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13. The PTH (400 μg/ml) was provided by G. Aurbach; OAF was obtained from normal activated human leukocytes (*10*); $1_{\alpha},25(OH)_2D$ ($10^{-8}M$) was provided by H. DeLuca; PGE_2 ($10^{-5}M$) was from Upjohn; SCT (100 milliunits per milliliter) was from Armour; and cortisol ($10^{-6}M$) was from Sigma.
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Apical Dichotomy Demonstrated in the Angiosperm *Flagellaria*

Abstract. *An equal dichotomy of an angiosperm vegetative apex, previously suspected on morphological grounds, is demonstrated both externally in whole apices and in histological preparations. Dichotomy is a normal developmental feature with complete continuity in growth from the simple to the bifurcated state. The accessibility of this rare phenomenon in this plant makes further, possibly experimental, approaches feasible.*

Recent collections from New Guinea have provided what is, to our knowledge, the first indisputable demonstration of equal dichotomy of the vegetative shoot apex as a normal developmental feature in an angiosperm, the monocotyledonous liane *Flagellaria indica* L. (Flagellariaceae). Branching in existing seed plants (gymnosperms and angiosperms) has been thought to occur exclusively by axillary branching, with a lateral meristem produced as an obvious appendage to the parent shoot apex, usually in the axil of a leaf. This lateral meristem is usually initiated relatively late and on the side of the parent apex, so disturbing its organization minimally. The converse of this, apical dichotomy, has been thought to be a primitive feature of vascular plants, although developmental details are lacking (*1*, *2*).

A number of recent reports, however, have produced circumstantial evidence for apical dichotomy of the vegetative axis in angiosperms, mostly in the monocotyledons—as in the Palmae (*Chamaedorea*) (*3*), (*Hyphaene*) (*4*), (*Nypa*) (*5*), Strelitziaceae (*Strelitzia*) (*6*), and Flagellariaceae (*Flagellaria*) (*7*)—but also in one dicotyledonous family, Cactaceae (*Mamillaria*) (*8*). Other examples relate to specialized lateral appendages, for example, *Asclepias* (*9*) and certain Alismatideae (*10*). In the examples of vegetative dichotomy, convincing demonstration of the process of dichotomy has always been difficult either because the apex is inaccessible, or because it is so small that dichotomous branching is not easily distinguished from early stages of leaf inception, or for both of these reasons. In some examples, material is difficult to obtain. This has meant also that it has not been possible to study changes in cytohistological zonation of apices during the process of bifurcation.

Flagellaria indica is an exception. It is a sprawling vine of wet and often disturbed sites common throughout the Old World tropics (*11*). Aerial shoots arise from a sympodial rhizome system and are supported by leaf-tip tendrils. Leaf arrangement is distichous, so that precise planes for sectioning are available and the shoot apex is conical and stands well above the youngest leaf primordium (Fig. 1A). Axillary branching is restricted to the underground rhizome that generates the aerial shoots, and to the paniculate inflorescence, which terminates each branch of the aerial system. Branching of the vegetative aerial shoots is by regular bifurcation at distant intervals. However, there are no axillary meristems in the aerial shoots. An earlier study (*7*) of limited material produced good evidence that bifurcation was the result of equal dichotomy of the vegetative apex, but it was deficient in convincing photographic documentation. A more recent extensive collection from a locality 4 miles east of Port Moresby, Papua, New Guinea, produced abundant material, which made good this deficiency. Material was fixed in formalin–acetic acid–alcohol, subsequently dissected in absolute ethyl alcohol and stained in acid fuchsin. A series of apices showing progressive stages in dichotomy were obtained and photographed by an epi-illumination technique (*12*). The same specimens were then processed by routine histological methods and sectioned in a median longitudinal plane. This allowed surface and sectional views of the same apex to be compared (Fig. 1, B–E). As well, some apices were pre-

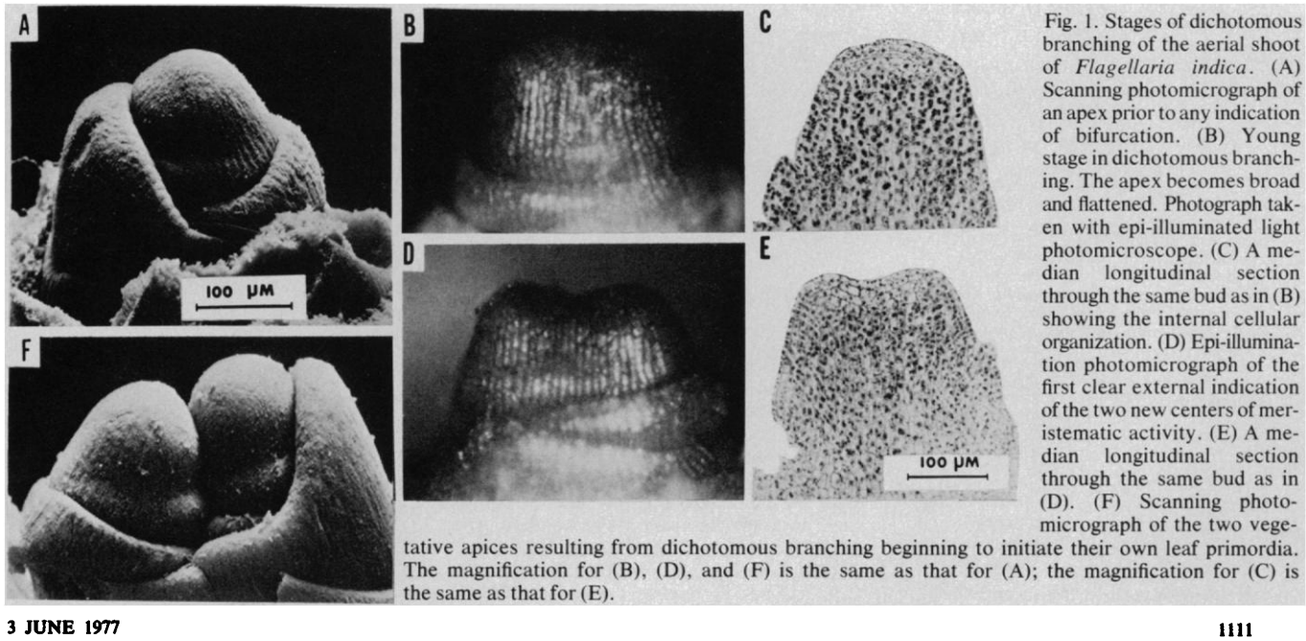

Fig. 1. Stages of dichotomous branching of the aerial shoot of *Flagellaria indica*. (A) Scanning photomicrograph of an apex prior to any indication of bifurcation. (B) Young stage in dichotomous branching. The apex becomes broad and flattened. Photograph taken with epi-illuminated light photomicroscope. (C) A median longitudinal section through the same bud as in (B) showing the internal cellular organization. (D) Epi-illumination photomicrograph of the first clear external indication of the two new centers of meristematic activity. (E) A median longitudinal section through the same bud as in (D). (F) Scanning photomicrograph of the two vegetative apices resulting from dichotomous branching beginning to initiate their own leaf primordia. The magnification for (B), (D), and (F) is the same as that for (A); the magnification for (C) is the same as that for (E).

pared by critical-point drying for observation with the scanning electron microscope, where greater depth of field was desired (Fig. 1, A and F). Dichotomy was shown to consist of enlargement of a single apex and its subsequent separation into two apices without any major change in cytohistological zonation (Fig. 1, A–F). Two to three tunica layers remain recognizable throughout the process, a group of central lightly staining initials enlarges and becomes two groups, and a basal rib meristem can be recognized at all stages (Fig. 1, C and E).

This observation implies that dichotomy is a continuous developmental process, an interpretation supported by the absence of any other evidence for rhythmic growth, such as fluctuation in internode length or leaf size. Leaf initiation continues throughout the bifurcation process, frequently producing organs with an unusual morphology, such as leaves with two blades attached to a single sheath. Since no axillary meristems are developed by the shoot during erect vegetative growth, there is no possibility of interpreting this branching process as precocious axillary branching.

The example illustrates that the angiosperm shoot apex, with a highly regularized cytological and histochemical zonation, has the morphogenetic capacity for equal dichotomy. It is unlikely that this dichotomy is primitive in view of the specialized organography of *Flagellaria*. This species is relatively easily grown and can be propagated clonally from rhizome segments. It could provide a useful experimental system for investigating morphogenesis in angiosperms. A fuller report will be published (*13*).

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Growth Hormone: Species-Specific Stimulation of Erythropoiesis in vitro

Abstract. *The effects of purified bovine and human growth hormone were tested in vitro with murine and human bone marrow by means of granulocyte-monocyte and erythroid progenitor cloning techniques. Nanogram concentrations of the growth hormones potentiated erythropoietin-stimulated erythropoiesis, but not granulopoiesis, in a species-specific manner.*

There is considerable evidence indicating that growth hormone is necessary in vivo for normal mammalian erythropoiesis, although the mechanism of its effect on red cell production is uncertain (*1*). There are few reports on the bioactivity of growth hormone in vitro in nanogram concentrations (*2*). Furthermore, it has been questioned whether growth hormone has a direct effect on tissues or whether it must operate through the obligate intermediate somatomedin (*3*). We report here a direct action of growth hormone on mammalian hematopoietic precursor cells in vitro revealed by assays of colony-forming activity. Our experiments demonstrate that growth hormone in nanogram concentrations stimulates erythropoiesis, but not granulopoiesis, in vitro in a species-specific manner.

Highly purified human growth hormone (*4*), bovine growth hormone (*5*), porcine prolactin (*6*), human chorionic somatomammotropin (*7*), and the plasmin-cleaved fragment [Cys(Cam)^{53}-HGH-(1-134)] of human growth hormone (*8*) were prepared as previously described. The hormones were dissolved in 0.1*N* NaOH and diluted in phosphate-buffered saline before addition to the cultures. Appropriate cultures were prepared without hormone to control for possible effects of the diluent material.

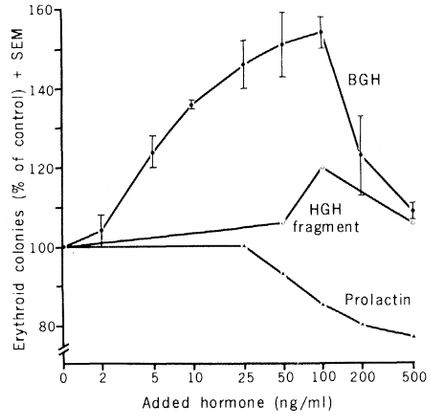

Fig. 1. Effect of bovine growth hormone (*BGH*), human growth hormone (*HGH*) fragment, and porcine prolactin on erythroid colony formation from mouse bone marrow. Each culture contained 0.5 unit of sheep plasma erythropoietin. The bovine growth hormone data are expressed as percentages of control and represent the mean ± the standard error of the mean of five experiments performed in duplicate. The mean cloning efficiency was 329 ± 19 per 10^5 nucleated marrow cells.

Bone marrow was obtained from 8- to 10-week-old male white Swiss-Webster mice by flushing out the femora. Normal human bone marrow was obtained from volunteers (with appropriate informed consent) by posterior iliac aspiration, and the nucleated cells were recovered after centrifugation in Wintrobe tubes. The methylcellulose plate technique was used to clone granulocyte-monocyte precursors and erythroid precursors capable of colony formation in vitro [measured as colony-forming units (CFU)-culture (C) and -erythroid (E), respectively] (*9*). Nucleated marrow cells (10^5) were cultured in 0.8 percent methylcellulose with alpha medium (Flow), 30 percent fetal calf serum, $10^{-4}M$ α-thioglycerol, and antibiotics. A partially purified extract of pregnant mouse uterus was used as the source of colony-stimulating activity to stimulate CFU-C in mouse cultures (*10*). This extract had an activity of 3000 to 6000 colonies per milligram of protein and was used in concentrations causing maximum colony formation (50 μl per culture). One-half unit of step III sheep plasma erythropoietin (Connaught, Toronto) was added to mouse erythroid cultures, and human urinary erythropoietin (approximately 80 units per milligram of protein) was used in the human marrow cultures in a concentration of 1 unit per milliliter.

Erythroid colonies consisting of eight or more cells containing hemoglobin were enumerated with an inverted microscope at 2 and 8 days, respectively, for the mouse and human studies. The erythroid nature of these colonies was confirmed by benezidine staining. Granulocyte-monocyte colonies of 50 cells or more were enumerated at 7 days. The bovine growth hormone content of the fetal calf serum was determined by radioimmunoassay, and the batch used in these experiments contained 3 ng/ml, thereby contributing a final content of approximately 1 ng per culture.