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.

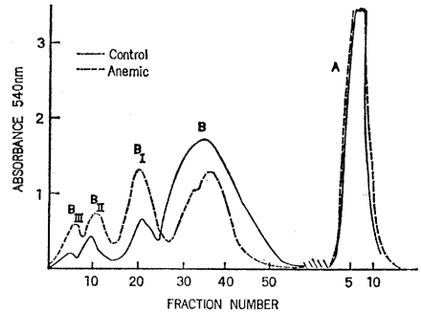

Fig. 1. Elution profiles of cat hemolyzates before and during anemia. Components were separated on a Bio-Rex 70 column (1 by 20 cm) equilibrated with 0.05*M* phosphate buffer (*p*H 6.4) (*6*). Solid line, hemolyzate from normal cat blood; broken line, hemolyzate from anemic cat blood.

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.

Table 1. Concentration of 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP) in blood samples from control and anemic cats. In each group there were 12 animals. A Gilford 240 spectrophotometer was used for the enzymatic assays as described in Sigma Chemical Co. circulars UV-35 and 366-UV. RBC, red blood cells; Hb, hemoglobin.

Sample	2,3-DPG		ATP	
	RBC (μmole/ml)	Hb (μmole/g)	RBC (μmole/ml)	Hb (μmole/g)
Control	1.26 ± 0.05	4.25 ± 0.20	0.36 ± 0.04	1.21 ± 0.16
Anemic	3.48 ± 0.12	12.03 ± 0.26	1.19 ± 0.29	4.12 ± 0.63

In healthy cats of this phenotype, B and B_1 constitute about 40 and 5 percent of total hemoglobin, respectively. During anemia these values may change to extremes of about 30 percent (B) and 12.5 percent (B_1). Reliable values of B_2 and B_3 are more difficult to obtain because of their low concentrations. The increase in B_1, B_2, and B_3 appears to be directly related to the length and severity of anemia. Recovery from anemia is accompanied by a return to the original levels of each component.

The effect that this change in hemoglobin levels has on the oxygen saturation curve of cat blood and the concomitant effect that it has on the ability of cat blood to deliver oxygen to peripheral tissues are of interest. Of particular concern is the significance of an increased amount of HbB_1 during anemia since its P_{50} is only one-third that of HbB (*8*). The increase in a high affinity hemoglobin component in an anemic state is difficult to reconcile with the usual assumption that a decrease in hemoglobin oxygen affinity increases the efficiency of tissue oxygenation. Presumably a state of anemia would be aggravated by an increased hemoglobin oxygen affinity.

Since studies on human and other blood have established that anemia invariably causes an increase of intraerythrocytic 2,3-diphosphoglycerate (2,3-DPG) (*9*), it seems reasonable to suggest that anemic cats adapt to anoxia by a similar mechanism. From the data in Table 1, it is apparent that a two- to threefold increase in the concentrations of 2,3-DPG and adenosine triphosphate (ATP) are observed in anemic cat blood. In fact, the magnitudes of these changes are greater than those previously reported for other species.

The net effect that drug-induced anemia has on the oxygen saturation of whole blood is shown in Fig. 2. Although there appears to be a slight increase in P_{50} during feline anemia, a more striking change evidenced in the shape of the curve is observed. This change of shape may be expressed as a decrease of the apparent Hill constant from 2.85 to 1.31 although the meaning of the Hill constant for such a system is uncertain. In other experiments, Hill constants for curves of anemic blood were consistently low but were more often closer to 2.0. The P_{50} of anemic cat blood, however, is subject to greater fluctuation. Normally, deoxygenated blood has a relatively low affinity for oxygen while oxygenated blood has a relatively high affinity. A unique feature of feline anemia is that this difference between the oxygen affinity of deoxygenated blood and that of oxygenated blood is substantially reduced.

The change in shape of the oxygen saturation curve of anemic cat blood presumably results from the unusual properties of the cat hemoglobins. The apparent decrease in affinity of the oxygenated blood (the shift to the right in the upper part of the curve) is apparently due to the effect that the increases in 2,3-DPG and ATP have on the organic phosphate–sensitive HbA. If this is true, it would seem that the relatively weak effect of 2,3-DPG on isolated HbA (*10*) does not preclude its efficacy in vivo. The apparent increased affinity of deoxygenated blood (the shift to the left on the lower part of the curve) presumably results from the replacement of the low affinity, phosphate-insensitive HbB with the high affinity, phosphate-insensitive HbB_1 and HbB_2. Hence, the cat hemoglobins possess the functional characteristics that might account for the anemia-induced changes in the shape of the whole blood oxygen saturation curve. However, under conditions of severe anemia, it is also possible that additional factors may be important in the change in whole blood oxygen saturation reported here.

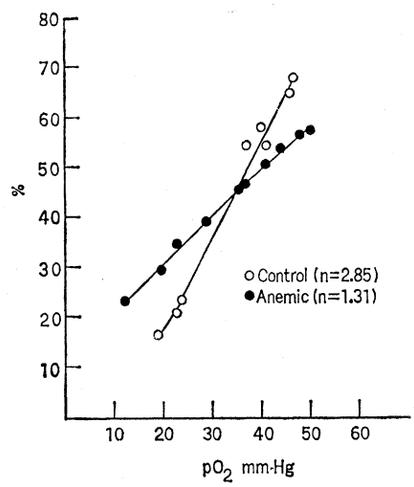

Fig. 2. Oxygen saturation curves determined at 37°C and 5 percent carbon dioxide. Open circles, blood from normal cats; closed circles, blood from anemic cats. Freshly obtained blood was equilibrated with oxygen and carbon dioxide at specified partial pressures, and the percentage of oxygenation was measured with a CO-oximeter (Instrumentation Laboratory).

Although the response of feline blood to an anemic state is fairly elaborate, it is not at all clear what advantage the animal derives from this. Some insight may be provided by recent work by Eaton *et al.* (*11*) who found that mice in which a high oxygen affinity was induced by cyanate were better able to withstand the rigors of a high altitude environment than were untreated controls. They conclude that while an increase in P_{50} may be of value to animals exposed to moderately anoxic conditions, a lower P_{50} may be advantageous for survival at more extreme degrees of oxygen deprivation. Apparently, the need to facilitate the loading of oxygen becomes paramount under these conditions. The increase in high oxygen affinity components in cats with anemia may be a related phenomenon because it provides a mechanism to ensure the ability to load as well as unload oxygen at low oxygen pressures. Such a mechanism may be necessitated by the fact that cat blood normally has a low oxygen affinity and would therefore be difficult to oxygenate under conditions of oxygen deprivation. The well-known observations that cats are peculiarly susceptible to anoxic conditions (*12*) could be explained on the basis of the peculiarities of the hemoglobin system which we described.

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Reversal of Morphine Tolerance after Medial Thalamic Lesions in the Rat

Abstract. *Tolerance, manifested by a diminished electroencephalographic response at cortical and subcortical recording sites, was found in rats subjected to repeated systemic injections of morphine sulfate. Reversal of tolerance to morphine resulted from destruction of the medial thalamus.*

After repeated administration of morphine, there comes a point when the same previously effective dose no longer has a soporific effect (*1*) on the organism. If the dosage is not increased, withdrawal symptoms frequently occur. In the rat, withdrawal is manifested by increased locomotor activity and "wet shakes." These symptoms are an indication of physical dependence on the drug.

Several hypotheses have been put forth to explain how drug tolerance develops. It has been generally assumed that morphine tolerance is manifested throughout the nervous system in a nonspecific manner. Wikler and Carter (*2*) studied the effect of repeated doses of morphine on spinal reflexes and demonstrated the development of tolerance in the caudal portion of the surgically sectioned spinal cord of the dog. Berkowitz and Spector (*3*) transferred tolerance from "addict" mice to previously untreated mice by injecting serum containing morphine immunogen. They suggested that these antibodies "reduced the concentration of morphine in critical sites in the brain." Pert and Snyder (*4*) showed that opiates do not bind equally to all cells in the central nervous system. For example, the caudate nucleus seems to have a greater affinity to opiates than does the cerebral cortex or cerebellum. Further evidence for a discrete locus of opiate action in the brain was provided by Wei *et al.* (*5*). In their experiments, withdrawal symptoms after morphine administration were precipitated by direct injections of minute amounts of naloxone (a potent antagonist) into a zone including the medial thalamus and midbrain. Naloxone injected at many other subcortical sites had no effect.

Despite biochemical evidence that morphine has a greater affinity for the basal ganglia of the brain than for other regions (*4*), there have been no reports of localized or selective bioelectrical changes in response to morphine injections. Changes in cortical electrical activity in response to morphine administration have been studied (*6*), but it is not known whether morphine response and morphine tolerance, manifested by electroencephalographic (EEG) responses to morphine, might show up in some regions of the brain earlier than in others. We compared the bioelectrical response of the caudate nucleus, medial thalamus, and cortex to repeated doses of morphine and studied the effect of medial thalamic lesions on naloxone withdrawal.

Ten male albino rats were anesthetized with sodium pentobarbital (50 mg per kilogram of body weight), and recording electrodes were implanted in the medial thalamus, caudate nucleus, and posterior cerebral cortex. After a 1-week recovery period, the animals were injected twice daily with morphine sulfate (30 mg/kg, intraperitoneally). At first, this regimen resulted in drastic alterations in brain bioelectrical activity and behavior. However, after repeated drug administration both the behavioral

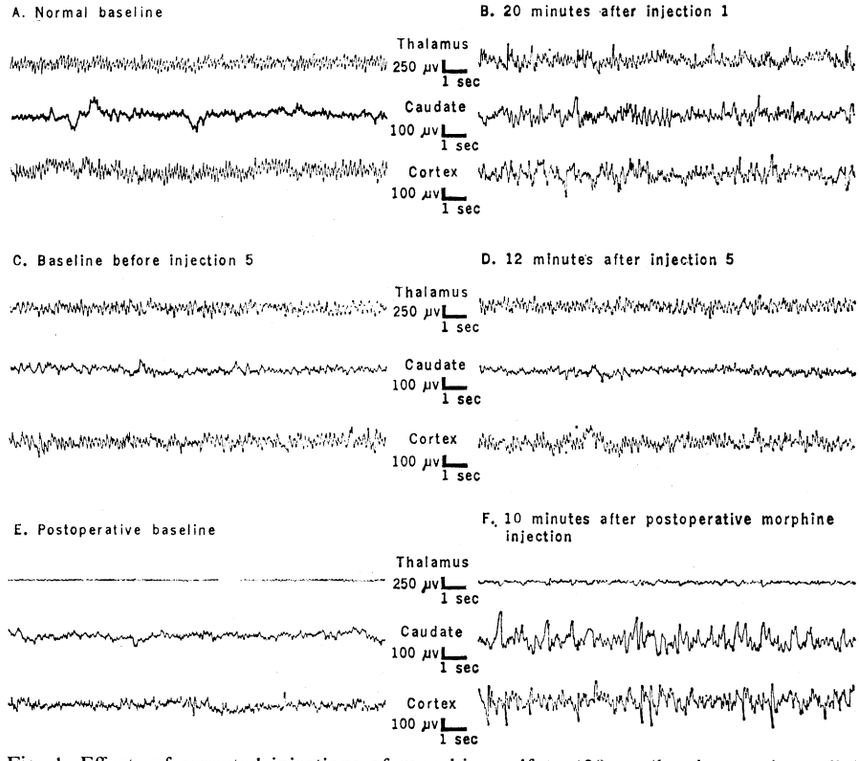

Fig. 1. Effects of repeated injections of morphine sulfate (30 mg/kg, intraperitoneally) on EEG tracings recorded from thalamic, caudate nucleus, and cortical sites in rat 256. Tolerance to morphine is demonstrated in (D). The medial thalamic lesion results in the reappearance of sensitivity to morphine (F).