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# STUDIES OF THE PERINATAL DIFFERENCES IN THE ACTIVITY OF HEPATIC δ-AMINOLEVULINIC ACID SYNTHETASE*

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**Abstract**—Hepatic δ-aminolevulinic acid (ALA) synthetase activity in prenatal rats, rabbits and guinea pigs is four to eight times the adult level. During the period of elevated activity, ALA synthetase is relatively much less responsive to induction by 3,5-dicarbethoxy-1,4-dihydrocollidine (DDC) or to repression by hemin than is observed in adult rats and rabbits. No induction of ALA synthetase could be demonstrated in fetal rats. In newborn rabbits, hemin caused no significant reduction in ALA synthetase activity, whereas the adult enzyme activity was reduced to 32 per cent of control. This difference in response to hemin was also demonstrated in fetal and newborn rats. Refractoriness to induction decreases as enzyme activity decreases to adult levels. These studies indicate that the neonate may not regulate the activity of hepatic ALA synthetase at the same level as the adult. It is suggested that increasing repression of the enzyme to lower basal levels occurs as the animals mature, bringing the activity and response to DDC and hemin to the levels observed in adults. Perinatal alterations in the regulation of hepatic ALA synthetase activity may account for some of the problems associated with heme metabolism in the newborn and the toxic effects of bilirubin and various drugs.

THE DECREASED capacity of perinatal animals to metabolize various drugs is well established[1,2] and represents a more general inability of the neonate to withstand pharmacologic and physiologic insults in comparison with adults. Deficiencies associated with the perinatal period include an immature hepatic drug-metabolizing system,[3–5] and excess plasma levels of bile pigments, which may result in hyperbilirubinemia. Both problems involve the hepatic production of non-erythropoietic heme-containing compounds and suggest alterations in the perinatal regulation of the rate-limiting enzyme in the heme biosynthetic pathway, hepatic δ-aminolevulinic acid (ALA) synthetase.[6]

While the control of ALA synthetase activity has been investigated in a variety of systems,[7–10] the regulation of hepatic heme synthesis in perinatal mammals has not been studied. The induction of hepatic ALA synthetase by numerous compounds in adult animals has been demonstrated.[11–15] Mutational or drug-induced alterations in the regulation of hepatic ALA synthetase activity have been shown to underlie inherited and experimental porphyria.[16] Various investigators have shown that both heme[17] and bilirubin[10] repress ALA synthetase in animals and prevent drug-induced

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increases in the activity of this enzyme. It has also been shown that heme and methemalbumin, administered to adult rats, are potent repressors of both drug-induced ALA synthetase and microsomal cytochromes.[18,19]

Studies of the sources of bile pigments in man[20,21] have shown that 10–20 per cent of the total bilirubin production in adults is derived from non-erythropoietic compounds. During the perinatal period, this level is 21–25 per cent in the full-term infant and constitutes up to 30 per cent of the total bilirubin load in the premature infant.[22] Significant increases in bile pigments derived from non-erythropoietic sources may occur under a wide variety of pharmacologic and pathologic conditions.[23]

Previous studies from this laboratory[24] have shown that hepatic ALA synthetase activity in perinatal animals is significantly greater than that of adults. Preliminary studies on the induction of this enzyme[25] indicated that hepatic ALA synthetase in neonatal rabbits may be relatively much less responsive to induction than is observed in adults. The current studies were undertaken to determine if differences in the activity of hepatic ALA synthetase observed during the perinatal period can be explained in terms of the response of the enzyme system to agents which are thought to induce or repress the levels of the enzyme in adult animals.

## MATERIALS AND METHODS

Hepatic ALA synthetase activities in adult and perinatal Dutch rabbits, Sprague–Dawley rats and guinea pigs were studied in these experiments. Rats were obtained from Charles River Breeding Laboratories, Inc., Boston, Mass., whereas rabbits and guinea pigs were purchased from local suppliers. Animals were housed in individual cages and were allowed food and water *ad lib.* In studies involving newborn rabbits, pregnant adults were obtained several days prior to the expected date of delivery and were housed individually in specially built cages equipped with removable nesting boxes.

### *Preparation of liver homogenates and enzyme assay*

Liver homogenates were prepared for incubation according to the method of Marver *et al.*[26] with slight modifications as previously described.[25] This method provides for the measurement of ALA synthetase activity in homogenates under conditions where ALA utilization is almost completely inhibited and the production of ALA is optimal. Under the conditions of this method, the production of aminoacetone (AA) in adult liver tissues is greatly suppressed. Thus, ALA levels measured in adult livers were considered to represent the total aminoketone level. The contribution of AA to the total aminoketone level in perinatal livers was determined as described below. The method of Mauzerall and Granick,[27] as modified by Davis and Andelman,[28] was used to separate porphobilinogen (PBG) and to concentrate ALA. Two columns were arranged so that a 10-ml aliquot of solution flowed from one to the other. The top column, containing an anion-exchange resin (Bio-Rad AG 1-X8), retained the PBG. The ALA was retained on the lower column, which contained a cation-exchange resin (Bio-Rad AG 50W-X4). After elution from the column with 1 M sodium acetate, ALA was determined colorimetrically. The amount of ALA present in millimicromoles per gram of liver per hour was calculated using a molar extinction coefficient of $6{\cdot}6 \times 10^4$, which was determined using pure ALA obtained from Calbiochem, Los Angeles, Calif.

*Preparation of bone marrow*

Marrow, extracted from femurs and tibias of adult rabbits, was weighed and delivered into 3 vol of cold saline containing 0·5 mM EDTA and 10 mM Tris buffer at pH 7·4. The marrow was then homogenized, and the ALA synthetase activity was determined as described for liver.

*Analysis of aminoacetone*

Aminoacetone production by immature mammalian liver has not been previously determined. It was therefore considered important to ascertain the contribution of this substance to the total aminoketone levels being measured when fetal and newborn livers were analyzed for ALA. In this case, a procedure which provided for the separation of AA and ALA was used.[29] PBG was separated from the incubation supernatant as previously described. The eluate was then collected instead of being placed on a second column. Both ALA and AA were then converted to pyrroles by condensation with acetylacetone. The solution containing both pyrroles was then placed on a second column of anion-exchange resin. The AA pyrrole, 2,4-dimethyl-3-acetyl pyrrole, was eluted with 10 ml of *n*-butanol containing 0·01 M ammonium hydroxide. The column was washed with 1 M acetic acid, and then the ALA pyrrole was eluted with a mixture (10 ml) of glacial acetic acid and methanol (1:2).

The eluate containing the AA pyrrole was mixed with an equal volume of Ehrlich's reagent and after 10 min was determined spectrophotometrically at 556 nm. The molar extinction coefficient of this color complex is $7{\cdot}6 \times 10^4$.

*Treatment of animals*

The two drugs used in this investigation were 3,5-dicarbethoxy-1,4-dihydrocollidine (DDC) and ferriprotoporphyrin chloride (hemin). DDC was obtained from Eastman Organic Chemicals, Rochester, N.Y., whereas hemin was provided by Calbiochem, Los Angeles, Calif.

All treatments of animals with DDC were made by subcutaneous injection of the drug dissolved in 95% ethanol. DDC was administered to rabbits in doses of 500 mg/kg, and to rats in doses of up to 250 mg/kg. These doses represent the levels of DDC required to induce a reproducible level of ALA synthetase activity in the adult of each species. In experiments with DDC, ethanol-treated animals served as controls. Hemin solutions were prepared by dissolving the drug in saline. Hemin was administered by intraperitoneal injections in dosages of 20 mg/kg. In experiments with hemin, animals treated with saline served as controls.

*Detection of DDC in tissues*

A gas chromatographic method was devised for the detection of DDC in fetal tissue after treatment of the mother. A Beckman GC-5 gas chromatograph (Beckman Instruments, Inc., Fullerton, Calif.) equipped with a flame ionization detector was used. The column was packed with 0·5% Hi-Eff-8B (cyclohexandimethanol succinate) on 100/120 mesh siliconized Gas Chrom Q. The carrier gas was helium. At a chart speed of 1 in./min a prominent peak representing DDC emerges after 1·5 min from the time of injection. Peak height is proportional to the concentration of DDC in the injected solution, as determined by a standard curve. The presence of DDC in biological tissues was determined in ethanol after chloroform extraction and evaporation.

## RESULTS

### *Activity of ALA synthetase during the perinatal period*

As an initial approach to the problem, basal levels of hepatic ALA synthetase of adult members of different species and sexes were determined. The results of these studies are presented in Table 1. It can be seen that enzyme activities of male and female rats do not differ significantly. The same is true of rabbits. While mice have a high level of enzyme activity, their fetuses and newborn were considered too small to be treated individually or to provide a quantity of liver tissue sufficient for enzyme analysis. The rat also has high enzyme activity levels and was considered to have fetuses, newborn and litters of sufficient size to be practical for use as an experimental animal. The rabbit has low basal enzyme activity, but offers the advantage of having relatively large newborn which can be individually treated over a period of days. The same features apply to the guinea pig. Rats and rabbits have relatively short gestation periods, 21 and 30 days, respectively, while that of the guinea pig is 60–70 days.

TABLE 1. HEPATIC ALA SYNTHETASE ACTIVITIES OF DIFFERENT SPECIES*

| Species | Activity |
|---|---|
| Female rat | 44·0 ± 13·2 (32) |
| Male rat | 44·5 ± 4·8 (10) |
| Female rabbit | 4·0 ± 1·6 (6) |
| Male rabbit | 4·0 ± 1·4 (5) |
| Male guinea pig | 7·9 ± 3·0 (6) |
| Male mouse | 47·4 ± 12·8 (9) |

* Adult animals were used for all determinations. The preparation of liver homogenates and the determination of ALA synthetase activity are described in Materials and Methods. In this and subsequent tables, values express metabolism as millimicromoles of ALA per gram of liver per hour and are expressed as means ± standard deviations. The number of animals individually analyzed is indicated in parentheses.

Preliminary studies conducted with adult and neonatal rabbit liver tissue indicated that ALA synthetase activity in neonatal rabbits is approximately eight times that of the adult. It was also shown that pretreatment with ethanol had no effect on the ALA synthetase activity of either age group. ALA synthetase activity in rat liver is also much higher during the prenatal period when compared to adult levels. Fetal enzyme levels at 16 ± 1 days of gestation, 4 days before expected delivery, are six to eight times those seen in the adult tissues. Subsequently, these levels decrease progressively until reaching adult activity about 2 days after birth.

ALA synthetase activity was measured in maturing rabbits over a 9-week period consecutively from 2 weeks prior to expected date of delivery to the seventh week after birth. At 2 weeks prior to delivery, ALA synthetase activity is approximately 50 times that of the adult. At 1 week before birth, the enzyme level is nearly 35 times that seen in the mature rabbit. At birth, ALA synthetase activity is six to eight times the adult level and within 2–3 weeks becomes equal to that of the mature animal.

When guinea pigs were studied, it was noted that the newborn animals had an ALA synthetase activity comparable to that of the adult. At approximately 15 days prior

to delivery, however, the prenatal enzyme level is seven to eight times that seen in the mature animals. This is the greatest activity that could be detected in the guinea pig. The decline in activity occurs within 2 weeks of delivery, reaching its nadir at the time of birth.

*Studies of the activity of perinatal hepatic ALA synthetase*

*Studies with rabbits.* The contribution of AA to the elevated basal aminoketone levels was determined in neonatal rabbit livers after incubation of homogenates as previously described. Aminoacetone was seen to constitute approximately 10 per cent of the total aminoketone production under the conditions used. Henceforth, aminoketone levels measured in perinatal livers were taken to represent levels of ALA. It was further noted that there were no alterations in AA production in either adult or newborn rabbits after DDC, hemin or ethanol treatment.

A comparison of the effects of DDC on the induction of ALA synthetase in adult and newborn rabbits is presented in Table 2. Animals were divided into four groups and were treated by subcutaneous injection with DDC dissolved in ethanol or with ethanol alone. Injections were made at daily intervals for 3 days, according to the experimental design presented in the table.

TABLE 2. COMPARISON OF THE EFFECTS OF DDC ON THE INDUCTION OF HEPATIC ALA SYNTHETASE IN ADULT AND NEWBORN RABBITS*

| Group | Treatment days prior to sacrifice | | | Adult | Control (%) | Newborn | Control (%) |
|---|---|---|---|---|---|---|---|
| | 3 | 2 | 1 | | | | |
| 1 | EtOH | EtOH | EtOH | 4·0 ± 1·1 (8) | | 30·9 ± 2·7 (9) | |
| 2 | EtOH | EtOH | DDC | 36·9 ± 9·9 (15) | 923 | 43·9 ± 17·7 (18) | 142 |
| 3 | EtOH | DDC | DDC | 68·2 ± 13·6 (10) | 1705 | 64·2 ± 14·6 (13) | 204 |
| 4 | DDC | DDC | DDC | 75·7 ± 19·5 (9) | 1893 | 66·3 ± 15·1 (10) | 215 |

* Animals were treated subcutaneously with DDC (500 mg/kg) or ethanol (EtOH) at times indicated. Ethanol was given in quantities equal to those used to administer DDC. Enzyme activities were determined 24 hr after the final treatment.

Adult animals which had received one dose of DDC showed an increase to 923 per cent of control. This increase was doubled in animals receiving a second treatment, producing a total activity greater than 17 times the control level. A third treatment produced a further increase in activity to almost 19 times that seen in the ethanol-treated controls. This last increase, however, was not significant ($P \leq 0{\cdot}05$). In contrast, newborn animals showed relatively much less response to DDC than did the adults. An increase to 142 per cent of the control level of activity was seen after one DDC treatment and to 204 per cent after a second dose. An additional treatment had very little effect in increasing the neonatal ALA synthetase activity.

A comparison of the effects of DDC and hemin, administered separately and in combination, on the activity of hepatic ALA synthetase in adult and newborn rabbits is presented in Table 3. Animals in each group were treated 24 hr prior to sacrifice with the agents indicated. In the adult, hemin alone caused a decrease to 38 per cent of control (ethanol and saline pretreated) enzyme activity. DDC treatment resulted

in an increase to 600 per cent of the control value. In the newborn a decrease to only 85 per cent of the control activity was observed after hemin treatment, while the increase induced by DDC was to approximately 133 per cent of the control level. In adults receiving the combined DDC/hemin treatment, an increase in enzyme activity to only 250 per cent of the control level was observed, reflecting a marked interaction of these two agents. Hemin treatment resulted in a decreased DDC induction of ALA synthetase in the adult by more than half when compared to the animals treated with DDC and saline. In the newborn, however, the interaction of DDC and hemin is much less apparent. Not only is DDC relatively ineffective in inducing ALA synthetase, but hemin appears to be much less effective in preventing the induction which does occur.

TABLE 3. COMPARISON OF THE COMBINED EFFECTS OF DDC AND HEMIN ON THE ACTIVITY OF HEPATIC ALA SYNTHETASE IN ADULT AND NEWBORN RABBITS*

| Group | Treatment | Adult | Control (%) | Newborn | Control (%) |
|---|---|---|---|---|---|
| 1 | EtOH + saline | 4·0 ± 0·7 (10) | | 29·8 ± 0·5 (12) | |
| 2 | EtOH + hemin | 1·5 ± 0·2 (10) | 38 | 25·3 ± 4·0 (9) | 85 |
| 3 | DDC + saline | 24·0 ± 5·3 (10) | 600 | 39·5 ± 6·5 (10) | 133 |
| 4 | DDC + hemin | 10·0 ± 3·3 (10) | 250 | 35·7 ± 7·3 (6) | 120 |

* Animals were treated with DDC (500 mg/kg) and hemin (20 mg/kg) 24 hr prior to sacrifice. Ethanol (EtOH) and saline were given in doses equal to those used to administer DDC and hemin respectively.

The possibility that elevated ALA synthetase activity in the neonatal liver might represent erythropoietic elements was considered by comparing the activities of neonatal hepatic ALA synthetase with that from adult bone marrow before and after treatment with DDC and hemin. The results are presented in Table 4. Adult hepatic enzyme levels are included for comparison. Marrow was extracted from the femurs and tibias of adult rabbits which had been treated 24 hr prior to sacrifice. The basal activity of bone marrow ALA synthetase is similar to the basal newborn hepatic activity. Adult hepatic activity undergoes a typical increase after DDC treatment.

TABLE 4. COMPARISON OF THE EFFECTS OF DDC AND HEMIN ON THE ACTIVITY OF BONE MARROW AND HEPATIC ALA SYNTHETASE IN ADULT AND NEWBORN RABBITS*

| Treatment | Activity | | |
|---|---|---|---|
| | Adult bone marrow | Adult liver | Newborn liver |
| EtOH | 25·0 ± 8·3 (6) | 4·0 ± 1·3 (5) | 28·3 ± 3·6 (8) |
| DDC | 20·5 ± 5·8 (6) | 31·6 ± 9·4 (6) | 41·2 ± 15·5 (11) |
| Hemin | 24·1 ± 3·4 (3) | 1·4 ± 0·8 (3) | 24·5 ± 2·6 (9) |

* DDC (500 mg/kg) or ethanol (EtOH) or hemin (20 mg/kg) was administered 24 hr prior to sacrifice. The preparation of bone marrow is described in Materials and Methods.

DDC showed no inducing effect on the activity of the bone marrow enzyme, but increased the activity of the neonatal hepatic synthetase by a small amount, as previously noted. Hemin, likewise, had no effect on the bone marrow ALA synthetase activity, but decreased slightly the activity of the newborn enzyme. Typical responses with hemin on the adult hepatic enzyme were again observed.

*Studies with rats.* These studies provide a species comparison of ALA synthetase activity and responses to DDC and hemin during the perinatal period. Fetuses were obtained after sacrifice of the mother. Livers of littermates were pooled for single determinations. Pregnancy did not affect the adult response to DDC pretreatment when ALA synthetase activity was compared after DDC treatment of pregnant and of nonpregnant rats.

Table 5 presents a comparison of the effects of different doses of DDC on ALA synthetase activity in adult and fetal rats. DDC and ethanol were administered to pregnant rats 24 hr prior to enzyme determinations. It can be seen that DDC at all doses increased the level of adult enzyme activity when compared with that of ethanol-treated controls. On the other hand, prenatal animals are not responsive to DDC induction *in utero*. No dose of DDC was able to increase the activity of hepatic ALA synthetase in animals 4 days before expected delivery. The same observation, no fetal response to DDC treatment of maternal animals, was made with fetal rats 5 days prior to expected birth and with animals treated the day before delivery. Neonatal rats had an ALA synthetase activity equal to 200 per cent of the adult level. When newborn rats were treated with DDC (31 mg/kg) 24 hr prior to enzyme determination, ALA synthetase activity was increased to 145 per cent of control levels. No further increase was observed in response to any higher dose of DDC up to 250 mg/kg.

TABLE 5. COMPARISON OF THE EFFECTS OF DIFFERENT DOSES OF DDC ON THE ACTIVITY OF HEPATIC ALA SYNTHETASE IN ADULT AND FETAL RATS*

| Dose of DDC (mg/kg) | Adult | Control (%) | Fetal (−4 days) | Control (%) |
|---|---|---|---|---|
| 0 | 59·1 ± 14·9 (8) | | 213·6 ± 29·8 (4) | |
| 31 | 114·2 ± 52·0 (8) | 193 | 165·3 ± 26·7 (4) | 77 |
| 62 | 140·0 ± 75·7 (7) | 237 | 211·6 ± 65·8 (4) | 99 |
| 125 | 138·2 ± 20·0 (6) | 234 | 194·0 ± 67·4 (4) | 91 |
| 250 | 250·5 ± 53·4 (6) | 424 | 185·5 ± 15·0 (4) | 87 |

* Pregnant rats were treated subcutaneously with the dose of DDC indicated 24 hr prior to sacrifice. Control animals received ethanol only. The number of animals (adult) or litters of pooled livers (fetal) individually analyzed is shown in parentheses.

DDC was demonstrated to cross the placenta readily. Gas chromatographic analysis of fetal blood and liver tissue demonstrated levels of DDC essentially the same as the quantities observed in maternal rat blood and liver tissue.

Table 6 presents a comparison of the effects of both DDC and hemin on the activity of ALA synthetase in adult and fetal rats 4 days prior to the expected date of delivery. Hemin alone decreased the level of ALA synthetase activity in adult rats to 68 per cent of the control value. [This decrease is not statistically significant ($P \leq 0{\cdot}05$) in light of the large standard deviation of the control value.] DDC treatment of adults

resulted in a more than 4-fold increase in ALA synthetase activity when compared with that of ethanol-treated controls. When DDC treatment was combined with hemin, the inductive effect of DDC was significantly reduced to only 145 per cent of control. These results are comparable to those observed in adult rabbits (Table 3). Hemin treatment of fetuses did not significantly decrease enzyme activity from the control level. Fetal animals demonstrated only a small increase in ALA synthetase activity after DDC treatment. The combined effects of DDC and hemin eliminated any increase in fetal enzyme levels which may have occurred when DDC was given alone. In no case, however, did the fetal enzyme activity differ significantly ($P \leq 0.05$) from the control value.

TABLE 6. COMPARISON OF THE COMBINED EFFECTS OF DDC AND HEMIN ON THE ACTIVITY OF HEPATIC ALA SYNTHETASE IN ADULT AND FETAL RATS*

| Group | Treatment | Adult | Control (%) | Fetal (−4 days) | Control (%) |
|---|---|---|---|---|---|
| 1 | EtOH + saline | 42·1 ± 17·8 | | 204·5 ± 45·7 | |
| 2 | EtOH + hemin | 28·5 ± 6·8 | 68 | 151·5 ± 57·2 | 74 |
| 3 | DDC + saline | 177·3 ± 42·9 | 421 | 225·5 ± 2·0 | 110 |
| 4 | DDC + hemin | 61·6 ± 26·7 | 145 | 162·5 ± 22·2 | 79 |

* Pregnant rats were treated with DDC (250 mg/kg) and hemin (20 mg/kg) 24 hr prior to sacrifice. Controls received ethanol (EtOH) and saline. Litters were combined for single determinations. Values are means ± standard deviations of five experiments.

## DISCUSSION

These studies have demonstrated that hepatic ALA synthetase activity in perinatal animals of three species is significantly higher than the adult enzyme level. During the period of elevated activity, ALA synthetase is relatively less responsive to induction and repression than is observed in the adult, although refractoriness to induction decreases as enzyme activity decreases to adult levels. These findings are consistent with either of two possibilities: (1) that increased ALA synthetase activity represents erythropoietic activity in perinatal livers, or (2) that alterations in ALA synthetase activity are a reflection of differences in the regulation of this enzyme in the immature liver cell.

The similarity of ALA synthetase activities in adult rabbit bone marrow and newborn liver suggests the possible contribution of hepatic erythropoietic activity to the high ALA synthetase levels observed in neonatal liver. Recent histological studies[30] have shown that, whereas hepatic hemopoietic activity in rats declines toward the end of gestation, a small number of developing blood cells remain in the liver during the first week of life. Thus, the elevated hepatic ALA synthetase activity observed in perinatal rats could, in part, reflect the gradual decline in the function of the liver as an erythropoietic organ. By contrast, erythropoiesis in fetal rabbit liver occurs from days 15–22 of gestation.[31] By day 19, bone marrow and lymph nodes are producing cells which will become erythrocytes, whereas hemopoiesis in the liver is negligible. It is concurrently reported that, at the time of birth in most mammalian species, there is little or no evidence of hepatic erythropoiesis.[31] If erythropoietic tissue were

responsible for the elevated neonatal ALA synthetase activities observed here, one would expect to find the same concentration of such tissue in neonatal liver as is found in adult bone marrow, since the basal ALA synthetase activities in these organs are comparable (Table 4). This, however, does not appear to be the case. The very low level of erythroblastic activity which may persist in the liver at the time of birth does not appear to be sufficient to account for the high level of ALA synthetase activity observed at that time.

On the other hand, these observations suggest that differences observed in adult and perinatal ALA synthetase activity reflect an alteration in the regulation of enzyme activity in the immature hepatic cell. One would expect that if adult and perinatal parenchymal liver cells were biochemically and physiologically the same, then the absolute changes in hepatic ALA synthetase activity should be about the same in all groups. The absolute changes in enzyme activity, however, are always greater in the adult livers. This fact suggests that in immature animals the regulation of ALA synthetase activity and the response to DDC and hemin are related to increasing repression of the enzyme to lower basal levels as the animals mature. Such a change is consistent with the concept that controls on inducibility of ALA synthetase in neonates may be developmentally determined or adaptively related to gestational influences.[32]

Such influences may be the result of some maternal–fetal interaction, as has been suggested to influence the development of hepatic drug-metabolizing enzymes.[33,34] Alternatively, naturally higher levels of endogenous steroid hormones present in the fetal environment[35,36] may influence enzyme regulatory activity in perinatal liver to the extent observed here. Perhaps the most intriguing possibility to explain the altered enzyme activity observed in these studies may be related to the proposed etiology of hepatic porphyria.[37] It is suggested that a developmentally altered operator gene is ineffective in exerting control over the structural gene coding for hepatic ALA synthetase. It is possible that such an alteration may also be present in the immature mammalian cell and may account for the altered responses of ALA synthetase noted in these studies. Such an alteration in genetic control may be resolved with changes which accompany maturation.

While these and other possible explanations for the altered enzyme activity in perinatal liver cells are under investigation, it may be concluded from these studies that hepatic ALA synthetase activity during the perinatal period is both elevated and refractory to induction and repression in comparison with adult levels. The enhanced enzyme activity may, in part, reflect erythropoietic foci which persist in the liver postpartum, but also appears to be due to a lack of repression of the enzyme to the adult levels. Refractoriness to induction and repression seems to be the result of some alteration in regulatory control associated with development of the liver cell.

It is suggested that alterations in hepatic heme production during the perinatal period may contribute to such problems as physiological jaundice, alterations in the production of hepatic heme-containing compounds, and changes in the ability to metabolize various drugs.

## REFERENCES

1. A. K. Done, *A. Rev. Pharmac.* **6,** 189 (1966).
2. F. Sereni and M. D. Principi, *A. Rev. Pharmac.* **8,** 453 (1968).
3. J. R. Fouts and R. H. Adamson, *Science* **129,** 897 (1959).

4. T. OMURA and R. SATO, *J. biol. Chem.* **239,** 2370 (1964).
5. L. G. HART, R. H. ADAMSON, R. L. DIXON and J. R. FOUTS, *J. Pharmac. exp. Ther.* **137,** 103 (1962).
6. R. D. LEVERE and S. GRANICK, *Proc. natn. Acad. Sci. U.S.A.* **54,** 134 (1965).
7. B. F. BURNHAM and J. LASCELLES, *Biochem. J.* **87,** 462 (1963).
8. J. LASCELLES, *J. gen. Microbiol.* **23,** 487 (1960).
9. S. GRANICK, *J. biol. Chem.* **241,** 1359 (1966).
10. N. HAYASHI, B. YODA and G. KIKUCHI, *J. Biochem.* **63,** 446 (1968).
11. S. GRANICK and G. URATA, *J. biol. Chem.* **238,** 821 (1963).
12. D. P. TSCHUDY, F. H. WELLAND, A. COLLINS and G. HUNTER, JR., *Metabolism* **13,** 396 (1964).
13. H. S. MARVER, D. P. TSCHUDY, M. G. PERLROTH and A. COLLINS, *Fedn Proc.* **24,** 721 (1965).
14. D. S. BEATTIE and R. N. STUCHELL, *Archs Biochem. Biophys.* **139,** 291 (1970).
15. S. R. GROSS and J. J. HUTTON, *J. biol. Chem.* **246,** 606 (1971).
16. D. P. TSCHUDY, M. G. PERLROTH, H. S. MARVER, A. COLLINS, G. HUNTER, JR. and M. RECHCIGL, JR., *Proc. natn. Acad. Sci. U.S.A.* **53,** 841 (1965).
17. A. D. WAXMAN, A. COLLINS and D. P. TSCHUDY, *Biochem. biophys. Res. Commun.* **24,** 675 (1966).
18. H. S. MARVER, *J. clin. Invest.* **47,** 66a (1968).
19. H. S. MARVER, R. SCHMID and H. SCHUTZEL, *Biochem. biophys. Res. Commun.* **33,** 969 (1968).
20. I. M. LONDON, R. WEST, D. SHEMIN and D. RITTENBERG, *J. biol. Chem.* **184,** 351 (1950).
21. C. H. GRAY, A. NEUBERGER and P. H. A. SNEATH, *Biochem. J.* **47,** 87 (1950).
22. M. F. VEST, in *Bilirubin Metabolism* (Eds. I. A. D. BOUCHIER and B. H. BILLING), p. 47. Blackwell, Oxford (1967).
23. S. H. ROBINSON, *J. Lab. clin. Med.* **73,** 668 (1969).
24. J. S. WOODS and R. L. DIXON, *Life Sci.* **9,** 711 (1970).
25. J. S. WOODS and R. L. DIXON, *Biochem. Pharmac.* **19,** 1951 (1970).
26. H. S. MARVER, D. P. TSCHUDY, M. G. PERLROTH and A. COLLINS, *J. biol. Chem.* **241,** 2803 (1966).
27. D. MAUZERALL and S. GRANICK, *J. biol. Chem.* **219,** 435 (1956).
28. J. R. DAVIS and S. L. ANDELMAN, *Archs environ. Hlth* **15,** 53 (1967).
29. H. S. MARVER, D. P. TSCHUDY, M. G. PERLROTH, A. COLLINS and G. HUNTER, JR., *Analyt. Biochem.* **14,** 53 (1966).
30. P. TH. HENDERSON, *Biochem. Pharmac.* **20,** 1225 (1971).
31. W. BLOOM, in *Handbook of Hematology* (Ed. H. DOWNEY), Vol. II, section XIII, p. 863. Hoeber, New York (1938).
32. A. KAPPAS, C. S. SONG, R. D. LEVERE, R. A. SACHSON and S. GRANICK, *Proc. natn. Acad. Sci. U.S.A.* **61,** 509 (1968).
33. G. FEUER and A. LISCIO, *Int. J. clin. Pharmac.* **3,** 30 (1970).
34. G. FEUER and A. LISCIO, *Nature, Lond.* **223,** 68 (1969).
35. M. MIZUNO, J. LOBOTSKY, C. W. LLOYD, I. KOBAYASHI and Y. MURASOWA, *J. clin. Endocr. Metab.* **28,** 1133 (1968).
36. D. B. VILLEE, *N. Engl. J. Med.* **281,** 533 (1969).
37. S. GRANICK, *Ann. N.Y. Acad. Sci.* **123,** 188 (1965).