In an attempt (*5*) to test the reasonableness of this hypothesis, computer calculations were made to predict the performance of two coupled sinusoidal oscillators having different amplitudes and frequencies. The assumption made was that the amplitudes of the two motions were to be superposed and that, whenever the amplitude reached a threshold value, eruption would be initiated. Such a model is yielding encouraging but not yet definitive results.

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1. I was a member of Dr. V. Schaefer's 1965 Yellowstone Field Expedition, supported in part by NSF.
2. Mitsuo Nogoshi and Yoshinobu Motoya, *Geophys. Bull. Hokkaido Univ.* **9**, 67 (1962), in Japanese.
3. These were supplied by G. W. Sielaff, West District Naturalist, Yellowstone National Park.
4. From a graph on the wall of the Old Faithful Ranger Station, Yellowstone National Park.
5. Dr. L. Alldredge, U.S. Coast and Geodetic Survey, suggested the model and helped carry through the calculations.

20 July 1965

Delta-Aminolevulinate Dehydratase Activity in Mice with Hereditary Anemia

Abstract. *Homozygous* (f/f) *but not heterozygous* (f/+) *mice of the highly congenic strain, FL/Re, manifest a severe transitory siderocytic fetal anemia. Adults of both* f/f *and* f/+ *genotype manifest decreased hepatic, splenic, and renal levels of* Δ*-aminolevulinate dehydratase (ALD) activity compared to homozygous* (+/+) *mice of the same strain. The degree of augmentation in splenic ALD activity following phenylhydrazine administration is high in* +/+, *intermediate in* f/+, *and low in* f/f *mice. These findings suggest that perhaps a deficiency in the fetal level of ALD may be responsible for the transitory fetal anemia.*

Detailed study of the effects of genic substitutions at the "flexed" (*f*) locus upon hematopoiesis has become possible through the development at the Jackson Laboratory of an inbred mouse strain, FL/Re, which is homozygous *f/f*, along with congenic FL/Re–+/+ and FL/Re–*f*/+ stocks (*1*). Homozygous *f/f* mice suffer from a transitory siderocytic anemia which is severe at fetal stages but disappears shortly after birth, whereas hematopoiesis appears normal in *f*/+ and +/+ mice at all ages (*2*). The anemia of *f/f* mice, first apparent on the 12th day of fetal development, is especially marked from the 13th to the 16th day, and seems to be especially associated with the first generation of nonnucleated red cells formed in the fetal liver (*1*). In 13- to 15-day *f/f* anemic fetuses, the new red cells are very deficient in hemoglobin (*1*) and contain large quantities of nonheme iron in the form of siderotic granules (*2*).

The activity of a key enzyme in the pathway of heme biosynthesis, Δ-aminolevulinate dehydratase (ALD), was investigated in adult mice of the FL/Re strain to determine whether the fetal accumulation of nonheme iron, resulting from the action of the gene *f*, might be associated with an enzymic lesion which impairs heme biosynthesis. A preliminary account of this work has been presented (*3*). Variations in hepatic level of ALD activity, apparently controlled by alleles at a single locus (Lv^a versus Lb^b), have been reported in normal adult mice (*4*). Also, the ALD activity in spleen, but not in liver, of adult rabbits is known to be susceptible to alteration by phenylhydrazine (*5*).

The ALD activity was determined according to a modification of the method of Russell and Coleman (*4*) at *p*H 6.2 in the presence of β-mercaptoethanol rather than glutathione during the preliminary incubation period. Hepatic, renal, and splenic levels of ALD activity were determined in 2- or 3-month-old +/+, *f*/+, and *f/f* mice. It may be seen in Table 1, rows 1–3 (for the mice not given phenylhydrazine treatment), that the ALD activity per gram in each of the three organs is about the same in *f*/+ and *f/f* mice, and that this level is substantially lower than that observed in the +/+ mice ($P < 0.01$). All probabilities quoted in this paper were calculated by the Wilcoxon rank sum test (*6*). The ALD activity in livers of Lv^a/Lv^b mice is intermediate between the high level in Lv^a/Lv^a mice and the low level in Lv^b/Lv^b mice (*4*). For these reasons, and because the gene *f* has previously been considered recessive in all its effects, it was surprising to find the same low level of ALD activity in the organs of heterozygous *f*/+ and homozygous *f/f* mice.

To test for possible effects of heterozygosity on the activity of this enzyme, anemia was induced in FL/Re mice by phenylhydrazine treatment. Adult +/+, *f*/+, and *f/f* mice were rendered anemic by five 1-mg intraperitoneal injections of neutralized phenylhydrazine at 12-hour intervals and were killed approximately 12 hours after the last injection. Microhematocrit determinations were performed on blood obtained from the retro-orbital sinus just prior to sacrifice. The hematocrit percentages of injected mice fell to little more than half of their pretreatment values (Table 1, column 5), and similar levels of reticulocytosis were observed in all mice (14 to 23 percent in *f/f*; 18 to 26 percent in +/+). Significant splenic enlargement was observed in mice of all three genotypes (Table 1, column 6), although the extent of enlargement was less in *f/f* than in *f*/+ and +/+ mice ($P < 0.01$). The ALD activity per gram of spleen increased above pretreatment levels in +/+ ($P < 0.01$) and *f*/+ ($P = 0.05$) mice, but

Table 1. Δ-Aminolevulinate dehydratase activity in various organs of mice of the FL/Re strain, without and with phenylhydrazine treatment, and hematocrit values and spleen weights.

Genotype	Δ-Aminolevulinate dehydratase*						Hematocrit (%)		Spleen weight (mg)	
	Liver		Kidney		Spleen					
	No phenylhydrazine treatment									
+/+	3.32	0.42†	1.43	0.27†	2.25	0.36†	49	1.6†	72	17†
f/+	1.20	.25	0.68	.09	0.80	.27	48	1.8	83	15
f/f	1.22	.16	.63	.10	.61	.20	50	2.5	70	14
	With phenylhydrazine treatment									
+/+	2.38	.15	1.02	.07	5.30	1.77	29	1.3	252	72
f/+	0.90	.20	0.43	.07	1.23	0.15	28	1.5	249	73
f/f	.86	.14	.44	.06	0.55	.32	24	2.4	155	34

* Activity of ALD is expressed in micromoles of porphobilinogen synthesized per hour per gram of liver (wet weight) ± standard deviation. † Values in these columns are standard deviations. The standard deviations of the hematocrits are expressed in percentage points, not percentages.

not in *f/f* mice. Total ALD activity per spleen increased 2.0-, 4.7-, and 8.5-fold in *f/f*, *f/+*, and +/+ mice, respectively, which demonstrates an intermediate heterozygous expression of the gene *f* in adult splenic tissue under conditions of severe hematopoietic stress.

The data in Table 1 imply that, in times of severe red cell breakdown, extra hematopoiesis may be stimulated in the spleen, and suggest that the action of the gene *f* modifies this response. During the period of fetal development when the anemia of *f/f* fetuses is most pronounced, the fetal red cell mass enlarges very rapidly in both +/+ and *f/f* fetuses (70- to 80-fold increases in cell number from the 12th to the 16th day of development) (*2*). This rapid increase in the number of red cells may constitute a severe stress to the fetal hematopoietic system. The appearance of hypochromic siderocytic anemia in *f/f* fetuses may be caused by an insufficiency of ALD, resulting from an inability of the hematopoietic system to respond adequately to the demand for this enzyme activity, an effect similar to the severely reduced ability of the spleens of phenylhydrazine-treated *f/f* adults to manifest increased ALD activity. Similarly, the absence of anemia in the heterozygous *f/+* fetuses may indicate that the level of ALD activity in this genotype is sufficient to meet the demands of the presumed hematopoietic stress, an effect similar to the moderate increase of ALD activity in the spleens of phenylhydrazine-treated *f/+* adults.

The gene *f* thus appears not only to control the level of ALD activity but also to modify the extent of response of this enzyme activity to conditions of hematopoietic stress.

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7. We thank P. Feigelson for helpful discussions and use of facilities. Supported in part by research grant P-338 from the American Cancer Society to the Jackson Laboratory, by PHS research grant R10 CA 02332 from the National Cancer Institute, and by NSF grant 20878 to the College of Physicians and Surgeons, Columbia University. One of us (F. L. M.) is a PHS postdoctroal trainee.

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Gibberellic Acid: Action in Barley Endosperm Does Not Require Endogenous Auxin

Abstract. *Endogenous auxin is not required for the increase in amylolytic activtiy induced by gibberellic acid in barley endosperm, as shown by the response of the system to anti-auxin. The action of gibberellic acid in this system is independent of the presence or absence of auxin.*

Treatment of barley endosperm with gibberellic acid results in *de novo* synthesis of α-amylase (*1*). This reaction is unique among the reactions induced by this acid, for auxin does not appear to be involved: exogenous auxin does not promote amylolytic activity in either the presence or absence of the acid (2). However, cereal seeds contain sizable amounts of endogenous auxin (*3*). The possibility exists that auxin is necessary for this reaction but that endogenous auxin satisfies the requirement. Such a situation exists in the elongation induced by gibberellic acid in *Avena* leaf sections (*4*). It is of particular interest to determine whether endogenous auxin is required for this process because several current theories postulate an obligate interaction between auxin and gibberellic acid (*5*).

The response of a system to anti-auxin can be used to assess the role of endogenous auxin. An obligate role of auxin is indicated if the inhibitor action is at least partially reversed by subsequent addition of auxin. This technique has been successfully used to study the role of endogenous auxin in the elongation of several tissues (*4*, *6*). We have now used it to examine the role of endogenous auxin in the increase in amylolytic activity in barley endosperm induced by gibberellic acid.

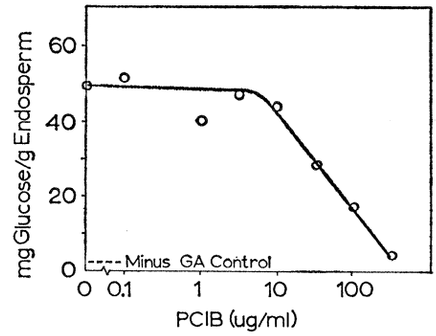

Fig. 1. Effect of the anti-auxin *p*-chlorophenoxyisobutyric acid (PCIB) on the increase in amylolytic activity induced by gibberellic acid (GA). Endosperm halves incubated in 3 ml of basic medium with varying concentrations of PCIB. Reducing sugars in medium determined after 22 hours at 30°C. In the absence of GA there were 1.7 mg of glucose equivalents per gram of endosperm.

The induction of amylolytic activity has been followed by the technique of Paleg (*7*). Seeds of barley, variety Himalaya, were first heated for 1 hour at 70°C to reduce the amount of endogenous amylolytic activity. After sterilization seeds were bisected transversely and lots of four endosperm halves were incubated in petri dishes with 3 ml of basic medium. The medium contained potassium maleate buffer (2.5) m*M*, *p*H 4.8), streptomycin (500 μg), gibberellic acid (1 μg/ml), and, where required, the anti-auxin, *p*-chlorophenoxyisobutyric acid, and any one of

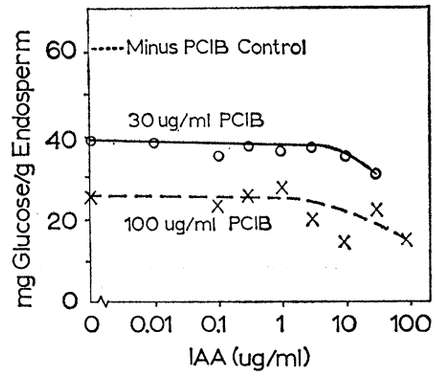

Fig. 2. Inability of the auxin indoleacetic acid (IAA) to reverse the PCIB-induced inhibition. Endosperms incubated in basal medium with 30 μg of PCIB per milliliter (circle, solid line) or 100 μg/ml (cross, broken line) and varying amounts of IAA. Reducing sugars determined after 22 hours at 30°C. In the absence of PCIB there were 60.2 mg of glucose equivalents per gram of endosperm.