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.

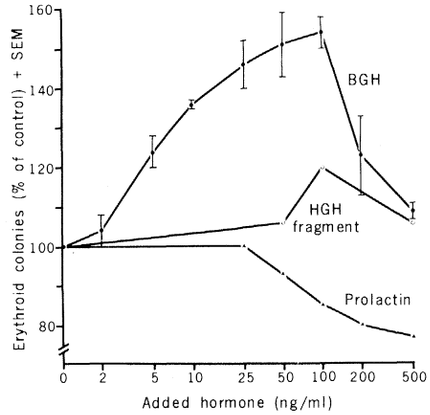

Fig. 1. Effect of bovine growth hormone (*BGH*), human growth hormone (*HGH*) fragment, and porcine prolactin on erythroid colony formation from mouse bone marrow. Each culture contained 0.5 unit of sheep plasma erythropoietin. The bovine growth hormone data are expressed as percentages of control and 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.

Bovine growth hormone consistently potentiated erythroid colony formation by mouse bone marrow cells in the presence of erythropoietin (Fig. 1). A clear effect was detectable with concentrations of added hormone as low as 5 ng/ml, and maximum stimulation occurred at 50 to 100 ng/ml. Higher concentrations showed reduced potentiation of cloning. This hormone augmented erythroid colony formation from mouse marrow at all levels of erythropoietin tested between 0.01 and 0.5 unit per milliliter. The colonies in cultures containing the hormone were larger, with most consisting of 16 to 32 cells as compared to the 8- to 16-cell colonies predominating in control cultures. Bovine growth hormone potentiated erythropoietin-stimulated erythropoiesis, but it could not substitute for erythropoietin. It had no effect on erythroid colony formation of human bone marrow cells when tested in five experiments. Figure 1 also shows that porcine prolactin did not potentiate erythropoiesis and was inhibitory at high concentrations. No effect of human or bovine growth hormone was demonstrable in the CFU-C assay for myeloid precursors (mouse and man) in at least five experiments for each hormone at concentrations up to 500 ng/ml.

Human growth hormone consistently potentiated erythroid colony formation of human bone marrow (Fig. 2) (*11*). Potentiation was detectable at concentrations of the human hormone as low as 25 ng/ml, and peak stimulation occurred at 100 ng/ml. Higher concentrations showed reduced potentiation. The human hormone also stimulated murine erythropoiesis. A mean augmentation in mouse erythroid cloning of 136 ± 6 percent of control was observed in four experiments at optimum concentrations of hormone. Porcine prolactin had no effect on human erythropoiesis in vitro in concentrations up to 500 ng/ml.

The peptide fragment [Cys(Cam)^{53}-HGH-(1-134)] stimulated both mouse and human erythroid colony formation, but with a potency less than half that of the intact hormone (Figs. 1 and 2). Earlier studies showed that the peptide fragment had 14 percent of the potency of human growth hormone as estimated by the tibia test and pigeon crop sac assay (*8, 12*). It is known that human growth hormone is active in both human subjects and experimental animals and that the bovine hormone is not active in man. Our observations reported here are consistent with the species specificity of growth hormone.

Human chorionic somatomammotropin (HCS) caused a 25 percent augmentation in erythroid cloning from human bone marrow at 100 ng/ml (Fig. 2). This is not surprising in view of the fact that HCS exhibits a 13 percent growth-promoting potency of human growth hormone as assayed by the tibia test (*13*). The chorionic hormone has low but significant activity in weight gain assays in immature hypophysectomized male rats (*14*). In addition, it is noteworthy that the primary structures of human growth hormone and HCS are 97 percent homologous (*7*).

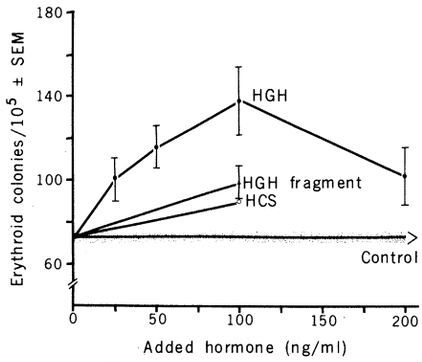

Fig. 2. Effect of human growth hormone (*HGH*), human growth hormone fragment, and human chorionic somatomammotropin (*HCS*) on erythroid colony formation from normal human bone marrow. Each culture contained 1 unit of human urinary erythropoietin. The human growth hormone data, expressed as erythroid colonies per 100,000 nucleated marrow cells, represent the mean ± the standard error of the mean of three experiments performed in duplicate.

Although a relation between pituitary growth hormone and normal mammalian erythropoiesis has been demonstrated for both animals and man, the precise role of growth hormone in hematopoiesis is uncertain. Growth hormone appears to be one of several hormones that can potentiate the cellular response to erythropoietin. Other hormones with potentiating activity include adrenocorticosteroids, thyroid hormones, and androgenic steroids (*9, 15*).

The relation between growth hormone effect on target cells and somatomedin has not yet been clarified, and it is believed by some investigators that all effects of growth hormone in vivo are mediated via somatomedin. However, our data, together with other observations (*2, 16*), show that growth hormone can have a direct in vitro biological action. The effect on erythroid progenitor cells in vitro is species-specific, concentration-dependent, and demonstrable in nanogram concentrations. Also, the potency of bovine growth hormone, HCS, human growth hormone, and Cys(Cam)^{53}-HGH-(1-134) in vitro parallels their known growth-promoting activity in vivo. With regard to other hematopoietic cells, growth hormone has been shown to influence T lymphocyte function and, recently, evidence has been presented for specific binding of growth hormone in physiologic concentrations to thymocytes (*17*).

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