Studies on the Mechanism of Induction of Tyrosine Aminotransferase in Neonatal Rat Liver*

P. G. Holt and I. T. Oliver†

ABSTRACT: Postnatal induction of tyrosine aminotransferase (L-tyrosine 2-oxoglutarate aminotransferase EC 2.6.1.5) in rat liver is prevented by adrenalectomy, and hydrocortisone induction in such animals is prevented by actinomycin D. Glucagon injection to fetal rats *in utero* induces enzyme only when endogenous adrenal steroid levels are high but catecholamines and 3′,5′-cyclic adenosine monophosphate do not induce activity.

Normal postnatal synthesis of the enzyme is slightly repressed by ergotamine tartrate, prostaglandin, insulin, and pyridoxine and is increased by repeated injection of glucagon or 3′,5′-cyclic adenosine monophosphate. Insulin and pyridoxine induce increased activity in 2-day postnatal animals. In 4-hr and 2–6-day postnatal animals induction of enzyme activity by 3′,5′-cyclic adenosine monophosphate is insensitive to actinomycin D and induction by epinephrine in 2–6-day animals is only marginally sensitive to the antibiotic. Thus, hormonal induction of tyrosine aminotransferase mediated through 3′,5′-cyclic adenosine monophosphate appears to operate at the level of translation in enzyme synthesis. It is suggested that apparent multiple inductive pathways for tyrosine aminotransferase in both fetal and adult animals may be explained in terms of the discrete induction of different forms of the enzyme.

The physiological induction of tyrosine aminotransferase (L-tyrosine 2-oxoglutarate aminotransferase EC 2.6.1.5) activity in neonatal rat liver appears to be triggered by changes in the animal resulting from birth, since enzyme activity develops within a few hours of delivery, both in naturally born full-term animals (Sereni *et al.*, 1959) and in premature animals delivered by uterine section (Holt and Oliver, 1968a; see also Litwack and Nemeth, 1965, for similar results in the rabbit). Actinomycin D and puromycin prevent the development of activity that follows both premature delivery (Holt and Oliver, 1968a) and natural birth (Greengard *et al.*, 1963) and hence the process can be regarded as induction of enzyme synthesis. The physiological mechanism of induction is undoubtedly complex since glucocorticoids (Sereni *et al.*, 1959; Yeung *et al.*, 1967) and glucagon (Greengard and Dewey, 1967; Holt and Oliver, 1968a) have been implicated in the process in neonatal animals; hydrocortisone, glucagon, insulin, epinephrine, and 3′,5′-cyclic AMP have been shown to have effects in organ cultures of fetal liver (Wicks, 1968a,b) and hydrocortisone, insulin, and glucagon have been shown to increase enzyme synthesis in adult rats (Kenney, 1963; Civen *et al.*, 1967; Csányi *et al.*, 1967; Holten and Kenney, 1967) and in the isolated perfused liver (Hager and Kenney, 1968). It was suggested by Greengard and Dewey (1967) that glucagon was the inducer of enzyme synthesis in the new-born rat and that release of the hormone was potentiated by postnatal hypoglycemia, but Holt and Oliver (1968a) showed in experiments with prematurely delivered animals that this simple hypothesis would not explain all the characteristics of the induction. In experiments on the induction of hepatic phosphopyruvate carboxylase (EC 4.1.1.32) in neonatal rat liver, Yeung and Oliver (1968a,b) have recently shown that 3′,5′-cyclic AMP is the intracellular inducer *in vivo*. In view of these results the role of 3′,5′-cyclic AMP in the postnatal development of tyrosine aminotransferase activity has been investigated. The cyclic nucleotide does not appear to be an inducer of enzyme synthesis at the gene level *in vivo* but rather to exert effects at a distal stage in protein synthesis, possibly at the level of translation. In addition, the complexity of the physiological system is further illustrated by age-dependent responses to some other agents.

Experimental Procedure

Chemicals. L-Tyrosine and sodium diethyldithiocarbamate were obtained from British Drug Houses Ltd., Poole, Dorset, England. Glucagon was obtained from Eli Lilly & Co., Indianapolis, Ind. Insulin was from Boots Pure Drug Co., Ltd., Nottingham, England. Epinephrine tartrate was from Burroughs Welcome & Co., Ltd., Sydney, Australia. Pyridoxine-HCl was from Nutritional Biochemicals Corp., Cleveland, Ohio; hydrocortisone hemisuccinate from the Upjohn Co., Kalamazoo, Mich.; prostaglandin PGE_1 was a gift from Professor S. Bergstrom, Karolinska Institute, Stockholm, Sweden.

N^6,2′-*O*-Dibutyryl 3′,5′-cyclic AMP and 3′,5′-cyclic

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† To whom to address reprint requests.

TABLE I: The Effect of Adrenalectomy on Postnatal Development of Tryosine Aminotransferase.[a]

Treatment	Tyrosine Aminotransferase Act. (μmoles of *p*-Hydroxyphenylpyruvate formed/g of Liver per hr)	
	Expt 1	Expt 2
None	92.0	37.7
Adrenalectomized	23.3	11.1
Adrenalectomized + hydrocortisone	88.0	38.8
Adrenalectomized + actinomycin D + hydrocortisone	26.7	12.0

[a] Litters of six animals were delivered by uterine section and after a few minutes placed in crushed ice. Three animals were adrenalectomized and the whole litter was then placed in a humidicrib at 37°; 4 hr later, the adrenalectomized animals were injected with hydrocortisone (0.25 mg in 25 μl of 0.145 M NaCl), the same dose of hydrocortisone together with actinomycin D (3.5 μg), or saline alone. All animals were killed 8 hr after delivery and the activity of tyrosine aminotransferase was determined in the liver. The livers from three animals which received no treatment were pooled for assay.

TABLE II: The Effect of Ergotamine Tartrate on the Postnatal Development of Tyrosine Aminotransferase.[a]

Expt	Test	Tyrosine Aminotransferase Act. (μmoles of *p*-hydroxyphenylpyruvate Formed/g of Liver per hr)	
		Control	% Repression
1	46.6	53.2	12
2	34.5	39.5	13
3	22.2	28.0	21
			Mean 15.3
			Std error 2.7

[a] Animals were injected at surgical delivery with 3 μg of ergotamine tartrate (test) in 15 μl of 0.145 M NaCl or with the same volume of saline (control). They were maintained for 5 hr in the humidicrib at 37° and then killed. Tyrosine aminotransferase activity was determined in pooled liver homogenates obtained from three animals. Each experiment was on a single litter.

forms of IMP, UMP, GMP, CMP, and TMP were obtained from C. F. Boehringer und Soehne, G.m.b.H., Mannheim, Germany; 3′,5′-cyclic AMP, 2′,3′-cyclic AMP, 5′-AMP, ergotamine tartrate, α-oxoglutarate, and *p*-hydroxyphenylpyruvate from the Sigma Chemical Co., St. Louis, Mo.

Animals. Rats of the Wistar albino strain of *Rattus norwegicus* were used. Fetal ages were established from a fetal weight–age curve on the colony (Yeung *et al.*, 1967). Surgical procedures, premature delivery by uterine section, and postnatal care of animals were as previously described (Yeung *et al.*, 1967; Yeung and Oliver, 1967). Premature animals were delivered within 1.5 days of term. Adrenalectomy was performed on neonatal animals through a ventral incision and anaesthesia was by hypothermia in ice.

Enzyme Assay. Livers were excised from animals killed by cervical fracture and washed in ice-cold 0.145 M NaCl. They were blotted on filter paper, weighed, and homogenized in ice-cold 0.25 M sucrose (3 ml/g of liver). Enzyme activity was assayed in 0.1-ml aliquots of the homogenate by the method of Sereni *et al.* (1959).

Results

Figure 1 shows the activity of tyrosine aminotransferase in the liver of fetal rats, 5 hr after the intraperitoneal injection of 25 μg of glucagon per fetus *in utero*. A detectable effect of glucagon is apparent only in the fetal weight range 2.0–4.0 g where 60% of the glucagon-treated litters exceed the value of the mean (plus 1 std dev) of sham-operated or normal control fetuses. In animals less than 2 g in weight there appears to be no effect, while in animals heavier than 4 g in weight the effect is only marginal. When the dose of glucagon was reduced to 12 μg or less no effect on the enzyme activity could be detected.

TABLE III: The Effect of Insulin on the Activity of Tyrosine Aminotransferase in Premature Rats.[a]

		Tyrosine Aminotransferase Act. (μmoles of *p*-Hydroxyphenylpyruvate/g of Liver per hr)	
		Insulin Treated	Control
Mean		24.7	35.7
Number of litters	11		
Mean of differences[b]	7.79 ± 2.90		
Paired-difference *t*-test	$0.02 < P < 0.025$		

[a] Animals were delivered by uterine section and maintained in a humidicrib at 37° after injection. Four animals from each litter were injected with 2 munits of insulin (intraperitoneal) and four more animals from the litter were used as controls; 5 hr after delivery the animals were killed and tyrosine aminotransferase activity was assayed in pooled liver homogenates prepared from the animals in each group. [b] The differences (control − insulin) were calculated for each litter and the significance of the deviation from zero was calculated by the paired-difference *t* test. The mean of the differences is given plus or minus standard error of the mean.

TABLE IV: The Effect of Pyridoxine on Postnatal Development of Tyrosine Aminotransferase.[a]

	Tyrosine Aminotransferase Act. (μmoles of *p*-Hydroxyphenylpyruvate Formed/mg of Protein per hr)			
	Pyridoxine before Delivery[b]		Pyridoxine at Delivery[c]	
hr after Delivery	Pyridoxine	Control	Pyridoxine	Control
0	0.06	0.06	0.12	0.12
5	0.30	0.51	0.31	0.35
10	0.48	0.99	0.38	0.61

[a] Fetuses were delivered by uterine section and maintained in the humidicrib at 37°. At the times stated they were killed and the liver was assayed for tyrosine aminotransferase activity. [b] Fetuses in one uterine horn received 3 mg of pyridoxine by intraperitoneal injection *in utero* 12 hr before delivery. The animals in the other horn received 0.145 M NaCl (control). Livers from two animals were pooled for each assay. [c] Half the animals in a litter delivered by uterine section received pyridoxine (3 mg); the other animals received saline (control). Livers from two animals were pooled for each assay. The results presented are representative of two experiments with each regime.

In fetal rats of 2.0–4.0 g weight, the intraperitoneal injection of 3′,5′-cyclic AMP, or N^6,2′-*O*-dibutyryl cyclic AMP, at doses between 0.14 and 0.42 μmole per fetus *in utero* was without effect on enzyme activity within 5 hr. The lower dose of 3′,5′-cyclic AMP permanently induces phosphopyruvate carboxylase activity in the liver of fetal rats (Yeung and Oliver, 1968b). Injection of a mixture of the 3′,5′-cyclic forms of the nucleotides IMP, UMP, GMP, CMP, and TMP (0.14 μmoles of each) was without effect on tyrosine aminotransferase activity under the conditions described above.

As first reported by Sereni *et al.* (1959), the data of Table I confirm that adrenalectomy of prematurely delivered animals largely prevents the postnatal synthesis of tyrosine aminotransferase and that the hydrocortisone-mediated increase in activity is prevented by simultaneous injection of actinomycin D. This experiment was carried out to confirm that the effect of steroid in this situation is a result of DNA-directed RNA synthesis and not of the translation of preformed message.

Table II shows a small repressive effect of ergotamine tartrate on the postnatal development of tyrosine aminotransferase in liver of premature rats. Figure 2 shows a similar small effect of prostaglandin PGE_1 on postnatal development of hepatic tyrosine aminotransferase. The anomalous results at higher doses have been observed with other liver systems (*e.g.*, see Yeung and Oliver, 1968b). Glucagon injected in a single dose (5 μg/g body weight) at delivery has no effect on the development of the enzyme but a repeated dose elevates the activity subsequently found (Figure 3).

The injection of 3′,5′-cyclic AMP into surgically delivered rats results in elevated enzyme activity and the dibutyryl derivative is even more effective (Figure 4). The mixture of cyclic nucleotides used in fetal animals was without effect on enzyme activity, as was 2′,3′-cyclic AMP and 5′-AMP.

Injection of either insulin or pyridoxine at delivery

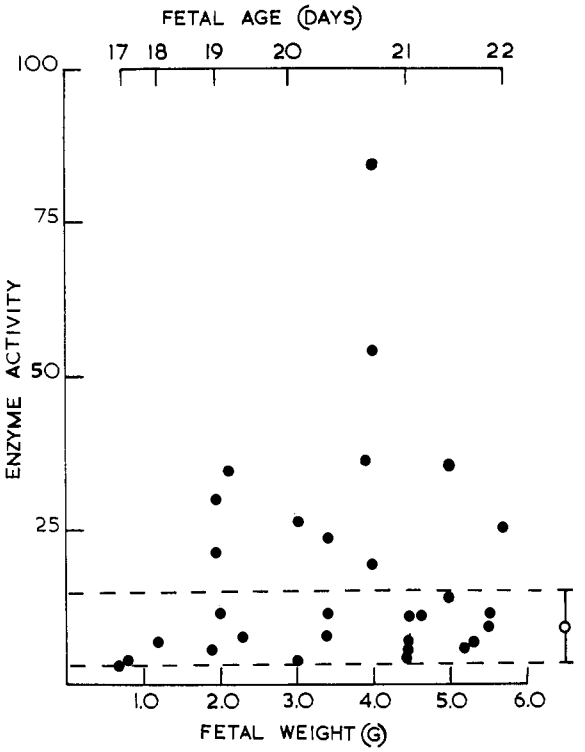

FIGURE 1: The effect of glucagon on tyrosine aminotransferase activity in the liver of fetal rats *in utero*. Fetal rats were injected intraperitoneally with 25 μg of glucagon in 25 μl of 1.6% glycerin–0.2% phenol. After 5 hr the fetuses were removed from the uterus and the enzyme was assayed in liver homogenates. Normal fetuses (○); the vertical bars represent ±1 std dev of the mean value obtained from 30 litters distributed over the age range 19–22 days. Glucagon treatment (●); each point was obtained from pooled livers from four to six animals of the same litter.

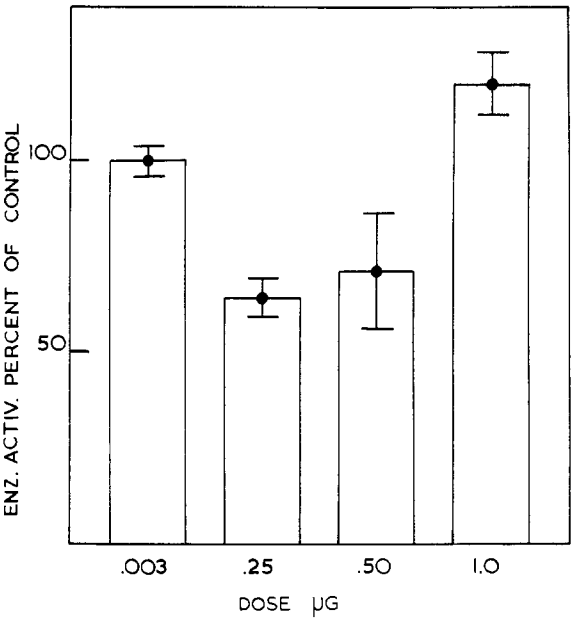

FIGURE 2: The effect of prostaglandin on the postnatal development of tyrosine aminotransferase in rat liver. Animals were injected at surgical delivery with prostaglandin PGE_1 and maintained in a humidicrib at 37° together with the saline-injected controls of the same litter; 5 hr after delivery all animals were killed and the enzyme was assayed in pooled liver homogenates obtained from four animals. The data were compared by assigning an arbitrary value of 100% to all controls. Experiments at each dose were repeated three times. The vertical bars represent ±1 std dev of the mean of the relative enzyme activity.

TABLE V: The Effect of Actinomycin D on the Increase in Tyrosine Aminotransferase Activity Mediated by Cyclic AMP[a] in Neonatal Rat Liver *in Vivo*.[b]

Treatment	hr after Delivery		Tyrosine Aminotransferase Act. (μmoles of *p*-Hydroxyphenylpyruvate/g of Liver per hr)	
	Injection	Assay	Expt 1	Expt 2
1. None		4	24.5	12.0
2. 0.145 M NaCl	4	7	55.6	27.5
3. Actinomycin D	4	7	24.5	12.0
4. Cyclic AMP	4	7	104.2	52.0
5. Cyclic AMP + actinomycin D	4	7	76.0	42.3
Activity Increment due to Cyclic AMP				
−Actinomycin D (4 − 2)			48.6	24.5
+Actinomycin D (5 − 3)			51.5	30.3

[a] In these experiments N^6,2′-*O*-dibutyryl 3′,5′-cyclic AMP was used. [b] In each experiment a litter of fetal rats was delivered by uterine section and maintained in a humidicrib at 37°. At 4 hr after delivery, duplicate animals were injected with 0.145 M NaCl, actinomycin D (3.5 μg), dibutyryl cyclic AMP (50 μg), or actinomycin D together with dibutyryl cyclic AMP. Another pair were killed for enzyme assay. The injected animals were maintained in the humidicrib for a further 3 hr and killed for enzyme assay.

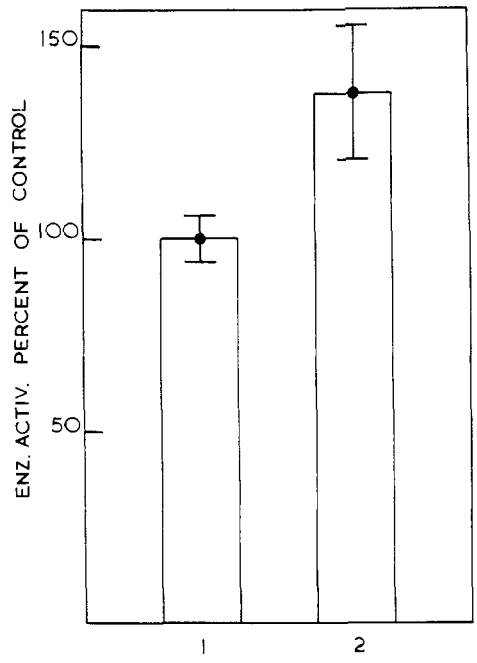

FIGURE 3: The effect of glucagon on the postnatal development of tyrosine aminotransferase in rat liver. Animals in expt 1 and 2 were injected at surgical delivery with 25 μg of glucagon, and in expt 2 the dose was repeated 2.5 hr later. Experimental and control animals from the same litter were maintained 5 hr in the humidicrib at 37° and then killed. Tyrosine aminotransferase activity was assayed in pooled liver homogenates from four animals. Data were calculated as in Figure 2. Each experiment was repeated three times and the vertical bars represent ± 1 std dev of the mean of the relative enzyme activity.

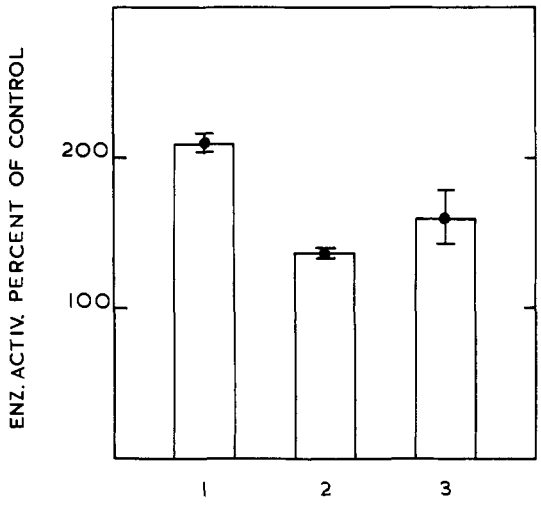

FIGURE 4: The effect of 3′,5′-cyclic AMP on the postnatal development of tyrosine aminotransferase in rat liver. In expt 1 and 2, surgically delivered animals were injected intraperitoneally with 50 μg of 3′,5′-cyclic AMP; in expt 1, this dose was repeated 2 hr later; in expt 3, N^6,2′-*O*-dibutyryl 3′,5′-cyclic AMP (50 μg) was used at delivery only. Experimental and litter-mate control animals were maintained for 5 hr in the humidicrib at 37° and killed. Tyrosine aminotransferase activity was determined in pooled liver from four animals. Data were calculated as in Figure 2. Each experiment was repeated four times; the vertical bars represent ± 1 std dev of the mean of the relative enzyme activity.

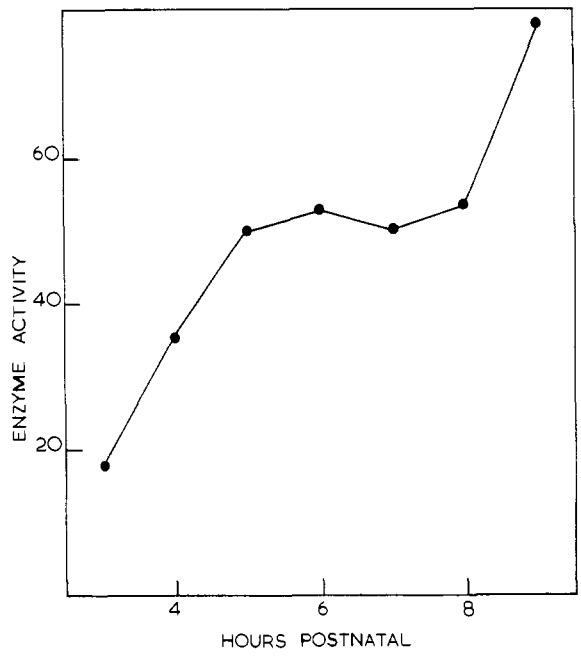

FIGURE 5: The time course of postnatal development of tyrosine aminotransferase in rat liver. A litter of animals was delivered by uterine section and maintained in the humidicrib at 37° and single animals were killed at various times after delivery. Tyrosine transferase activity was determined in the liver homogenates.

brings about repression of enzyme synthesis (Tables III and IV) but the effect of insulin is extremely variable in different experiments. The insulin effect is significant at the 2% level when the data are analyzed by the paired-difference *t* test.

In Table V it is shown that actinomycin D has no effect on the increase in tyrosine aminotransferase activity brought about by dibutyryl cyclic AMP in the neonatal rat. The results from only two experiments are shown but essentially similar results were obtained in a total of four experiments. Experimental procedures were begun 4 hr after delivery in order to allow the initiation of DNA-directed enzyme synthesis. There is a pronounced but variable lag phase in the appearance of enzyme activity in postnatal rat liver (Sereni *et al.*, 1959) and previous experiments with actinomycin D have shown that a similar lag occurs in the DNA-directed synthesis of RNA (Holt and Oliver, 1968a).

The effects of insulin and pyridoxine on enzyme synthesis in newborn rats (Tables III and IV) were further tested as a function of postnatal age; Table VI shows that as early as the second day postpartum these effects are reversed.

Epinephrine injection in newborn rats at delivery is without detectable effect on the development of enzyme activity but in 2–6-day-old rats 3–10-fold increases in activity rapidly follow injection of the hormone (Table VII). When actinomycin D is administered 30 min prior to epinephrine, there still follows a 2.5–6-fold rise in activity. Actinomycin D alone did not reduce the enzyme activity in these experiments. Previous experiments have shown that actinomycin D is effective in

TABLE VI: The Effect of Insulin and Pyridoxine on the Level of Tyrosine Aminotransferase in the Liver of 2-Day Rats.[a]

		Tyrosine Aminotransferase Act. (μmoles of *p*-Hydroxyphenylpyruvate/g of Liver per hr)	
Expt	Control	Insulin Treated	Pyridoxine Treated
1	6.7	10.1	37.9
2	7.8	17.1	32.3
3	20.0	37.9	45.7

[a] Rats (2-day old) were maintained in a humidicrib at 37°. Groups of three animals were injected with insulin (2 munits), pyridoxine (5 mg), or saline and killed 5 hr later for enzyme assay. Tyrosine aminotransferase was assayed in pooled liver homogenates prepared from each group. Each experiment used a single litter of animals.

TABLE VII: Effect of Epinephrine and Actinomycin D on Tyrosine Aminotransferase Activity in Liver of Postnatal Rats.[a]

		Tyrosine Aminotransferase Act. (μmoles of *p*-Hydroxyphenylpyruvate/g of Liver per hr)	
Postnatal Age (days)	Control	Epinephrine	Epinephrine + Actinomycin D
2	42.3	134	
2	11.1	58	60
2	17.8	44	40
2	15.6	111	56
3	25	107	60
5	15.6	138	65
6	20	80	56

[a] Litter-mate animals were injected intraperitoneally with actinomycin D (0.7 μg/g) 30 min prior to epinephrine (2 μg/g) or epinephrine alone (2 μg/g). Controls received no treatment; 5 hr after the epinephrine injection all animals were killed for enzyme assay. A total of 16 experiments with litters of the age range shown were performed but representative results only are presented. Tyrosine aminotransferase activity was assayed in liver homogenates prepared from two animals in each group.

preventing enzyme synthesis for periods well in excess of 5 hr when injected into newborn rats. This experiment was also repeated in 16 litters over the same age range with epinephrine replaced by dibutyryl 3′,5′-cyclic AMP (0.04 μmole/g body weight). The cyclic nucleotide resulted in a mean increase in enzyme activity of 92 ± 17% (standard error of the mean) and this was reduced to only 83 ± 17% when actinomycin D was also administered.

The time course of enzyme development in postnatal animals has been followed at close intervals using individual litters which were age-synchronized by surgical delivery. With this technique an entire litter can be delivered in about 10 min and thus it is to be expected that all animals in the litter will proceed through the physiological events which trigger enzyme synthesis at about the same time. In at least 50% of such litters, the time course of enzyme development proceeds in a biphasic fashion. Figure 5 shows a typical case.

Studies on the intracellular distribution of the enzyme in homogenates prepared in 0.25 M sucrose and 0.15 M KCl showed that 20–30% of the total activity was in the pellet obtained after centrifugation at 25,000*g* for 1 hr or 100,000*g* for 1 hr. Sonication of the homogenate did not improve the yield of supernatant enzyme. The percentage of particulate enzyme does not change during postnatal development (newborn, 14-days postnatal). All assays were done on homogenates.

Discussion

The postnatal, birth-related appearance of tyrosine aminotransferase activity in rat liver appears to be due to induction of enzyme synthesis *de novo*. The appearance of activity can be blocked by both puromycin and actinomycin D (Greengard *et al.*, 1963; Holt and Oliver, 1968a) and is thus due to protein synthesis involving both translation on the polysome and the transcription of DNA into RNA specific for enzyme synthesis whether this be mRNA, rRNA, tRNA, or all

three. The physiological inducer has been characterized as a corticosteroid since adrenalectomized newborn rats fail to produce enzyme unless given hydrocortisone (Sereni *et al.*, 1959). Furthermore, the hydrocortisone-stimulated synthesis is completely prevented by actinomycin D (Table I), indicating that the hormone affects DNA-directed RNA synthesis, essential for the production of enzyme. Triamcinolone injection to fetuses results in development of enzyme activity *in utero* (Yeung *et al.*, 1967), hydrocortisone stimulates enzyme production in fetal liver explants in organ culture (Wicks, 1968a), and the same hormone stimulates enzyme production *in vivo* in adult rat liver (Lin and Knox, 1957; Kenney, 1963). Furthermore, in the newborn rat, Holt and Oliver (1968b) have demonstrated a rapid rise in the level of plasma corticosterone which occurs within 5 hr of delivery. However, in the rat fetus the plasma levels of corticosterone during gestational days 19–20.5 are also high (Holt and Oliver, 1968b), and it is therefore difficult to account for the absence or at best very low levels of enzyme in the fetal liver. However, if the enzyme inductive process responds to a threshold level of corticosterone this may not be reached in the fetal animal. Alternatively the level of hormone may not be prolonged above the threshold for a sufficient period to trigger induction due to placental removal of the endogenous hormone. Induction by triamcinolone occurs in the fetal animal only after long exposure (Yeung *et al.*, 1967).

Wicks (1968a) has suggested that the enzyme may be repressed during uterine development by growth hormone, but in this laboratory no effect on postnatal enzyme development was found after injection of growth hormone (3 μg/animal).

In recent papers it has been reported that glucagon stimulates the production of tyrosine aminotransferase activity in fetal rat liver (Greengard and Dewey, 1967; Holt and Oliver, 1968a) and in fetal liver explants in culture (Wicks, 1968a). However, in parallel experiments on the glucagon induction of phosphopyruvate carboxylase activity in fetal rat liver (Yeung and Oliver, 1968a) it became apparent that the effect on the tyrosine aminotransferase system was much less consistent and that it could be evoked only at high hormone dosage. This led to a reexamination of the glucagon effect and the data of Figure 1 and of Holt and Oliver (1968b) show that the effect occurs only when the circulating levels of corticosterone are high and even then with some inconsistency. This feature of the results makes it unlikely that glucagon is involved in the primary induction of the enzyme *in vivo*, but the apparent effect may occur through elevation of the corticosteroid level.

The fetal adrenal gland contains high concentrations of corticosterone during the glucagon-sensitive period of uterine life (Kamoun *et al.*, 1964). Although the fetal adrenal gland in the rat is refractory in its response to exogenous ACTH[1] (Levine *et al.*, 1967) high doses of polypeptides such as glucagon may be more effective than ACTH on the immature gland and thus promote the release of its accumulated steroids. Elevation of steroid hormone above a threshold level may then lead to enzyme induction *in utero* and some further cooperative effect with glucagon could occur. In adult animals, insulin injection has been shown to promote elevated secretion of adrenal steroids (Matsui and Plager, 1966) and glucagon injections lead to a 3–4-fold elevation of serum insulin levels (Campbell and Rastogi, 1966). Although the insulin–steroid interactions are complex, elevated steroid levels may result from glucagon treatment by this mechanism. Alternatively, elevated insulin levels may result in enzyme induction since Holten and Kenney (1967) have demonstrated induction by insulin (and glucagon) in intact adult rats. However, the interactions of the type above demand cautious interpretation of results on hormonal induction in intact animals.

It is of interest that epinephrine has no effect on serum insulin levels (Campbell and Rastogi, 1966) and the catecholamines are ineffective as inducers of tyrosine aminotransferase in the intact rat fetus *in utero* (Holt and Oliver, 1968a).

The steroid induction of the enzyme in fetal liver *in utero* cannot be due to steroid-potentiated release of glucagon since steroids fail to induce phosphopyruvate carboxylase (Yeung *et al.*, 1967) which is induced by glucagon, catecholamines, and 3′,5′-cyclic AMP (Yeung and Oliver, 1968a,b).

Since glucagon is known to elevate the levels of 3′,5′-cyclic AMP in the liver (see review by Sutherland and Robinson, 1966) this latter compound and its more potent derivative, the dibutyryl ester, were tested in the fetal rat. They both failed to bring about tyrosine aminotransferase production even during the period when the endogenous steroid levels are high. Despite this result, 3′,5′-cyclic AMP does appear to have an effect on enzyme synthesis in postnatal rats but, as will be shown, this is not at the level of the induction of enzyme-specific DNA-directed RNA synthesis.

Ergotamine tartrate and prostaglandin PGE_1 both result in a reduction in the postnatal development of tyrosine aminotransferase when administered to surgically delivered rats. Ergotamine is known to block epinephrine-stimulated production of cyclic AMP in the liver (Berthet *et al.*, 1957), while prostaglandin has been suggested as an inhibitor of adenyl cyclase (Bergstrom, 1967). Thus, the effects of both compounds are on the level of 3′,5′-cyclic AMP in the tissue. Similarly, when insulin is administered to newborn rats, the postnatal development of tyrosine aminotransferase activity is reduced and insulin is known to lower the 3′,5′-cyclic AMP level in perfused adult rat liver (Exton *et al.*, 1966). Repeated injection of glucagon in newborn rats results in only a slight increase in enzyme activity over the controls (*cf.* Csányi *et al.*, 1967, in adult rats) and 3′,5′-cyclic AMP and its dibutyryl ester give rise to a moderately large increase in activity. However, the mode of action of cyclic AMP becomes apparent only when the effect of actinomycin D is seen.

The fact that injection of 3′,5′-cyclic AMP to fetuses *in utero* does not result in enzyme production, while its

[1] Abbreviation is adrenocorticotropin.

postnatal administration increases enzyme activity, suggested that a necessary prerequisite for the effect is the initiation of mRNA synthesis. Since it has been shown previously that the postnatal lag in enzyme production involves a lag in DNA transcription (Holt and Oliver, 1968a), the strategy of the experiments in Table V was to allow the initiation of transcription and enzyme synthesis and then to identify the locus of action of cyclic AMP by the use of actinomycin D. The results show that enzyme synthesis indeed began during the initial 4-hr postnatal period and that the cyclic AMP-mediated increase in enzyme activity that was subsequently evoked was not at all affected by actinomycin D. Hence cyclic AMP cannot be acting as an inducer at the DNA level and must be involved in the translational process. In short-term experiments *in vitro* no effect of 3′,5′-cyclic AMP on the activity of tyrosine aminotransferase could be elicited and it is thus unlikely that the results can be explained by enzyme activation, although Tryfiates and Litwack (1964) reported a small stimulation of enzyme activity by 3′,5′-cyclic AMP incubated for 90 min in a cell-free system from rat liver. The effect of 3′,5′-cyclic AMP *in vivo* cannot be reproduced with 2′,3′-cyclic AMP, with 5′-AMP, or with other cyclic nucleotides at the same dose level, and thus appears to be specific. Wicks (1968a,b) has recently presented evidence that the enzyme is induced by glucagon and epinephrine in fetal liver explants in culture, the effects being mediated through 3′,5′-cyclic AMP. However, several features of the experiments require comment. Firstly, the enzyme activity, although very low, increased 3–4-fold during 42 hr of preincubation of the tissue explants and then by a factor of 2 with addition of glucagon and 3-fold with epinephrine and 3′,5′-cyclic AMP. That is, enzyme synthesis appeared to have been initiated before the addition of the inducers to the medium. Secondly, although the inhibitor of protein synthesis, cycloheximide, blocked the effect, experiments with actinomycin D were not reported and the acute effect of cycloheximide is the inhibition of polypeptide synthesis on the polysome (Ennis and Lubin, 1964; Wettstein *et al.*, 1964). Hence, the experiments do not constitute evidence for induction at the genome level.

In experiments with 2–6-day postnatal rats reported here, epinephrine injection increased hepatic tyrosine aminotransferase 3–10-fold, but in the presence of actinomycin D, epinephrine was still effective in producing a 2.5–6-fold rise in activity (Table VII). The minor repressive effect of actinomycin D when the epinephrine-mediated increase in activity was very large can possibly be attributed to general cytotoxicity of the drug.

Dibutyryl cyclic AMP also resulted in small increases in activity but actinomycin D had no effect. It is not yet known whether the actinomycin-insensitive increase brought about by epinephrine and 3′,5′-cyclic AMP occurs in the adult animal, but the results in the young and newborn animals indicate that neither of these agents act by induction at the DNA level.

These results are consistent with a translational locus of action for 3′,5′-cyclic AMP in this system and Perlman and Pastor (1968) have also implicated 3′,5′-cyclic AMP in translational control during induction of β-galactosidase and tryptophanase in *E. coli*. Holten and Kenney (1967) showed that both glucagon and insulin administration to adult rats increased tyrosine aminotransferase activity in the liver. The effects of both hormones were prevented by simultaneous administration of actinomycin D and the fact that the effects were additive to that of hydrocortisone but not to each other led the authors to suggest that the glucagon effect may be due to insulin release in the intact animal. However, later experiments with isolated perfused liver showed that each hormone acted independently on the tissue. Continuous infusion of glucagon or insulin resulted in a transient increase in activity which in each case was sensitive to actinomycin D (Hager and Kenney, 1968). Since hydrocortisone infusion led to a continuous increase in enzyme activity the authors suggested independent inductive mechanisms for hydrocortisone and pancreatic hormones and assigned a "secondary" route of induction to the latter group. Wicks (1968a,b) has also suggested multiple inductive mechanisms for the enzyme.

Anomalous effects which are not consistent with a unitary theory of induction are also apparent from experiments with young rats; for example, the data of Tables III, IV, and VI show that repressive effects of insulin and pyridoxine in the newborn rat are reversed in 2-day-old rats. A further anomalous phenomena shown in Figure 5 is of a biphasic time course of enzyme development in surgically delivered rats, which suggests the sequential operation of different inductive mechanisms.

However, alternative suggestions to account for the apparent complexity of inductive mechanisms for tyrosine aminotransferase can be made. While multiple routes of induction may exist for a single enzyme, it appears simpler to consider a multiplicity of enzymes with distinct inductive mechanisms. Tyrosine aminotransferase has been highly purified from rat liver by several authors but Hayashi *et al.* (1967) reported the finding of three enzymic bands in polyacrylamide electrophoresis of the enzyme purified from the liver of hydrocortisone-induced rats. Although it is possible that such bands were due to polymerized enzyme components, preliminary experiments in this laboratory have revealed multiple enzyme peaks after polyacrylamide gel electrophoresis of crude liver extracts prepared from rats at various ages. The experiments utilized the specific determination of enzyme activity in sequential gel sections since staining procedures were found to be unspecific for tyrosine aminotransferase. Furthermore, the electrophoretic pattern can be specifically altered by the *in vivo* administration of several hormones shown to elevate the enzyme activity in rat liver. These experiments give preliminary evidence for the existence of multiple forms of tyrosine aminotransferase which may eventually explain the apparent complexities of the inductive mechanism.

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