Plasma Corticosterone Concentrations in the Perinatal Rat

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1. Plasma corticosterone concentrations were determined in the foetal rat during the gestational period from day 18.5 to term and in postnatal rats over the first few hours after delivery. 2. The plasma corticosterone concentrations in foetal rats are as high as six times maternal values at day 19 of gestation and are approximately equal to maternal values from day 20 to term. 3. In postnatal rats the plasma corticosterone concentrations rise 3.5-fold on average within 5 hr. of delivery. 4. The results are discussed in relation to the function of adrenal steroids in postnatal liver development.

It was reported by Lin & Knox (1957) that the parenteral administration of hydrocortisone to intact or adrenalectomized adult rats was followed within 5 hr. by a substantial increase of tyrosine aminotransferase (EC 2.6.1.5) activity in the liver. Subsequently the hormone was shown to be ineffective in induction of activity in foetal rat liver in the same time-period, though the presence of functional adrenal glands was necessary for the normal postnatal development of the enzymic activity (Sereni, Kenney & Kretchmer, 1959). It has since been shown that the enzyme can be induced *in utero* by the fluorinated steroid analogue triamcinolone, provided that the time of exposure exceeds 5 hr. (Yeung, Stanley & Oliver, 1967). Premature delivery of rabbit and rat fetuses also results in the induction of tyrosine aminotransferase activity in the liver (Litwack & Nemeth, 1965; Holt & Oliver, 1968). In the light of these facts the plasma corticosterone concentrations in foetal and postnatal rats were determined to test whether the postnatal development of enzyme activity is associated with the release of adrenal steroids.

METHODS

Wistar albino rats were used. Foetuses were prematurely delivered as described by Yeung & Oliver (1967), and their foetal ages were established by weight (Yeung *et al.* 1967). Litters used for determination of the prenatal plasma corticosterone concentrations ranged in gestational age from 18.5 days to term.

To standardize as far as possible the conditions of blood collection, each litter was treated in exactly the same way. Under ether anaesthesia, the uterus was exposed by laparotomy and kept warm with a lamp while foetuses were sequentially delivered. Each foetus, still attached to the placenta, was rapidly exsanguinated by jugular puncture.

The blood from the whole litter was pooled in a heparinized tube and the plasma was collected and stored frozen. On average each foetus was bled within 1 min. of removal from the uterus. The average time for blood collection from the whole litter was about 10 min.

For the determination of steroid concentrations within the first few hours after delivery, animals close to term were prematurely delivered and maintained in the humidicrib at 37° for various times before blood collection. Where the litter size was 12 or more, half the animals were bled immediately on delivery and the rest of the litter was treated as described above. The maternal plasma corticosterone concentration at several stages of pregnancy was also determined, blood being collected by heart puncture under ether anaesthesia. Corticosterone, which comprises approx. 50% of the steroids released from the adrenal cortex in the rat, was determined on plasma volumes of 0.5–1.0 ml. The blue tetrazolium method of Elliot, Birmingham, Schally & Schönbaume (1954) as modified by Peron (1962) was used. This method determines the total non-conjugated corticosterone, including protein-bound hormone that is split from the protein and extracted by the organic solvent.

RESULTS

Fig. 1 shows the plasma corticosterone concentrations of foetal rats varying in gestational age from 18.5 to 22 days. Animals at about day 19 have high plasma corticosterone concentrations, but by day 20 these have fallen precipitously and the concentration is then maintained close to the maternal values (0.5 µg./ml.) until term.

The postnatal values (Table 1) were obtained from groups of six to ten littermate animals killed between 1 and 5 hr. after normal or surgical delivery. The data suggest that in different litters the plasma corticosterone concentrations rise at different times after delivery and for statistical assessment all the postnatal data were combined and compared with

Fig. 1. Plasma corticosterone concentrations in foetal rats. Pooled blood plasma was prepared from litters of animals at various ages and assayed for corticosterone as described in the text.

Table 1. *Plasma corticosterone concentrations in neonatal rats*

Values for foetal rats were obtained from pooled litters of gestational age 20–22 days (calculated from the data in Fig. 1). Newborn rats were surgically delivered and the blood was pooled from at least six rats. 'Postnatal' rats were delivered surgically (six litters) or by normal vaginal delivery (four litters), the time of natural birth being observed as accurately as possible. Animals were maintained in a humidicrib at 37°, killed in groups of six to ten at various times after delivery and the blood was pooled from each group. The means $\pm$ S.D. were calculated, with the number of determinations in parenthesis for the late-foetal rats. The difference between the means of foetal and 'postnatal' values is highly significant ($t = 4.61$, 16 degrees of freedom, $P < 0.001$).

Animals	Plasma corticosterone concentration ($\mu\text{g./ml.}$)
Late foetal (20–22 days)	0.61 ± 0.20 (8)
Newborn	$0.56, 0.64, 0.72$
Mean	0.64
‘Postnatal’	
1 hr.	1.60
2 hr.	2.30
2.5 hr.	3.20, 1.60, 1.10
5 hr.	4.75, 2.00, 1.70, 1.50, 1.40
Mean	2.12 ± 1.10

the late-foetal data. Newborn animals have the same corticosterone concentration as the late-foetal animals, but values for postnatal animals are on average 3.5-fold higher and the difference is highly significant ($P < 0.001$).

DISCUSSION

The small size of blood samples obtainable from individual animals made it necessary to measure corticosterone concentrations in pooled blood, since the more sensitive fluorimetric methods were not available to us. Technical difficulties in collecting blood from animals younger than 18.5 gestational days (foetal wt. 1g. or less) made it impossible to show whether the high corticosterone concentration at day 19 was a peak value compared with earlier ages. Kamoun, Mialhe-Voloss & Stutinsky (1964) showed that the maximal concentration of corticosterone in the adrenal glands of foetal rats occurs between days 18 and 19 of gestation, but the high foetal plasma corticosterone concentrations may be derived from the maternal circulation. It has been shown that plasma corticosterone concentrations are higher in pregnant than in non-pregnant females (Saba & Hoet, 1963), and in the animals used here the mean plasma corticosterone concentration was $0.5 \mu\text{g./ml.}$ in pregnant females and $0.2 \mu\text{g./ml.}$ in non-pregnant females of the same age. However, corticosterone does not readily cross the placenta (Hagerman & Vilee, 1960), and in nearly all litters sampled at about day 19 the foetal plasma corticosterone concentrations were higher than the maternal values.

The rat placenta has been suggested as a source of foetal steroids since at day 18 of gestation it contains 10–15% more corticosterone per unit fresh weight and 450% more total corticosteroids than the maternal adrenals (Ratsimamanga, Costes-Sodigne, Boisselot, Rabinowicz & Jacquand, 1962). However, the passage of these steroids into the foetal circulation is unlikely (Hagerman & Vilee, 1960). It is thus reasonable to assume that the high plasma corticosterone concentrations at day 19 are due to release of hormone from the steroid-loaded foetal adrenal gland, and Jost (1960) has presented biochemical evidence for pronounced pituitary–adrenal interactions during foetal days 18–19.

Holt & Oliver (1968) discussed problems associated with the hypothesis of glucocorticoid induction of tyrosine aminotransferase activity in the newborn animal that are raised by the data presented in this paper. Though the postnatal increase in corticosterone concentration (Table 1) lends support to such a hypothesis, other factors such as the release of glucagon stimulated by postnatal hypoglycaemia have been suggested as important additional factors in enzyme induction in the normal postnatal animal (Greengard & Dewey, 1967; Holt & Oliver, 1968). It has been suggested that the binding of corticosterone to its specific corticosteroid-binding globulin results in biologically inactive steroid (Slaunwhite & Sandberg, 1959; Daughaday, 1958;

Gala & Westphal, 1965), and in the present work it was only possible to measure total (unconjugated) corticosterone concentrations. However, Gala & Westphal (1965) have reported that the corticosterone-binding activity of rat plasma is 10–20% of the adult activity in the postnatal period 3–12 days. Thus increases in corticosterone concentration in the immediate postnatal period are probably reflected by increased tissue availability of the hormone.

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REFERENCES

- Daughaday, W. H. (1958). *Arch. intern. Med.* **101**, 286.
- Elliot, F. H., Birmingham, M. K., Schally, A. V. & Schönbäume, E. (1954). *Endocrinology*, **55**, 721.
- Gala, R. R. & Westphal, U. (1965). *Endocrinology*, **76**, 1079.
- Greengard, O. & Dewey, H. K. (1967). *J. biol. Chem.* **242**, 2986.
- Hagerman, D. D. & Vilee, C. A. (1960). *Physiol. Rev.* **40**, 313.
- Holt, P. G. & Oliver, I. T. (1968). *Biochem. J.* **108**, 333.
- Jost, A. (1960). *Harvey Lect.* no. 55, p. 201.
- Kamoun, A., Mialhe-Voloss, C. & Stutinsky, F. (1964). *C. R. Soc. Biol., Paris*, **158**, 828.
- Lin, E. C. & Knox, W. E. (1957). *Biochim. biophys. Acta*, **26**, 85.
- Litwack, G. & Nemeth, A. M. (1965). *Arch. Biochem. Biophys.* **109**, 316.
- Peron, F. G. (1962). In *Methods in Hormone Research*, vol. 1, p. 223. Ed. by Dorfman, R. I. New York and London: Academic Press Inc.
- Ratsimamanga, A. R., Costes-Sodigne, G., Boisselot, J., Rabinowicz, M. & Jacquand, S. (1962). *C. R. Soc. Biol., Paris*, **156**, 820.
- Saba, G. C. & Hoet, J. J. (1963). *Ann. Endocr., Paris*, **24**, 737.
- Sereni, F., Kenney, F. T. & Kretchmer, N. (1959). *J. biol. Chem.* **234**, 609.
- Slaunwhite, W. R., jun. & Sandberg, A. A. (1959). *J. clin. Invest.* **38**, 384.
- Yeung, D. & Oliver, I. T. (1967). *Biochem. J.* **105**, 1229.
- Yeung, D., Stanley, R. S. & Oliver, I. T. (1967). *Biochem. J.* **105**, 1219.