GENETIC CONTROL OF HEPATIC δ-AMINOLEVULINATE DEHYDRATASE IN MICE[1]

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THE enzyme δ-aminolevulinate dehydratase (5-aminolevulinate hydro-lyase E C 4.2.1.24) effects the condensation of two molecules of δ-aminolevulinate (ALA) to form porphobilinogen. It has been suggested that this enzyme may regulate the rate of porphyrin biosynthesis by allowing increased amounts of ALA to form porphyrins rather than letting it be degraded by the succinate glycine cycle (ONISAWA and LABBE 1962). In a study of the hepatic activity of this enzyme in mice with hereditary anemias (R. N. SCHIMKE and D. L. COLEMAN, unpublished results), it became apparent that several normal, nonanemic strains of mice shows reproducible inter-strain differences in hepatic activity of the enzyme. The various inbred strains tested could be classified into three groups, high, low, and intermediate with respect to their ALA dehydratase activities. Experiments demonstrating the induction of this enzyme with substrate have been reported using noninbred mice which had ALA dehydratase activities somewhat lower than the group which was found to be low in our studies (ONISAWA and LABBE 1962). This report deals with a genetic analysis of the factors involved in the control and development of this enzyme and with attempts to induce this enzyme in two inbred strains of mice.

MATERIALS AND METHODS

The inbred strains of mice used in these studies were obtained from the Production Department of the Roscoe B. Jackson Memorial Laboratory. The (AKR/J × C57BL/6J) F_1 hybrid mice and the backcross of these hybrid mice to C57BL/6J were obtained from the research colony of DR. ELIZABETH S. RUSSELL. The other crosses were carried out in the author's own colony. Except where indicated otherwise, all mice assayed were females five to seven weeks of age.

For determination of ALA dehydratase activity, the livers from mice were removed and rinsed in ice-water. An appropriate weight (usually 0.5 g) was homogenized with two volumes of ice-cold 0.15 M KCl in a Potter-Elvehjem ho-

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mogenizer, and exactly 0.3 ml of this homogenate was added to a flask containing 0.8 ml of 0.2 M sodium phosphate buffer, pH 6.8, and 0.1 ml of 0.3 M reduced glutathione. After a one-hour preincubation at 37° under N_2 in a Dubnoff Metabolic Shaking Incubator, 0.3 ml of 0.1 M neutralized ALA was added and the incubation continued under the same conditions for an additional one-half hour. The reaction was then stopped with 1.5 ml of ten percent trichloroacetic acid (TCA) which was 0.1 M with respect to $HgCl_2$. After centrifugation, 0.5 ml of the supernatant, diluted to 1.5 ml with five percent TCA, was assayed for porphobilinogen by the modified Ehrlich's method of MAUZERALL and GRANICK (1956), using 4N perchloric acid. Since control flasks in which ALA was omitted showed no activity, a mixture of 1.5 ml each of five percent TCA and of modified Ehrlich's reagent was routinely used as a reagent blank.

In experiments on induction, mice were injected intraperitoneally with neutralized ALA (0.1 ml of 0.1 M ALA per 10 g body weight) at various intervals before the livers were removed for enzyme assay. The assay was conducted as described above with the exception that flasks to which enzyme, but no substrate, had been added served as controls. This modification was found to be necessary to correct for the large amounts of endogenous ALA present in the livers of mice which had received ALA injections only a short time before enzyme assay.

RESULTS AND DISCUSSION

The activities of ALA dehydratase in the livers from several inbred strains of mice are shown in Table 1. Mice of strains, AKR/J, C57BR/cdJ, C57L/J and C3H/J (not shown) had the highest levels of enzyme activity while C57BL/6J had the lowest. A preliminary survey indicated that mice of the A/J, 129/J, SJL/J, SWR/J, BALB/cJ, CBA/J, DBA/1J, and DBA/2J strains had intermediate values of enzyme activity. It is of interest that animals of both the C57L/J and C57BR/cdJ strains have high activities, when compared with those of the C57BL/6J strain. All three strains originally stemmed from the same stocks;

TABLE 1

Liver δ-aminolevulinate dehydratase activity in four strains of mice

Strain	Liver ALA dehydratase activity* (μmoles/g/hr)	
AKR/J	5.13 ± .09	(20)
AKR/J (pregnant)	8.16 ± .45	(4)
C57BR/*cdJ*	5.58 ± .21	(6)
C57L/J	5.70 ± .24	(6)
C57BL/6J	1.98 ± .09	(22)
C57BL/6J (pregnant)	2.97	(2)
C57BL/6J (bearing tumor)†	1.47 ± .03	(20)

* Figures represent μmoles of porphobilinogen synthesized per gram of liver per hour ± standard error of the mean. The number of animals is in parentheses. All mice were females.

† These animals had been inoculated with B-16 melanoma, and at the time of assay the tumor represented about 20 percent of the total body weight.

however, the C57BL/6J strain was separated from the other two at a very early stage in the development of these lines.

Pregnancy appears to increase the activity by 50 to 60 percent (8.16 *vs.* 5.13 for AKR/J, 2.97 *vs.* 1.98 for C57BL/6J) which may reflect the increased hematopoiesis which occurs in pregnancy. Hormonal control may also be important since in the strains studied nonpregnant females had levels 10 to 20 percent higher than those of their male siblings. Tumor-bearing C57BL/6J mice showed about 50 percent reduction in enzymatic activity, a result which appears in accord with the lower hematopoietic activity of such mice. Similar results with tumors have been reported by TSCHUDY and COLLINS (1957) only in their case the C57BL/6 strain had a hepatic enzyme activity lower than ours and was the only strain which did not show decreased ALA dehydratase activity in the presence of a tumor. This discrepancy may result from the different tumors used (a glioma rather than a melanoma) or from metabolic differences in their C57BL/6 subline.

About 20 percent more enzyme activity than normal was seen in livers which had been frozen prior to homogenation. After the initial freezing the activity remained constant even after several months of storage. The increase in activity following freezing is probably due to more complete physical disruption of the cells than is possible with the Potter-Elvehjem homogenizer.

Genetic analysis: The AKR/J and C57BL/6J strains were chosen for genetic analysis of the factors involved because of the availability of these mice and because mice of F_1 hybrid and the first generation backcross of this hybrid to the C57BL/6J parent were available as surplus animals from another study. The results of the genetic analysis are shown in Table 2. All of the (AKR/J × C57BL/6J) F_1 (line 3) animals showed a level of enzyme activity intermediate between

TABLE 2

Liver δ-aminolevulinate dehydratase activity resulting from crosses of high and low strains of mice

	Strain	Liver ALA dehydratase activity*		
		High ($Lv^a\ Lv^a$)	Intermediate ($Lv^a\ Lv^b$)	Low ($Lv^b\ Lv^b$)
1.	AKR/J	5.13 ± .09 (20)	.....	.....
2.	C57BL/6J	.....	.....	1.98 ± .09 (22)
3.	(AKR/J × C57BL/6J) F_1	.....	3.06 ± .02 (25)	.....
4.	F_2	4.83 ± .18 (10)	3.30 ± .09 (18)	1.86 ± .03 (10)
5.	(F_1 × C57BL/6J)BC_1	.....	3.48 ± .06 (11)	1.86 ± .03 (9)
6.	(F_1 × AKR/J)BC_{1a}	4.53 ± .12 (10)	3.30 ± .09 (14)	.....
7.	(BC_{1a} × AKR/J)BC_2†	4.71 ± .06 (12)	.....	.....
8.	(BC_{1a} × AKR/J)BC_2‡	4.83 ± .06 (14)	2.80 ± .06 (17)	.....

* Figures represent μmoles of porphobilnogen synthesized per gram of liver per hour ± standard error of the mean. The number of animals is in parentheses. All activities tabulated were from female mice.

† This BC_2 generation represents the female progeny of the two male mice of the BC_1 generation which had high levels of the enzyme, and which appear to be homozygous for the controlling gene ($Lv^a\ Lv^a$).

‡ This BC_2 generation represents the female progeny of the three male mice in the BC_1 generation which had intermediate levels of the enzyme and which appear to be heterozygous for the controlling gene ($Lv^a\ Lv^b$).

animals of the two parental strains although somewhat closer to the C57BL/6J parent, suggesting partial dominance. The evidence which suggests that the activity of this enzyme is controlled by a single genetic factor is apparent upon examination of the remainder of Table 2. The F_2 animals (line 4) fell into three distinct classes with respect to hepatic enzyme activity, corresponding closely in activity levels to those values seen in the two parental strains and the F_1 hybrid animals. The proportions did not deviate significantly ($P = 0.94$) from a 1:2:1 ratio. The backcross of the F_1 hybrid to C57BL/6J (line 5) produced offspring of two distinct classes, one corresponding to the F_1 and the other to the C57BL/6J parent. Again the proportions did not differ significantly ($P = 0.65$) from a 1:1 ratio. A similar backcross to the AKR/J parent (line 6) again produced two distinct classes of offspring, one with enzyme activities corresponding to the F_1 and the other corresponding to the activities found in the AKR/J parent, in a nearly 1:1 ratio ($P = 0.42$). A test for transmission of the controlling factor through a second generation was performed by randomly selecting ten males from the backcross of the F_1 hybrid mice to mice of the AKR/J strain and mating each of these males to two female AKR/J mice. When both females were ascertained to be pregnant, the males were sacrificed and the livers assayed for enzyme activity. Again the enzyme values obtained from these males like those found for their female siblings (used to obtain the data in line 6 of Table 2) fell into two classes with respect to enzyme activity, one high group of six mice (3.92 ± 0.22 μmoles/g/hr) and one low group of four mice (2.45 ± 0.09 μmoles/g/hr). As can be seen these values for males are about 20 percent lower than the corresponding values seen for female siblings (line 6). The female progeny from matings with two of the males which were now shown to be high with respect to enzyme activity and those from three of the males which had intermediate enzyme activity were saved. These were assayed for ALA dehydratase activity when they reached five to seven weeks of age. Table 2 (7th line) shows that the progeny of the females which had been mated to high group males showed high enzyme activity, while line 8 shows that the progeny from each of the females mated with low group males fell into two discrete groups, one corresponding to the F_1 and the other to the AKR/J parent. These groups appeared in proportions not deviating significantly ($P = 0.48$) from a 1:1 ratio.

The evidence presented in Table 2 strongly suggests that the hepatic level of ALA dehydratase is under the control of a single genetic factor. The gene symbol *Lv* is suggested for this locus with Lv^a for the allele causing high enzyme activity and Lv^b for the allele causing the lower enzyme activity. Since the initial high and low levels for the two parental strains were never again attained in backcross generations, the presence of other modifying genes may be indicated.

Effects of age: Previous studies on fetal liver, which is rich in hematopoietic tissue, had shown that the fetal level of ALA dehydratase markedly exceeds that of newborn mice (SCHIMKE and COLEMAN, unpublished). The lower levels seen in the C57BL/6J adults might reflect a failure in postnatal synthesis of the enzyme after its drop from the high fetal level to the lower level seen immediately after birth. The results of an age study to examine this possibility

are shown in Figure 1. It is readily apparent that mice of the C57BL/6J strain at no age had enzyme activity approaching that of AKR/J mice. A high enzyme activity was observed in fetal livers from AKR/J mice at the earliest time studied (13th day of gestation). This activity dropped off rapidly until two to six days after birth, after which it rose again until the adult value was reached at about one month of age. The C57BL/6J strain had a similar pattern of development only on a reduced scale demonstrating that the reduced enzyme levels found in the adult were not a consequence of a failure of the postnatal development of this enzyme. Although the fetal level of enzyme in the C57BL/6 mice was markedly reduced (one fifth that of AKR/J mice) it apparently was enough to maintain normal hematopoiesis since there are no blood abnormalities associated with this strain of mice. The decrease in enzyme activity around the time of birth probably reflects the shift from liver to bone marrow as the major site of porphyrin synthesis. The reason for the rapid postnatal rise in activity to the adult level which is even higher than that found *in utero* is not apparent at this time. These results suggest a similar genetic control of enzyme activity throughout development in both strains of mice.

Induction: The normal enzyme activity for the AKR/J strain (5.13 μmoles/g/hr) is approximately three times that reported for maximally induced Swiss mice (Onisawa and Labbe 1962), which suggested that the AKR/J strain might be operating at all times as though fully induced. To test this possibility, AKR/J and C57BL/6J mice were injected with ALA and their enzyme activities assayed at various times after injection. The enzyme activities in mice of both strains were found to be inducible. The AKR/J strain showed a maximum increase of 20 percent (6.15μmoles/g/hr *vs.* 5.13 for noninduced) in enzyme

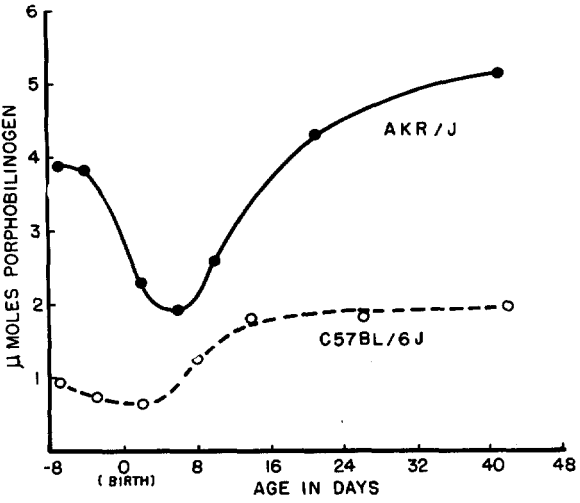

Figure 1—Effect of age on the amount of ALA dehydratase in the livers of mice of the AKR and C57BL/6J strains. Enzyme activity is expressed in μmoles porphobilinogen produced by one gram of liver per hour.

activity 12 to 18 hours after injection of substrate, and dropped off slowly thereafter. A similar time sequence was shown by the C57BL/6 mice but the maximum response to injected substrate was only about 10 percent (2.22 μmoles/g/hr *vs.* 1.98 for noninduced). Thus contrary to expectations the enzyme in the C57BL/6J mice was not even as readily inducible as the enzyme in AKR/J mice. Even with higher concentrations of ALA the enzyme activity in C57BL/6J mice did not approach the normal AKR/J level. Induction in both strains appeared to be a relatively slow process, in contrast to the rapid rise in enzyme level found by ONISAWA and LABBE (1962) in Swiss mice. A spurious rapid rise could be observed in our experiments, if the data were not corrected for the large amounts of endogenous substrate converted to porphobilinogen during the one-hour preincubation period. This rise fell off rapidly after three or four hours, reflecting the rapid decrease in endogenous substrate concentration.

Comparison of these induction studies with the age studies presented above indicates that the C57BL/6J strain and the AKR/J strain differ in three respects, 1) the level of ALA dehydratase in liver throughout life, 2) the amount which this level rises after reaching its postnatal low, and 3) the response of this level to ALA as an inducer. While evidence for single-factor control has been presented for only the first of these differences, it seems possible that the second and third differences are closely related and may be under the control of the same factor.

Anemic mice: Previous studies on the rates of incorporation of various heme precursors into hemoglobin (ALTMAN, RUSSELL, SALOMON and SCOTT 1953) suggested that the hereditary anemia determined by alleles at the *W* locus (E. S. RUSSELL 1955) in mice could be due to a relative deficiency of ALA dehydratase in the hematopoietic tissues of the anemic mice. To test this possibility by direct assay of the enzyme level, livers from *W*-anemic and normal fetuses were removed at appropriate times before birth and assayed for ALA dehydratase. Little difference was found between *W*-anemic and normal mice in the level of ALA dehydratase in fetal liver. This finding seems to argue against determination of the anemia by lowered ALA dehydratase levels, at least when fetal liver is the site of hematopoiesis. The possibility remains, however, that in the adult anemics, where the bone marrow is the main hematopoietic site, lowered ALA dehydratase is responsible for the anemia. Preliminary studies to test this possibility have been undertaken by removing whole femurs, stripped of muscle and connective tissues, from normal and anemic mice and grinding them with two volumes of cold 0.15 M KCl. The resulting homogenate was treated in the same fashion as the liver homogenates described previously, with the exception that the 0.3 ml of homogenate added to the assay flasks did not contain any of the bony fragments which remained after grinding. This assay was based on weight of femur rather than marrow itself. Although the two types of mice may have similar quantities of marrow, it is recognized that the proportion of erythroid precursor cells in normal and anemic mice may not be the same. The results of this study although only preliminary suggest that the anemic mice (W W^v) had somewhat more enzyme

(6.0 μmoles/g/hr) than did normal mice (*w w*, 3.7 μmoles/g/hr). This further suggests that ALA dehydratase is not a limiting factor in this type of anemia.

SUMMARY

An interstrain difference in the hepatic activity of δ-aminolevulinate dehydratase in mice appears to be under the control of a single genetic factor (*Lv*). The enzyme activity is high in fetal liver, falls to a low shortly after birth, and rises again to the adult level at three to six weeks. A strain (C57BL/6J) with a low adult level of enzyme had enzyme activities lower than those of a strain (AKR/J) with high adult enzyme level in all stages of development. The enzyme activity in the high (Lv^aLv^a) strain could be more readily induced by injection of the substrate δ-aminolevulinate than could that of the low strain (Lv^bLv^b). Preliminary studies on *W*-anemic mice indicated little difference between anemics and normal mice in enzyme activity, both in fetal liver and in adult marrow.

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