milliliter of supernatant, an average of 87 ± 17 ng of material resembling hCG or LH or approximately 1 to 1.5 ng per blastocyst. These estimates are tentative, but they provide unequivocal evidence for the presence of material similar to hCG or LH in the rabbit blastocyst prior to implantation.

In the plasma of pregnant rabbits on days 5 and 6 after mating the concentration of material resembling hCG or LH was 6 to 8 ng/ml, values significantly higher than the < 3 ng/ml found in the plasma of nonpregnant animals. Since the radioreceptor assay does not discriminate between hCG and LH, the release of pituitary LH after fertilization was also considered. However, the pituitary as a single source of LH would probably not explain the tenfold difference in concentrations of the gonadotropins between blastocyst and serum. These observations are unique in view of the existing concept that hCG is produced by syncytiotrophoblast after the implantation of the blastocyst. There is some evidence for the secretion of LH-like material by the rabbit blastocyst as measured by radioimmunoassay (*4*), as well as for high concentrations of hCG around the time of implantation as measured by a specific radioimmunoassay of the hormone-specific β subunit (*5*). In our study, the radioreceptor assay recognized only native hormone and thus interference by nonspecific immunoreactive material was eliminated. Hence the gonadotropin in the blastocyst may be similar to both hCG and LH. It is known that rabbit blastocyst is capable of synthesizing certain steroids and enzymes (*6*). Since trophoblast is differentiated as early as at the 17-cell stage (*6*), it is conceivable that these cells may be capable of producing a gonadotropin at very early stages of the development of the rabbit blastocyst. An hCG- or LH-like material has also been detected in the uterine fluid. Hence, the actual site of synthesis of gonadotropins before implantation remains to be established. However, this finding suggests the role of preimplantation gonadotropin in the maintenance of corpus luteum function in early pregnancy (*7*). The hCG has been shown to be a potent and reversible inhibitor of the response of human lymphocytes to phytohemagglutinin (*8*). Also activity similar to that of blood group A in the α subunit of hCG, before and after the removal of carbohydrate moiety, has been reported (*8*). These findings may have bearing on the possible role of hCG-like gonadotropin in the suppression of lymphocyte function during implantation.

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Dedifferentiated Guard Cells in Magnoliaceous Leaves

Abstract. *Evidence has been obtained that guard cells and other epidermal cells as well as mesophyll cells undergo division during wound repair of mature leaves in 26 magnoliaceous taxa in the genera* Kmeria, Elmerrillia, Magnolia, Manglietia, Michelia, Paramichelia, *and* Talauma. *Division of epidermal cells is believed to be rare in mature leaves, and division of guard cells is particularly unusual in most species previously studied.*

Epidermal cells do not usually divide during regeneration after wounding in mature leaves. Internal cells of the leaf blade commonly exhibit an immediate response to injury. Cells along the wound die, and adjacent living cells are induced to undergo rapid physiological changes (*1*). Subsequent stages of wound repair may include cell proliferation, callus development, periderm formation, and cork differentiation. Most commonly, epidermal participation is nil (*2*); other responses reported include division only in immature epidermal cells (*3*), or division in epidermal components under pathological conditions only, as in gall formation (*4, 5*). Dehnel (*6*) and Wylie (*2*) are the only workers who have reported cell division in epidermis in wound repair zones of otherwise healthy, mature leaves. Von Mohl (*7*) cited periderm formation in *Buxus* leaf epidermis, which implies cell division. Reports of cell division in guard cells are even more rare (*4, 8*).

I have studied leaf regeneration following experimental wounding in two magnoliaceous species and following natural wounding in herbarium specimens of about 52 magnoliaceous species, representing 10 of the 11 genera in the family. Leaves were prepared for study by bleaching in 5 percent sodium hydroxide, clearing in saturated aqueous chloral hydrate, and staining in safranin (*9*), which makes it possible to observe the wound area at all levels of the leaf thickness and to compare it with healthy tissue in other parts of the same leaf. Results indicate that the mature epidermis dedifferentiated (underwent cell division and returned to a less differentiated state) during wound repair in leaves of 65 percent of the species studied; in the remaining 35 percent only internal leaf tissues participated in regeneration. In all the taxa studied, the mesophyll was the primary site of regeneration, rather than the epidermis or vascular tissue. The mesophyll cells adjacent to the necrotic zone along the wound divided repeatedly and in various planes to produce a callus of uniformly small, isodiametric cells. Intercellular spaces were greatly reduced compared to those in normal leaf tissue. In some instances the mesophyll cells eventually began to divide primarily periclinally to the wound surface.

There is indirect evidence that cell

division had occurred in epidermal cells along the wound. These cells were smaller and had thinner walls than those of mature epidermis nearby, and the cell outline was angular, in contrast to the crenate, lobed, or stellate shapes commonly encountered in healthy portions of the same leaves. Because clearing removes the cell contents, direct evidence of mitosis was not obtainable from such preparations. To obtain direct evidence, experimental wounding techniques were used. Experimental wounds, consisting of incisions with a sharp razor blade to remove pieces of leaf blade about 4 mm square, were made on attached mature leaves of two plants grown outside in Baton Rouge, Louisiana. The plants were specimens of *Magnolia grandiflora* L. and *Michelia figo* (Lour.) Spreng. On 2 February 1972 12 wounds were made on different leaves of each plant; this was repeated, with previously unwounded leaves, on 16 May 1972. At intervals after the wounding (1, 2, 4, 7, 14, 28, and 42 days) pieces 4 mm square were removed from around wounds on six randomly selected leaves of each plant. Stomata and other structural features were evenly distributed over the abaxial leaf surface, so that the leaf pieces were essentially uniform except for the time variable. Needle punctures were also tried, but were abandoned because they were nonuniform and yielded variable results. However, neither of the two species used showed epidermal participation in wound repair of mature leaves, either in the experiments or in leaves with natural wounds. It is possible that other methods of wounding might produce different results in these species.

The fate of guard cells along the naturally occurring wounds, in species showing epidermal dedifferentiation, was more easily followed than that of other epidermal cells. Divisions had occurred in identifiable guard cells within the wound scar area in 26 magnoliaceous taxa in seven genera: *Elmerrillia pubescens* (Merr.) Dandy, *Kmeria chaoana* Cheng, *Magnolia cubensis* Urb., *M. dealbata* Zucc., *M. ekmanii* Urb., *M. guatemalensis* D. Sm., *M. liliflora* Desr., *M. officinalis* Rehd. & Wils., *M. splendens* Urb., *M. thamnodes* Dandy, *M. virginiana* L., *Manglietia blaoensis* Gagnep., *M. glauca* Bl., *M. hookeri* Cub. & W. W. Sm., *Michelia champaca* L., *M. compressa* (Maxim.) Sarg., *M. doltsopa* Buch.-Ham. *ex* DC., *M. hypolampra* Dandy, *M. martini* (Lév.) Dandy, *M. masticata* Dandy, *M. nilagirica* Zenk, *Paramichelia baillonii* (Pierre) Hu, *Talauma dodecapetala* (Lam.) Urb., *T. gitingensis* Elmer, *T. gloriensis* Pitt., and *T. ovata* A. St.-Hil. Evidence for degeneration of guard cells, reported in other plants after wounding (*6*), was not observed among the magnoliaceous plants. The guard-cell pairs remained recognizable for a time because of their paired arrangement and because of the differentially thickened walls, particularly those bordering the stomatal pore. After wounding and callus initiation, the guard cells first enlarged in the wound margin area. Cell shape often changed, and the thick anticlinal walls became thinner. In a broad wound-scar, the cellular components of the epidermis appeared uniform in size and meristematic. Hence, it was deduced that the previously enlarged guard cells had undergone division, together with surrounding epidermal cells.

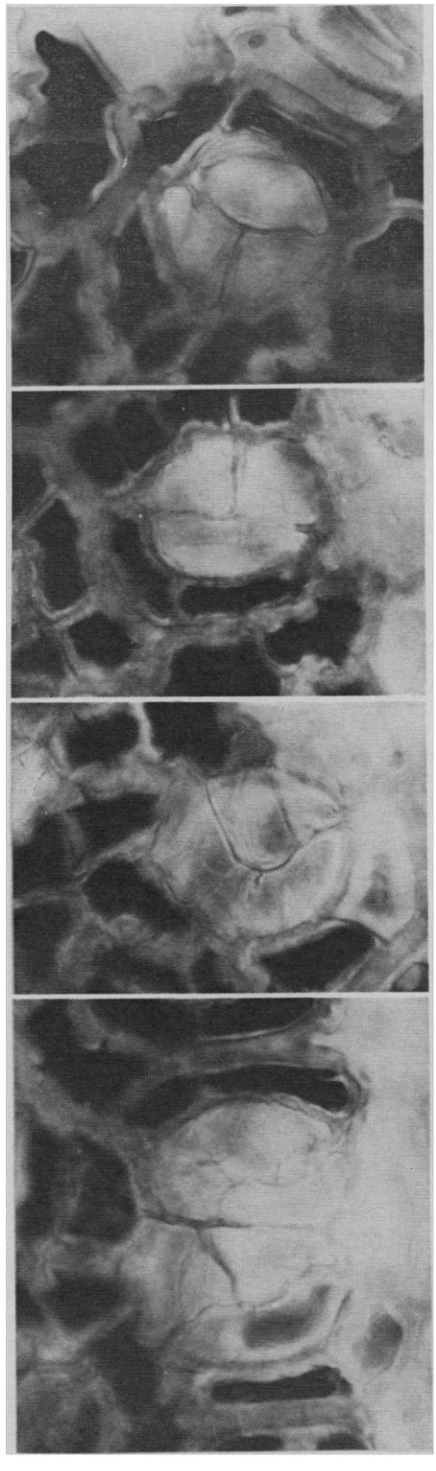

Fig. 1. Guard cell pairs in the leaf epidermis of *Kmeria chaoana* which have undergone cell divisions. The epidermal cells stain darkly in the wound area and lightly in the adjacent healthy tissue (at the top or right in each picture) back of the wound zone. The newly divided cells have thin walls, compared to the thick, sinuous, pitted walls characteristic of epidermal cells (× 450).

The epidermis of *Kmeria chaoana* is particularly useful for demonstrating division of guard cells. The epidermal components all have unusually thick, pitted walls, and the epidermal cells of the wound-scar area adjacent to healthy tissue stain darkly with safranin, while guard cells do not. In the living wound margin (about 350 to 450 μm wide) each guard cell appeared to have enlarged and then divided one to several times (Fig. 1). The first new wall was essentially perpendicular to the pore. New walls remained thinner than those of the parent cells. Pits were evident in walls of guard cells as well as those of other epidermal cells. The wound influence on the guard cells was very localized; normal undivided guard cells were present within 60 μm of the affected cells, just back of the wound callus region.

The presence of prominent pits between guard cells and other epidermal cells in *Kmeria chaoana* and many other magnoliaceous taxa suggests that plasmodesmata may persist into the mature condition in the epidermis. Cleared preparations do not retain cytoplasm, so the plasmodesmata cannot be shown. Plasmodesmata occur only infrequently between guard cells, or between a guard cell and an adjacent epidermal cell, in other taxa. They have been reported from immature leaves (*10*), cotyledons (*11*), mature epidermis of grasses (*12*) and other monocotyledons (*13*), and mature epidermis of dicotyledons (*8*, *14*). The usual loss of regenerative potentiality of guard cells is probably correlated

with the loss of plasmodesmatal connections to adjacent cells as the epidermal cells mature. The regenerative response in mature guard cells of some magnoliaceous leaves may indicate an exception to this tendency. The Magnoliaceae should be sampled further for members with guard cells capable of dedifferentiation, to seek definitive proof of plasmodesmatal retention between guard cells, or between guard cells and adjacent epidermal cells. Since most of the magnoliaceous plants showing dedifferentiative epidermis and guard cells are tropical or semitropical, many with coriaceous evergreen leaves, the unusually elaborate wound response may be characteristic of such plants, but rare among the temperate zone plants which have been the subject of most wounding experiments.

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Anemia in Domestic Cats: Effect on Hemoglobin Components and Whole Blood Oxygenation

Abstract. *Phenylhydrazine-induced anemia in the domestic cat results in an increase in minor, high oxygen affinity hemoglobin B components and an accompanying decrease in the major, low affinity B component. This change is accompanied by an unusually large increase in erythrocytic adenosine triphosphate and 2,3-diphosphoglycerate, a slight decrease in the oxygen affinity of whole blood, and a large decrease in the Hill constant.*

The types of proteins synthesized by an organism can be determined by the types of environment or stress to which the organism is exposed. An example of this phenomenon is the change in the types of hemoglobins synthesized by various animals during exposure to anoxic conditions. Specifically, when made anemic by various means certain sheep (*1*), goats (*2*), ducks (*3*), and mice (*4*) demonstrate altered patterns of hemoglobin synthesis. The nature of this change is specific for each species, but it generally involves the increased production of a hemoglobin usually present in low concentrations at the expense of a normal major component (sheep and goats), or it involves a change in the relative amounts of normal major components (ducks).

A similar phenomenon has now been observed in the blood of domestic cats. Normal cat blood contains two major hemoglobin components, HbA and HbB, both of which have low oxygen affinity relative to most other mammalian hemoglobins (*5*). In addition, at least three other minor components, designated HbB_1, HbB_2, and HbB_3, closely related to HbB in electrophoretic and chromatographic properties but with higher oxygen affinities are also present (*6*). Preliminary amino acid analyses and peptide maps indicate that the structural differences between B components are small. Components B, B_1, and B_2 possess very similar β chains that are acetylated at the amino terminals. Hybridization experiments reveal that B_2 has a unique α chain. It is not yet known if the minor B components are genetically determined or arise from postsynthetic modification. Phenylhydrazine-induced anemia has now been found to change the relative amounts of these B components and to produce a striking change in the whole blood oxygen equilibrium. The latter involves a change in the shape of the oxygen saturation curve that may be related to the special characteristics of the mixture of hemoglobins in the feline erythrocyte.

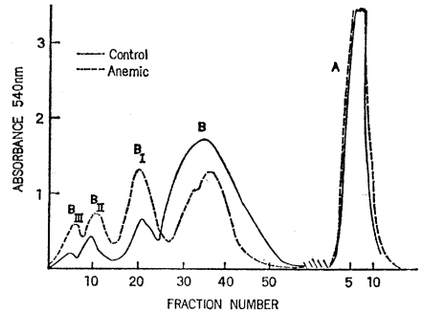

Fig. 1. Elution profiles of cat hemolyzates before and during anemia. Components were separated on a Bio-Rex 70 column (1 by 20 cm) equilibrated with 0.05*M* phosphate buffer (*p*H 6.4) (*6*). Solid line, hemolyzate from normal cat blood; broken line, hemolyzate from anemic cat blood.

Mongrel domestic cats possessing an equal amount of HbA and HbB were used (*7*). The term HbB in this context (referring to ratios of A to B) is used broadly to include HbB, HbB_1, HbB_2, and HbB_3. Control samples of nonanemic blood were obtained in heparin by cardiac puncture, and hematocrits and reticulocyte counts were recorded. Hemolyzates were prepared and hemoglobin components were analyzed (*6*). The cats were then made anemic by daily subcutaneous injections of phenylhydrazine (6.5 mg per kilogram of body weight) for 4 to 5 days and then were given injections of the same dose every 2 to 3 days thereafter for 2 to 3 weeks. Blood was drawn by heart puncture 2 days after the last dose of phenylhydrazine.

Typical elution profiles obtained by ion-exchange chromatography of hemolyzates of normal cats and cats made anemic with phenylhydrazine are shown in Fig. 1. The hematocrit of the blood from the sample from the normal cat was 37 percent and the reticulocyte count was practically zero, whereas the corresponding values for samples from the anemic cat were 12 and 70 percent, respectively. Comparison of the two profiles shows that the HbB_2 and HbB_1 are increased and HbB is decreased in the anemic condition. The amount of HbA as well as the ratio of the amount of HbA to the combined total of HbB, HbB_1, HbB_2, and HbB_3 remained essentially unchanged. Only the relative amounts of the B components appear to change, the increase in concentrations of the higher oxygen affinity components, HbB_1 and HbB_2, being compensated by a corresponding decrease in the lower affinity component, HbB.