The Perinatal Development of Haem Biosynthesis in Rats, Guinea Pigs and Humans

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It is known that δ -aminolaevulinate synthetase (EC 2.3.1.13) activity is elevated in the neonatal rat and during the perinatal period in rat, rabbit and guinea-pig liver (Woods & Dixon, 1970). In mice Freshney & Paul (1971) have shown that hepatic δ -aminolaevulinate synthetase, δ -aminolaevulinate dehydratase (EC 4.2.1.24) and ferrochelatase (EC 4.99.1.1) activities are elevated maximally at 14, 15 and 17 days of gestation respectively. These elevated activities fall to normal values shortly after birth.

In the present study the activity of δ -aminolaevulinate synthetase (the initial and normally the rate-limiting enzyme of haem biosynthesis), the activity of ferrochelatase (the terminal enzyme of haem biosynthesis) and the concentration of porphyrins were measured in the perinatal period in rat and guinea-pig liver. δ -Aminolaevulinate synthetase activity was measured by determining the formation of δ -aminolaevulinate from glycine in a whole liver homogenate (Moore, 1970), ferrochelatase activity by measuring the incorporation of ^{59}Fe into protoporphyrin to form haem (Goldberg *et al.*, 1956), porphyrins by the method of Rimington (1961). In the prenatal period the activities of the two enzymes in the foetal liver are sequentially greater than those in the maternal livers, and these increased activities are associated with elevated concentrations of hepatic porphyrins. These porphyrin concentrations are again sequentially elevated, with that of uroporphyrin elevated first about 10 days before birth, then that of coproporphyrin about 5 days before birth and finally that of protoporphyrin about 2 days before birth. All of these elevated concentrations fall to the maternal values shortly after birth. When the mother rats were treated with 2-allyl-2-isopropylacetamide (a potent porphyrogenic drug; Goldberg & Rimington, 1954) it was found that, although there was a large elevation of the activity of the maternal δ -aminolaevulinate synthetase, there was little increase in that of the foetal enzyme. In newborn rats there was a similar lack of effect.

In humans, erythrocyte porphyrins were measured in maternal blood and in umbilical-cord blood. It was found that there was a significantly higher concentration of protoporphyrin in the newborn babies than in the maternal blood and that this was increased in premature births. These results suggest that in the perinatal development of haem biosynthesis there is a sequential induction of the enzymes of the pathway,

each becoming rate-limiting at a given period, this all being associated with a de-repressed activity of δ -aminolaevulinate synthetase and leading to an excessive porphyrin production.

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Lead, Ethanol and δ -Aminolaevulinate Dehydratase

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It has long been accepted that lead has a direct effect on haem biosynthesis (Goldberg, 1968), and in particular it has been shown, both in direct studies and in measurement of excreted δ -aminolaevulinate in the urine, to depress severely the activity of δ -aminolaevulinate dehydratase (the second enzyme of haem biosynthesis; Haeger-Aronsen *et al.*, 1971) both *in vitro* and *in vivo*. It has further been shown that ethanol also depresses δ -aminolaevulinate dehydratase activity (Moore *et al.*, 1971). For this reason it was decided to examine the effects of these two substances, separately and together, on δ -aminolaevulinate dehydratase activity in rats.

A significant depression of δ -aminolaevulinate dehydratase activity at a mean blood lead concentration of $122 \pm 48 \mu\text{g}/100\text{g}$ of blood and at a blood ethanol concentration of $192 \pm 51 \text{mg}/100\text{ml}$ of whole blood was observed. When these are combined at the similar concentrations of $133 \pm 50 \mu\text{g}$ of lead/100g of blood and 199mg of ethanol/100ml of blood, the activity of δ -aminolaevulinate dehydratase is significantly elevated over the two separate depressed activities, although lower than the control value. In a subsequent experiment *in vitro*, with rat liver as a source of the enzyme, it was found that δ -aminolaevulinate dehydratase activity was again depressed by both ethanol and lead separately but elevated by these compounds together. The linking factor in both of these experiments seems to be the concentration of GSH in the system. Lead is known to bind to thiol groups, and other workers have shown that the addition of GSH to systems in which δ -aminolaevulinate dehydratase activity is being measured

minimizes the depression of activity due to lead (de Barriero, 1969). Conversely, although GSH is an essential cofactor for δ -aminolaevulinate dehydratase, excessive concentrations of GSH lower the activity of the enzyme (Moore *et al.*, 1971). Ethanol oxidation substantially alters the redox potential of the cell and, in doing so, alters the [GSH]/[GSSG] ratio in the cell. It is therefore suggested that, although lead depresses GSH concentrations and thus lowers δ -aminolaevulinate dehydratase activity, and ethanol raises GSH concentrations and lowers the activity, these two in conjunction act in opposite directions and lead to an effective maintenance of activity.

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The Increased Anti-Tumour Activity of Chlorambucil after Pretreatment with Phenobarbitone

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Differences in the utilization of chlorambucil (4-[4-bis-(2-chloroethyl)aminophenyl]butyric acid) after its uptake by tumour cells *in vitro* have been demonstrated (Harrap & Hill, 1970): cells of an alkylating-agent-resistant strain of the Yoshida ascites sarcoma are capable of hydrolysing the chloroethyl groups and modifying the aromatic ring of the drug more rapidly than are alkylating-agent-sensitive cells. Phenobarbitone, which is known to induce a number of microsomal drug-metabolizing enzymes (Kuntzman, 1969), was used in the present studies. Rats bearing the drug-sensitive strain of the Yoshida sarcoma were treated with phenobarbitone 12h before the administration of chlorambucil ($\frac{1}{2}$ LD₅₀ 8mg/kg body wt.). This combination resulted in a greater tumour-cell kill than was observed in tumour-bearing animals treated with chlorambucil alone, but was not more toxic to peripheral leucocytes. However, the growth rate of the drug-resistant tumour, maintained by routine passage in rats treated with an alkylating agent, was unaffected by this combination of phenobarbitone and chlorambucil.

These differing responses to phenobarbitone pretreatment were also observed in the two Yoshida cell lines growing in suspension culture. In addition, with a line of L5178Y cells sensitive to alkylating agents it has been shown, by assay of colony-forming

ability, that phenobarbitone pretreatment increases the tumour-cell-killing effect of chlorambucil three- to four-fold. 3-Methylcholanthrene and benz[*a*]anthracene, hydrocarbons that are also capable of inducing drug-metabolizing enzymes (Nebert & Gelboin, 1968), have similarly been shown to enhance the toxic effects of chlorambucil in these drug-sensitive cells.

The administration of phenobarbitone to experimental animals is known to enhance the overall rate of hepatic protein synthesis (Conney, 1967). Exposure of L5178Y and drug-sensitive Yoshida cells in culture to phenobarbitone resulted in a doubling of their rates of protein synthesis. This effect was not observed in drug-resistant Yoshida cells.

Although chlorambucil is generally thought to be a direct-acting alkylating agent (Ross, 1962), these results suggest that metabolic activation may be involved in the anti-tumour activity of this compound in drug-sensitive cells. Some possible implications of these findings and of the lack of response of alkylating-agent-resistant cells to enzyme inducers will be discussed.

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Lysosomal Enzymes and Degradation of the Proteoglycans of the Human Intervertebral Disc

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Previous work has indicated (Lyons *et al.*, 1964, 1966; Davidson & Woodhall, 1959) that the proteoglycans in prolapsed discs are degraded compared with those in normal tissue, and a similar change occurs on aging. Lysosomal enzymes may be involved, but their presence in the disc has received little attention. A particulate fraction obtained by differential centrifugation from homogenates in 0.25M-sucrose contained several forms of acid phosphatase (Osborn, 1971) that were released by treatments known to disrupt lysosomes. Acid protease and at least three exoglycosidases (Happey *et al.*, 1971) were present, but hyaluronidase was not detected in the particulate fraction or in proteoglycan-free supernatants prepared from concentrated homogenates of nucleus pulposus.

The degradation of the proteoglycans is likely to be