf primordia and (2) owing to the minute size of leaf primordia and their relatively small number of cells, fragmentary losses are a serious source of error when counting by hæmocytometer slide after dilution.

A method is suggested here which mainly overcomes these difficulties and has been used successfully for the second to the sixth primordia of tomato (*Lycopersicon esculentum*) and tobacco (*Nicotiana tabaccum*) stem apices. It has greater ease and rapidity of counting than the squash method originally used by Sunderland and Brown[1] and can be directly integrated with values obtained by other techniques (for example, chromic acid) for larger leaflets[3].

Primordia were excised under a dissecting microscope using a small splinter from a safety razor blade gripped in a holder. Immediately after excision the primordia were placed in acetic acid/ethanol, 1 : 1 (v/v), for at least 10 min. at room temperature and then treated with *N* hydrochloric acid for 8–15 min. at 60° C. in an oven, depending on the size of the primordia[1]. The primordia were immersed in the liquid in wells constructed by placing on a slide up to three rings cut in lengths of 3 mm. from polyvinyl chloride tubing (10 and 15 mm. diameter) with a razor. During the treatments the slide rested on the rim of a watch glass, which stood in a Petri dish containing the same liquid as the slide in order to reduce evaporation of the liquid[1]. After the removal of each reagent by applying filter paper strips to the base of the outside of the well, the polyvinyl chloride rings were removed with a pair of forceps without disturbing the primordia. The primordia were then removed from the slide under the dissecting microscope, using a fine glass rod or a filter paper strip, and transferred to 10 × 0·6 cm. (approx.) specimen tubes containing a precisely known volume



Figs. 1 and 2. Tomato, ○, hæmocytometer; ●, squash; tobacco, ⊡, hæmocytometer; ■, squash

—between 0·3 ml. and 1·0 ml.—of 0·1 *M* ethylenediaminetetraacetic acid in 5 per cent (v/v) aqueous butanol mixture at *p*H 8 (ref. 4) using ammonia solution to adjust the *p*H. The tubes were tightly stoppered and the primordia in the ethylenediamine tetraacetic acid/butanol mixture were incubated overnight at 30° C. The primordia were then macerated gently with a small syringe (1·0 ml.) to which a finely drawn out piece of glass capillary tubing was attached with surgical polythene tubing. After maceration, 0·1 ml. of 1 per cent (w/v) malachite green in water was added to each tube to produce a minimum of 0·4 ml. for the smallest primordia; for larger primordia the volume was made up to 1 or 2 ml. and the volume of the stain increased proportionally. The suspension was again drawn a few times through the syringe to ensure uniform distribution of cells and 0·2 ml. was transferred to a hæmocytometer slide (Fuchs–Rosenthal; depth, 0·2 mm. area, 1/16 mm.^{2}) and counted. Duplicate estimations on separate aliquots were made for each primordium.

Preliminary results obtained with this method for the second or third and older primordia of tomato and tobacco plants aged 8 weeks in November and grown in water culture are shown in Figs. 1 and 2, in relation to those obtained for the first and second primordia by the squash method of Sunderland and Brown[1]. For the particular purpose of these comparisons plants about 8 weeks old were selected when the youngest primordium visible on the apex (numbered 1) had attained what was judged to be almost the maximum size before the next was due to appear. In Fig. 2, all primordia except the first were measured by the hæmocytometer method.

The numbers of cells counted for each aliquot of 0·2 ml. ranged between 60 and 600 according to primordial position and species. The standard error of the means of 18 pairs of counts was ± 3·7 and the mean difference between pairs did not differ significantly from zero. These counts were about 0·5 or 1 per cent of the total cell number for the smaller primordia (Nos. 3 or 2) and 0·1 per cent of the total for the sixth primordium. The extension of counts to more mature leaves using chromic acid maceration[3] showed a linear relationship for log cell number up to about the eighth or ninth leaf in both species.

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