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Factors Affecting the Premature Induction of Tyrosine Aminotransferase in Foetal Rat Liver

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1. Premature delivery of foetal rats by uterine section results in the rapid appearance of tyrosine aminotransferase activity in foetal liver, after an initial lag period of 3–6hr. 2. The premature induction of activity is completely repressible by actinomycin D given soon after delivery and partially repressible by puromycin and amino acid analogues. 3. Glucagon injections into foetal rats *in utero* lead to production of tyrosine aminotransferase in the foetal liver, but adrenalin and noradrenalin are without effect. 4. Injections of glucose, galactose, fructose and mannose into prematurely delivered rats repress the development of tyrosine aminotransferase activity about 50% when they are given 2hr. after delivery, but glucose has no significant effect when injected at delivery. 5. The results are discussed in relation to current hypotheses on the role of hormones in enzyme induction in foetal development.

Tyrosine aminotransferase (EC 2.6.1.5) activity is normally absent from the foetal rat liver until shortly after birth and its postnatal development is dependent on the presence of the adrenal glands (Sereni, Kenney & Kretchmer, 1959). The enzyme activity can be induced in adrenalectomized newborn animals by injection of hydrocortisone.

Yeung, Stanley & Oliver (1967) have shown that tyrosine aminotransferase activity can be also induced in foetal liver *in utero* by direct administration of the fluorinated glucocorticoid analogue triamcinolone. The activity appears 5–10hr. after intraperitoneal administration of hormone to the foetal animals. Litwack & Nemeth (1965) reported that the development of tyrosine aminotransferase can be precociously induced in neonatal rabbit liver by premature delivery of the animals, and in the present paper it is shown that the same procedure applied to the rat foetus results in the appearance of high activity of the enzyme in the liver. The effects of actinomycin D, puromycin and amino acid analogues indicate that the premature enzyme development is the result of enzyme synthesis, and some aspects of the kinetics of development were investigated. Induction of the enzyme by administration of glucagon *in utero* has been reported by Greengard & Dewey (1967). Though these results have been confirmed in a qualitative sense, some properties of the system are at variance with the simple concept of glucagon as an initiator of enzyme synthesis in the physiological postnatal period, as proposed by Greengard & Dewey (1967).

MATERIALS AND METHODS

Chemicals. L-Tyrosine and sodium diethyldithiocarbamate were obtained from British Drug Houses Ltd. (Poole, Dorset) and the latter compound was recrystallized from water before use; α-oxoglutaric acid was from Sigma Chemical Co. (St Louis, Mo., U.S.A.); pyridoxine hydrochloride was from Nutritional Biochemical Corp. (Cleveland, Ohio, U.S.A.). *p*-Hydroxyphenylpyruvic acid was synthesized as described by Yeung *et al.* (1967). Hexoses, antibiotics, drugs and hormones were obtained from the same sources and dissolved as described by Yeung & Oliver (1968).

Animals. Wistar albino rats were used. Foetal ages were established by weight (Yeung *et al.* 1967), and surgery, experimental procedures and postnatal care were as described by Yeung & Oliver (1968).

Assay of tyrosine aminotransferase. Livers were washed in cold 0·9% NaCl, blotted on filter paper, weighed and homogenized in ice-cold 0·25M-sucrose (3ml./g. of liver). Enzyme activity was assayed in 0·1ml. samples of the homogenate by the method of Sereni *et al.* (1959), except that incubations were carried out for 0 and 15min. at 37°. In some experiments in which parallel determinations of phosphopyruvate carboxylase (EC 4.1.1.32) were made, enzyme activity was determined in 0·1ml. samples of the supernatant prepared by centrifugation of the homogenate at 210000*g* for 30min. at 4° in rotor no. 50 of the Spinco model L–2 preparative ultracentrifuge.

Determination of protein. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Samples were diluted 50-fold in 2% (w/v) Na_2CO_3–0·1N-NaOH and incubated overnight at 37° before assay.

RESULTS

Fig. 1 illustrates the rapid development in the liver of tyrosine aminotransferase activity that follows premature delivery of foetal rats. Though not evident in these data, there is a lag period in development that varies in duration with different litters. The lag period is always at least 3hr. and is sometimes as long as 5–6hr.

Table 1 illustrates the effect of starvation on the development of tyrosine aminotransferase in the liver of normally delivered rats. In the starved animals the enzyme activity appears more slowly than in their normally fed littermates.

The premature development of tyrosine aminotransferase in rat liver can be prevented completely by intraperitoneal injection of actinomycin D and partially prevented by puromycin and amino acid analogues given at the time of delivery (Table 2). A rather high dose of puromycin is necessary to obtain a significant effect.

When actinomycin D is injected at various times after delivery, its effectiveness in preventing enzyme development varies in a systematic way (Table 3). After an initial period during which actinomycin D completely stops enzyme production there is a second period in which the drug is less effective the later it is given.

The intraperitoneal injection of glucagon into foetal rats causes increased tyrosine aminotransferase activity in the foetal rat liver within 5hr. (Table 4). The average tyrosine aminotransferase activity in liver of normal or sham-operated foetuses is 7 (S.D. ± 3) μmoles/hr./g. wet wt. However, in about 15% of cases values as high as 20 μmoles/hr. were observed. For this reason saline-injected foetuses in one uterine horn were used as modified controls for hormone-injected animals in the other horn. Using similar experimental designs Yeung *et al.* (1967) and Yeung & Oliver (1968) have shown that some hormonal transfer occurs from the test to control group. Despite this disadvantage, the use of the paired-difference *t* test allows the statistical significance of the data to be assessed. The mean of the differences

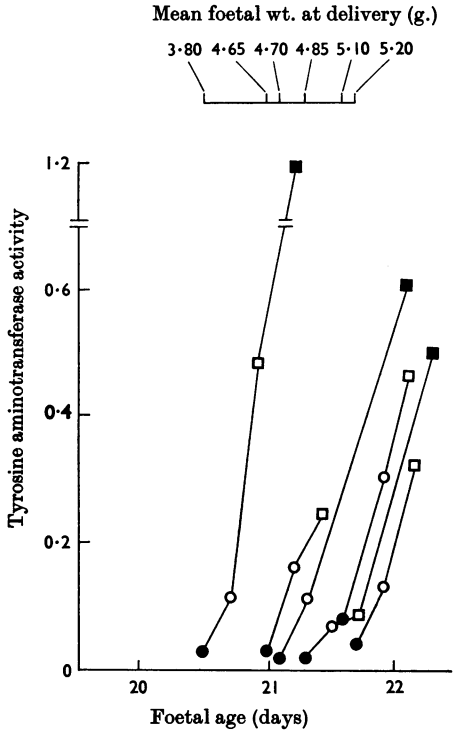

Fig. 1. Induction of hepatic tyrosine aminotransferase by premature delivery of foetal rats. Foetal animals were delivered by uterine section under ether anaesthesia of the mother, weighed and maintained in a humidicrib at 37° for various times until they were killed. They were killed in groups of two or three and tyrosine aminotransferase activity was determined in homogenates of the pooled livers. The values up to 24hr. after delivery were obtained from a single litter. The points corresponding to delivery are plotted against the age at delivery (shown at the bottom) and the weight (shown at the top). Enzyme activities are expressed as μmoles of *p*-hydroxyphenylpyruvate produced/hr./mg. of protein at 37°. ●, Activity at delivery; ○, activity 5hr. after delivery; □, activity 10hr. after delivery; ■, activity 24hr. after delivery.

Table 1. *Normal postnatal development of tyrosine aminotransferase in rat liver and the effect of starvation*

Animals were delivered by normal vaginal delivery. Half of each litter was removed from the mother after delivery of the whole litter was complete and maintained in the humidicrib without feeding. From each litter two animals were killed immediately after total delivery was complete and the pooled livers assayed for enzyme. Pairs of animals were killed at the times indicated and the enzyme activity was assayed. Six litters were used. Enzyme activities are expressed as mμmoles of *p*-hydroxyphenylpyruvate formed/hr./mg. of protein at 37°, and are presented as means ± S.E.M. of the numbers of determinations given in parentheses.

Time after birth (hr.)	Tyrosine aminotransferase activity			
	Fed		Starved	
0	81 ± 5·6	(9)	81 ± 5·6	(9)
5	447 ± 27	(5)	212 ± 29	(4)
10	313 ± 51	(5)	440 ± 39	(4)

Table 2. *Effects of puromycin, actinomycin D and amino acid analogues on the premature development of tyrosine aminotransferase in rats*

Animals were injected with drugs (test) or the same volume of 0·9% NaCl (control) at surgical delivery and maintained at 37° in the humidicrib until they were killed for liver assay. At least three livers were pooled for each assay. Enzyme activities are expressed as μmoles of *p*-hydroxyphenylpyruvate formed/hr./mg. of protein at 37°.

Treatment	Dose (μg./animal)	Time of assay (hr. after delivery)	Tyrosine aminotransferase activity		'Repression' (%)
			Test	Control	
Puromycin	175	4·0	0·49	0·56	13
	350	4·0	0·34	0·40	15
	350	5·5	1·07	1·09	0
	350	5·5	0·44	0·73	40
	700	6·0	0·20	1·00	80
	700	6·0	0·35	0·86	57
	700	6·0	0·46	0·77	40
Actinomycin D	3·5	5·0	0	0·58	100
	3·5	5·0	0	0·86	100
	3·5	5·0	0	1·15	100
Ethionine	1000	5·0	0·13	0·23	43
	1000	5·0	0·11	0·28	61
	2000	5·0	0·33	0·58	43
	2000	5·0	0·39	0·61	36
	3000	5·0	0·18	0·55	66
	3000	5·0	0·19	0·50	62
p-Fluorophenylalanine	2500	5·0	0·09	0·13	31
	2500	5·0	0·19	0·27	30
	2500	5·0	0·27	0·40	33
	2500	5·0	0·12	0·18	25
	2500	5·0	0·18	0·20	10

Table 3. *Kinetics of repression of tyrosine aminotransferase synthesis in liver of premature rats by actinomycin D*

For each experiment littermate foetuses were delivered by uterine section and injected in pairs with 20μl. of actinomycin D solution (3·5μg.) at the times stated. Two control animals received 20μl. of 0·9% NaCl at delivery. All animals in the litter were maintained continuously from delivery in a humidicrib at 37° and were all killed 2hr. after the final injection of actinomycin D. Specific activities of tyrosine aminotransferase were determined in the pooled livers from each pair of animals and the percentage repressions calculated as:

$$\left(1-\frac{\text{activity in test liver}}{\text{activity in control liver}}\right)\times 100$$

Time of injection (hr. after delivery)	Repression (%)			
	Expt. 1	Expt. 2	Expt. 3	Expt. 4
0	100	100	100	—
1	—	100	100	—
2	55	100	100	—
3	34	87	100	—
4		44	70	—
5			35	73
6·5				41
8				14

(11·3) is significant at the 2% level ($t = 3·049$, 10 degrees of freedom, $0·01 < P < 0·02$). A logarithmic transform to allow for different foetal ages yields a mean log(difference) value of 0·22, which is significant at the 1% level.

Intraperitoneal injection of adrenalin (5–10μg./foetus) and noradrenalin (10–20μg./foetus) failed to promote production of tyrosine aminotransferase in foetal rat liver *in utero*. The foetuses were exposed to the catecholamines for between 3 and 10hr. in the experiments.

Glucose injected immediately after birth and again 4hr. later has no effect on the premature development of tyrosine aminotransferase. This regime for glucose administration is similar to that used by Greengard & Dewey (1967), and the data of Yeung & Oliver (1968) indicate that the procedure prevents postnatal hypoglycaemia. However, when glucose is given 2hr. and 6hr. after delivery only about 50% of the activity found in control animals develops. The other sugars show similar effects when given in this regime (Table 5). Galactose and fructose were without effect on enzyme development when given immediately after delivery, whereas mannose was just as effective in this regime when given immediately as later.

DISCUSSION

Yeung *et al.* (1967) showed that the administration of triamcinolone to foetal rats *in utero* results in the development of tyrosine aminotransferase in the foetal liver provided that the gestational age exceeds 19 days and the time of exposure to the hormone exceeds 5hr. It was suggested that the activation of a fundamental trigger mechanism at that time is necessary for hormonal induction of enzymic activity. As reported here, premature delivery of animals as young as 20·5 days also results in the development of enzymic activity, but it was not possible to test the trigger hypothesis further by this technique because younger animals do not survive after surgical delivery. The amount of activity that develops after premature delivery is quantitatively similar to that appearing in normally delivered full-term animals. When full-term animals are starved, as are the premature animals, the enzyme activity develops more slowly but still reaches normal values.

The effects of actinomycin D, puromycin and amino acid analogues (Table 2) indicate that the premature appearance of enzyme activity is the result of enzyme synthesis. Greengard, Smith & Acs (1963) also found that the normal postnatal development of tyrosine aminotransferase is blocked by actinomycin D given soon after birth (see also Greengard, 1963), and Kenney (1963) has most rigorously shown that the corticosterone-induced activity in the adult animal is a result of new enzyme synthesis.

Parallel studies with puromycin have been made on the premature development of phosphopyruvate carboxylase (Yeung & Oliver, 1968). At the lowest dose described by these authors little effect on the tyrosine aminotransferase system could be found, whereas the phosphopyruvate carboxylase system was maximally sensitive. This was clearly seen when both enzymes were measured in the same puromycin-treated animals and controls. At higher doses of puromycin the development of tyrosine aminotransferase activity is markedly blocked, and thus the two liver enzyme systems seem to possess different orders of sensitivity to puromycin.

The variable lag period in the appearance of tyrosine aminotransferase activity in premature

Table 4. *Premature induction of tyrosine aminotransferase in foetal rats* in utero *by glucagon*

Foetuses in one uterine horn were injected intraperitoneally with 25 μg. of glucagon in 25 μl. of solution (test) and animals in the other horn with the same volume of 0·9% NaCl (control). After 5hr. the animals were removed from the uterus and killed. Enzyme assays were carried out on pooled liver homogenates from each group of at least four animals. Enzyme activities are expressed as μmoles of *p*-hydroxyphenylpyruvate produced/hr./g. wet wt. of liver.

Mean foetal wt. (g.)	Approx. foetal age (days)	Tyrosine aminotransferase activity: Test	Control
1·95	19	24·5	5·2
2·1	19	35·0	33·5
4·0	20·8	19·0	11·1
4·0		54·0	27·7
4·0		84·0	49·5
4·45	21	5·6	7·0
4·5		11·1	7·8
4·6		10·0	7·8
5·0	21·5	35·0	12·2
5·0		14·3	7·7
5·2		5·5	4·5
	Mean	27·1	15·8
	Mean of differences	11·3	
	Paired-difference *t* test	$0·01 < P < 0·02$	

Table 5. *Effects of sugars on the premature development of tyrosine aminotransferase in rats*

Littermate foetuses were delivered by uterine section and injected intraperitoneally with 50mg. of the sugar in 100 μl. of water (test) or 100 μl. of 0·9% NaCl (control) at the times given. They were maintained in the humidicrib at 37° for 8hr. after delivery and then killed. Tyrosine aminotransferase activity was measured in liver homogenates prepared from individual animals. Enzyme activities are expressed as μmoles of *p*-hydroxyphenylpyruvate formed/hr./g. wet wt. of liver and are presented as means ± S.E.M. of the numbers of determinations given in parentheses.

Sugar	Time of injection (hr. after delivery)	Tyrosine aminotransferase activity 8hr. after delivery: Test		Control
Glucose	0 and 4	26·2 ± 1·9 (9)	$P \simeq 0·70$	27·1 ± 1·8 (10)
Glucose	2 and 6	35·8 ± 3·0 (16)	$P < 0·005$	56·9 ± 5·8 (17)
Galactose	2 and 6	26·6 ± 2·0 (9)	$P < 0·001$	49·8 ± 3·1 (9)
Fructose	2 and 6	39·3 ± 4·2 (8)	$P < 0·001$	84·5 ± 9·2 (8)
Mannose	2 and 6	24·1 ± 4·6 (9)	$P < 0·01$	65·3 ± 12·2 (9)

animals is similar to the lag period that occurs during the normal postnatal development of the enzyme (Sereni *et al.* 1959). The time-dependence of the effect of actinomycin D given after delivery of the animals shows that drug-sensitive events essential for enzyme synthesis occur during the lag period (Table 3). Actinomycin D inhibits DNA-directed RNA synthesis (see Reich & Goldberg, 1964), but in rat liver and some other animal tissues the drug is a more potent inhibitor of synthesis of ribosomal RNA than of DNA-like RNA, presumably messenger RNA (Harel, Harel, Boer, Imbenotte & Carpeni, 1964; Tata, 1966, 1967). Recent work reviewed by Tata (1966) suggests that messenger RNA is transported from the cell nucleus in association with ribosomal precursors and that precoded polysomes appear in the cytoplasm. The formation of such complexes would thus be inhibited by actinomycin D. If such entities specific for the synthesis of tyrosine aminotransferase exist in foetal rat liver before the arrival of the birth-associated inductive stimulus, then actinomycin D should have little effect on subsequent enzyme synthesis unless it also inhibits translation on the polysome. However, actinomycin D completely suppresses enzyme synthesis when given at or soon after birth and it is less effective the later it is given. It seems unlikely that pre-existing polysomes specific for tyrosine aminotransferase exist and their synthesis must be subsequent to the enzyme-inductive stimulus. It also follows from this deduction that specific polysome synthesis is unlikely to be associated with the 19-day trigger discussed above. Actinomycin D is rapidly concentrated in liver (in Reich & Goldberg, 1964), and results of Yeung & Oliver (1968) suggest that in premature rats this takes no more than 1hr. after intraperitoneal injection. The results of Table 3 cannot therefore be due to the time of absorption and accumulation of the drug in the liver.

Greengard & Dewey (1967) reported that intraperitoneal injection of glucagon to foetal rats *in utero* results in the appearance of tyrosine aminotransferase activity and increases in the activities of glucose 6-phosphatase (EC 3.1.3.9) and serine dehydratase (EC 4.2.1.13). Similar results with tyrosine aminotransferase are presented in Table 4. Yeung & Oliver (1968) report a similar induction of phosphopyruvate carboxylase *in utero*. However, adrenalin and noradrenalin induce the carboxylase in foetal liver *in utero*, but fail to induce the aminotransferase, despite prolonged exposure. Greengard & Dewey (1967) suggested that postnatal hypoglycaemia stimulates glucagon release from the pancreas and that glucagon then induces enzyme activity. This hypothesis was strengthened by their report that postnatal injection of glucose decreases the normal postnatal development of both tyrosine aminotransferase and serine dehydratase. Yeung & Oliver (1968) showed that various sugars repress the premature development of phosphopyruvate carboxylase activity. The glucose regimes used by them to prevent the transient postnatal phase of hypoglycaemia were also investigated here for their effects on the tyrosine aminotransferase system (Table 5). No effect on the development of enzyme was demonstrated in animals injected immediately after delivery. However, if the animals were allowed to reach their maximal postnatal hypoglycaemia (2hr. after delivery) before receiving glucose, then subsequent enzyme development was strongly repressed. Further, galactose and fructose, which Yeung & Oliver (1968) showed to have no significant effect on postnatal blood glucose concentrations, also repress tyrosine aminotransferase development when given 2hr. after delivery, and have little or no effect when given immediately on delivery. The results of Greengard & Dewey (1967) were obtained on normal newborn animals with the result that some of the animals were at least 2hr. postnatal when injected with glucose. This may explain the discrepancy because a whole litter of premature animals can be delivered and injected within 15min. It is thus difficult to accept the hypothesis of Greengard & Dewey (1967) in unmodified form. The complete suppression of hypoglycaemia with glucose leads to no repression of enzyme development. The other sugar regimes, though they do not prevent the initial hypoglycaemia, perhaps suppress the release of glucagon. Even if this is so further difficulties with the glucagon hypothesis are the induction of tyrosine aminotransferase activity *in utero* by triamcinolone (Yeung *et al.* 1967), the failure of adrenalectomized newborn rats to produce the enzyme and the induction of the enzyme by hydrocortisone in adrenalectomized animals (Sereni *et al.* 1959).

In foetal plasma, the corticosterone concentration is high at day 19 of gestation but low from day 20 to term; it is highly probable that concentrations before day 19 are quite as low as in the immediate prenatal period (Holt & Oliver, 1968). The data of Greengard & Dewey (1967) show that glucagon does not induce tyrosine aminotransferase in foetuses younger than 19 days, and the data in Table 3 suggest that glucagon induction is most effective when the corticosterone concentrations are high at about day 19 or 20 and is less effective after day 21. The corticosterone concentrations rise again soon after birth and the physiological induction of the enzyme may be due to synergistic actions of steroid hormones and glucagon.

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REFERENCES

Greengard, O. (1963). In *Advances in Enzyme Regulation*, vol. 1, p. 61. Ed by Weber, G. London and New York: Pergamon Press Ltd.

Greengard, O. & Dewey, H. K. (1967). *J. biol. Chem.* **242**, 2986.

Greengard, O., Smith, M. A. & Acs, G. (1963). *J. biol. Chem.* **238**, 1548.

Harel, L., Harel, J., Boer, A., Imbenotte, J. & Carpeni, N. (1964). *Biochim. biophys. Acta*, **87**, 212.

Holt, P. G. & Oliver, I. T. (1968). *Biochem. J.* **108**, 339.

Kenney, F. T. (1963). In *Advances in Enzyme Regulation*, vol. 1, p. 137. Ed by Weber, G. London and New York: Pergamon Press Ltd.

Litwack, G. & Nemeth, A. M. (1965). *Arch. Biochem. Biophys.* **109**, 316.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.

Reich, E. & Goldberg, I. H. (1964). *Progr. Nucleic Acid Res. molec. Biol.* **3**, 183.

Sereni, F., Kenney, F. T. & Kretchmer, N. (1959). *J. biol. Chem.* **234**, 609.

Tata, J. R. (1966). *Progr. Nucleic Acid Res. molec. Biol.* **5**, 191.

Tata, J. R. (1967). *Biochem. J.* **104**, 1.

Yeung, D. & Oliver, I. T. (1968). *Biochem. J.* **108**, 325.

Yeung, D., Stanley, R. S. & Oliver, I. T. (1967). *Biochem. J.* **105**, 1219.