Quantitative Relations between Stages of Leaf Development and Differentiation of Sieve Tubes

The differentiation of xylem cells in vegetative apices of *Coleus* shoots was recently studied (*1*). Physiological techniques were combined with anatomical ones with the aim of getting anatomical information which would be both quantitative and regularly reproducible. By means of large-sample, round-the-clock collecting, this aim was achieved. One of the most striking results was the discovery that there was a very close quantitative relation between the length of the leaves and the particular stage of xylem differentiation occurring within the leaves. This report (*2*) presents similar data, obtained from the same and later collections, on differentiation of sieve tubes in young leaves of *Coleus*.

Materials and methods were the same as those used by Jacobs and Morrow (*1*), except that no clearing was used in the confirming collections.

The size of a *Coleus* leaf (as measured by its length) was as strictly related to the initiation and subsequent differentiation of sieve tube elements as was previously found for xylem. The first sieve tube appeared in the leaves of the second pair below the apex, when these leaves reached a characteristic length.

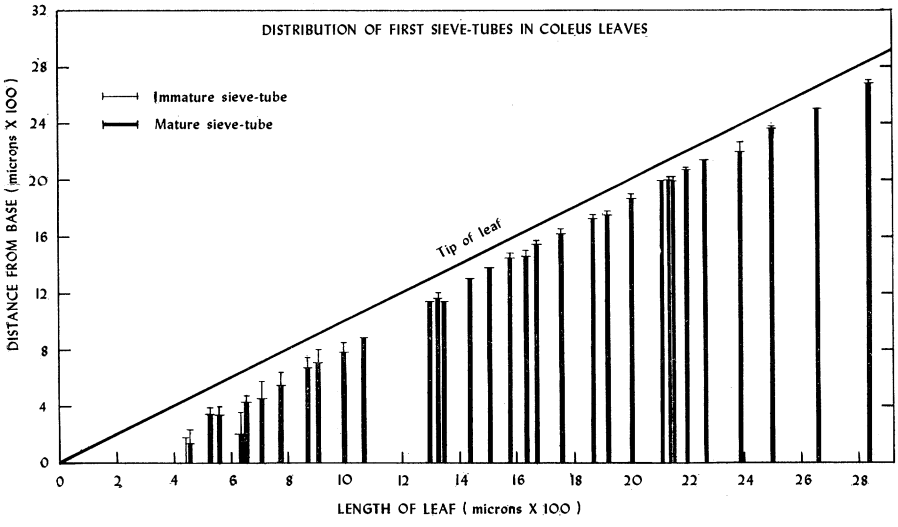

Fig. 1. Longitudinal distribution of sieve tubes within the main vein of young *Coleus* leaves. Each solid vertical line represents the sieve tube of a different leaf.

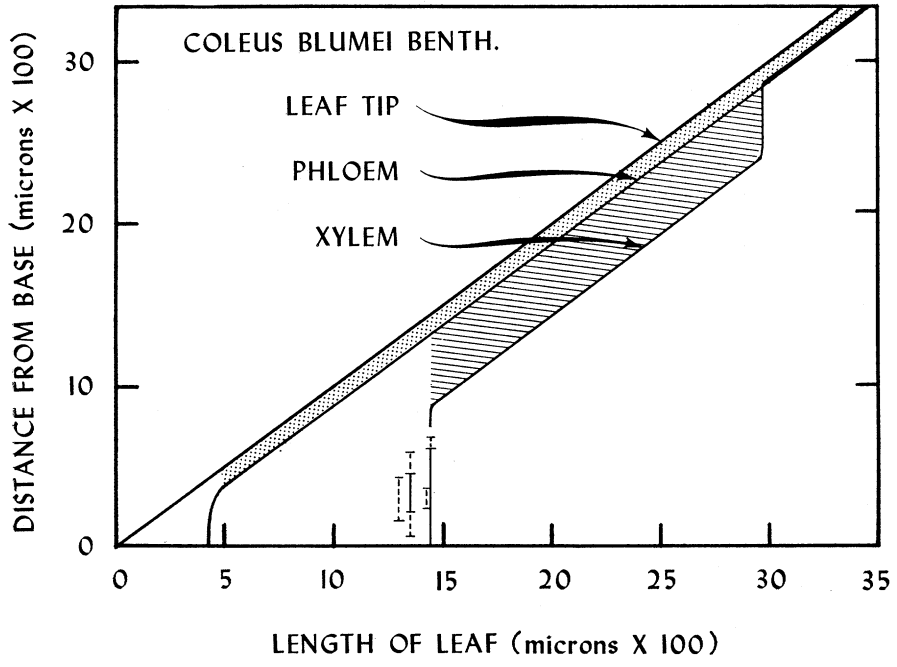

Fig. 2. Graph showing, in diagrammatic form, the relation between the length of young *Coleus* leaves and the differentiation of the first xylem and sieve tubes in these leaves.

In a given leaf, the first sieve tube differentiated quickly, in terms of increasing leaf length, to within 142 μ of the leaf tip—a position which it maintained with remarkable constancy while the leaf grew thousands of microns more (Fig. 1) and became, successively, a member of the third and then of the fourth leaf pair from the apex of the shoot. Examination of many leaves shorter than 400 μ showed no sieve tubes in any one of them. In contrast to the xylem, which first appears in the leaf at an isolated locus (Fig. 2), sieve tubes differentiate continuously upward from the stem (Fig. 1).

We found no sudden jump in differentiation of the sieve tubes at any leaf length studied. In this respect, sieve tubes were unlike the xylem, which showed a sudden acropetal jump at the time the isolated xylem strands first connected basipetally with the mature vascular tissue of the main stem. This acropetal jump of the xylem, which occurs at a leaf length of about 3000 μ, brings the xylem a few microns distal to the sieve tubes. The relative position and differentiation of these two cell types in leaves of increasing length are shown in Fig. 2.

Much evidence has been presented (*2*) that auxin from the young leaf is the normal limiting factor for the differentiation of xylem cells. The contrasting pattern of differentiation of sieve tubes suggests that the latter are normally limited by a factor moving upward from the more mature portions of the shoot. It is appropriate that it is in leaves 0.4 and 1.3 mm long that these cells, specialized for the transport of foods and mineral salts, should start to differentiate, since this is roughly the range of lengths beyond which ordinary physical diffusion would inadequately provide for the growth of the leaf primordium. This last consideration suggests that these results with *Coleus* will apply, with no change in order of magnitude, to vascular differentiation in leaves of other genera.

Large-sample collections made in subsequent years have confirmed the above-mentioned findings. In our clone of *Coleus blumei,* the first sieve tube differentiates in a young leaf when the leaf is from 400 to 450 μ long.

With respect both to xylem elements and sieve tubes, it is now evident that cells in specific stages of differentiation can be collected simply by measuring and collecting leaves of specific lengths. This discovery provides the experimental anatomist with a particularly powerful research tool.

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References and Notes

→ W. P. Jacobs and I. B. Morrow, *Am. J. Botany* 44, 823 (1957).

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Changes in Tryptophan Peroxidase Activity in Developing Liver

Some enzymes found in adult mammalian liver are absent from fetal liver. These enzymes are present in low activity at the end of gestation, and they increase rapidly to adult levels in a matter of hours or days after birth. This developmental pattern was first demonstrated for glucose-6-phosphatase (*1*); more recently it has been shown for phenylalanine hydroxylase, tyrosine-oxidizing enzymes, and uridine diphosphoglucuronic acid transferase (*2*). The questions arise: What mechanisms control the appearance of these enzymes late in gestation? What factors control the dramatic increase in activity which occurs after birth? One suggested mechanism is that of substrate induction. Knox reported that injection of L-tryptophan into normal adult rats or into adrenalectomized animals caused a transient increase in activity of liver tryptophan peroxidase (*3*). Knox presented evidence that the effect depended upon substrate induction.

To assess the possible role of substrate induction in the development of enzymes, we studied liver tryptophan peroxidase in the guinea pig, measuring levels of activity and the effect of L-tryptophan injection in fetal and postnatal stages up to the adult (*4*). This survey of tryptophan peroxidase revealed that, as with glucose-6-phosphatase, two distinct changes occur during development: an initial appearance of activity late in gestation and a rapid increase to adult levels after birth (Fig. 1). We found that injection of L-tryptophan into the fetus or into the mother before term had no effect on fetal liver tryptophan peroxidase activity. Injection of L-tryptophan in combination with ACTH or ACE also had no effect. Injection of a term fetus, *in utero* or after delivery by section, resulted in a small increase in fetal liver activity. A newborn guinea pig, after injection, showed a similar small increase. Twenty-four hours after birth, when liver tryptophan peroxidase activity had attained adult levels, the increase in activity following injection was as great as in the adult (Fig. 1).

As Knox has shown, the controlling mechanism increasing tryptophan peroxidase activity in adult liver after L-tryptophan injection is substrate induction (*3*). The relative refractoriness of fetal liver to L-tryptophan suggests that substrate induction is not the rate-limiting mechanism controlling the changes in enzyme activity observed during development.

In brief, the observations discussed above were made in the following manner. Assay of tryptophan peroxidase activity was carried out in 12.5-percent homogenates, as described by Knox (*5*). All assays were done on single livers. Conversion of L-tryptophan to kynurenine was measured. This is a two-step reaction; the first step is catalyzed by tryptophan peroxidase, the second, by formylase. Formylase is not rate-limiting in the over-all reaction in adult or fetal guinea-pig liver homogenates, for formylkynurenine, the product of the first step, does not accumulate. Also kynurenine is not metabolized in such homogenates, so the over-all conversion of L-tryptophan to kynurenine is a measure of tryptophan peroxidase activity.

To study the effect of L-tryptophan on liver tryptophan peroxidase activity, an animal was given 1 mmole of L-tryptophan intraperitoneally for each 200 g of weight. After 5 hours the animal was sacrificed by cervical dislocation, and the liver was assayed. Subcutaneous injection of L-tryptophan in the adult was as effective as intraperitoneal injection in raising tryptophan peroxidase activity. Injection of saline was without effect.

L-Tryptophan was injected into fetuses *in utero* in amounts proportional to the estimated weight. The mother was anesthetized with diethyl ether, a paramedian abdominal incision was made, and the fetus was injected intraperitoneally through the uterine wall. After 5 hours the mother was sacrificed, the fetuses were delivered by hysterotomy, and the livers were assayed. Anesthesia and laparotomy alone did not increase maternal liver tryptophan peroxidase activity. Intraperitoneal injection of L-tryptophan into the pregnant animal or into the fetus increased maternal liver tryptophan peroxidase activity about fivefold without affecting fetal liver activity. In all cases the injected fetuses seemed to be normal at the time of sacrifice.

It should be emphasized that the deficit of tryptophan peroxidase activity observed in fetal liver is due to absence of the active enzyme and not to a deficiency of a heat-stable cofactor or to the presence of an inhibitor in the homogenate. This conclusion was reached because the addition of fetal liver homogenate to adult liver homogenate had no effect on the activity found in the latter. Also, addition of a heated extract of adult liver failed to stimulate activity in fetal liver homogenates.

Some enzymes which have no activity in fetal liver until the end of gestation have been found to have little or no activity in certain hereditary diseases (*6*). It has been suggested (*1*) that such a disease can be viewed as a persistence of the fetal condition into postnatal life. One might predict that an inherited deficit will be discovered for each enzyme with a developmental pattern like tryptophan peroxidase. However, an inherited deficit of an enzyme necessary for fetal metabolism would probably elude discovery

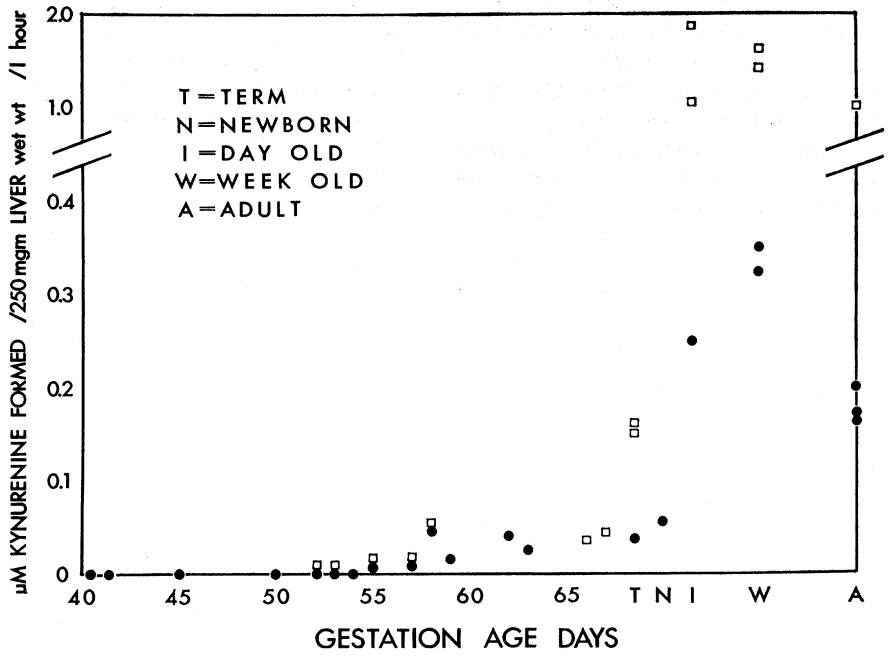

Fig. 1. (Solid circles) Tryptophan peroxidase activity; (squares) tryptophan peroxidase activity 5 hours after injection of 1 mmole of L-tryptophan per 200 g of weight.