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Development of Tyrosine Aminotransferase in Perinatal Rat Liver: Changes in Functional Messenger RNA and the Role of Inducing Hormones

Stephanie T. Perry, Robin Rothrock, Kenneth R. Isham, Kai-Lin Lee, and Francis T. Kenney

University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences (S.T.P., R.R.) and the Biology Division, Oak Ridge National Laboratory (K.R.I., K-L.L., F.T.K.), Oak Ridge, Tennessee 37830

Expression of the hepatic enzyme tyrosine aminotransferase was analyzed in the perinatal period of development in the rat, when this expression undergoes significant changes associated with hepatocyte differentiation. In late prenatal liver both enzyme and functional mRNA gene products are present at levels 10- to 15-fold below those in the fully differentiated adult liver. This low level of expression in fetal liver is refractory to induction by glucocorticoids, but both gene products are increased to a limited extent by cyclic AMP. This induction by cyclic AMP (cAMP) does not confer glucocorticoid-responsiveness on expression. By 3 hr after birth both functional mRNA and enzyme levels are significantly increased, an increase which continues until a peak is reached at 12 hr that is appreciably above the adult levels. Both gene products then decline until adult levels are reached by 24 hr. The postnatal shift in aminotransferase expression is accompanied by acquisition of the capacity to respond to glucocorticoids. Treatment of newborns with an antiglucocorticoid steroid or with glucose suppresses the postnatal overshoot of expression, but neither treatment affects the increase from fetal to adult levels of expression. The results indicate that prior to birth, expression of the aminotransferase gene is partially repressed, a repression that is lifted essentially immediately upon birth. The hormones capable of inducing aminotransferase synthesis have no apparent necessary role in this process.

Key words: hepatocyte differentiation, tyrosine aminotransferase, functional mRNA, hormonal regulation

The tyrosine aminotransferase of rat liver (L-tyrosine:2-oxoglutarate aminotransferase, E.C. 2.6.1.5) has been studied intensively as a model for the study of regulation of gene expression in mammalian cells, owing to properties such as the

Stephanie T. Perry's present address is Department of Pediatrics, University of North Carolina School of Medicine, Chapel Hill, NC 27514.

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sensitivity of its synthesis to several hormonal regulators and rapid turnover of the gene products. In an early study of developmental regulation of this enzyme, Sereni, Kenney, and Kretchmer found that aminotransferase levels were low and not responsive to glucocorticoid control in fetal rat livers prior to birth, but increased rapidly and became glucocorticoid-sensitive within the first few hr after birth [1]. These results have since been confirmed and extended by a number of investigators, leading to a number of observations relevant to our present study. Among these are the demonstration that precocious delivery elicits precocious appearance of the enzyme, while delayed delivery delays its appearance [2,3]; the prenatal enzyme can be increased by glucagon or its intracellular mediator, cAMP [3–5]; the placement of fetal livers into organ culture results in a slow increase in basal enzyme levels and attainment of the capacity to respond to glucocorticoids as well as to cAMP [6–9]. In the present study we have analyzed this differentiation-associated shift in expression of tyrosine aminotransferase, complementing earlier enzyme measurements with translational assays of functional mRNA, and with the aim of defining the pattern of change in both functional gene products and the role of inducing hormones.

MATERIALS AND METHODS

Animals

Virgin female Sprague-Dawley rats (150–200 g from Charles River Breeding Laboratories) were placed with males overnight. The following morning, females were tested by vaginal smears for the presence of spermatozoa. Gestational age of fetuses was determined from the time of detection of spermatozoa in the vaginal tract and is accurate to 12 hr. Precise age of newborns was established by direct observation of their birth. Hormonal administration to fetuses in utero following maternal laparotomy was as described before [1]. All substances were dissolved in 0.14 M NaCl and injected intraperitoneally.

Characterization of Fetal Tyrosine Aminotransferase

Livers from 18-day fetuses and from adult male rats previously induced with hydrocortisone were homogenized in four vol (w:v) of buffer containing 50 mM potassium phosphate, pH 7.6, 100 mM KCl, 5mM α-ketoglutarate, and 1 mM EDTA. The homogenates were centrifuged at 15,000g for 15 min, and the supernatants taken and centrifuged again at 80,000g for 30 min. The extracts were supplemented with pyridoxal phosphate to 30 μg/ml and α-ketoglutarate to 10 mM and heated to 62°C with constant stirring in a water bath at 85°C. The heat-treated extracts were rapidly cooled in an ice-ethanol bath and denatured protein removed by centrifugation. These preparations were then applied to 5 ml columns of DEAE-cellulose, previously equilibrated with buffer A (50 mM potassium phosphate, pH 7.6, 5 mM α-ketoglutarate, 1 mM EDTA, and 1 mM 2-mercaptoethanol), and washed with four column volumes of buffer A + 100 mM KCl + 3 μg/ml pyridoxal phosphate. The enzyme was then eluted with buffer A + 400 mM KCl + 3 μg/ml pyridoxal phosphate, and the fractions containing the bulk of the enzyme activity were pooled and precipitated with $(NH_4)_2SO_4$ to 75% saturation. The precipitate was collected by centrifugation and dialyzed against buffer A + 3 μg/ml pyridoxal phosphate. These procedures resulted in purification of about 100-fold of the enzyme from both sources, and concentrated both for immunological analysis. To equivalent aliquots of both prepa-

rations, increasing amounts of antibody prepared against homogeneous aminotransferase were added. After incubation at 37°C for 15 min, prewarmed reaction mixture was added and the amount of enzyme activity remaining was determined by the method of Diamondstone [10]. The unit of activity is the amount catalyzing the formation of 1 nmol product per min. Proteins were estimated by the method of Lowry [11].

For routine determination of enzyme levels in fetal and postnatal livers the supernatants of homogenates centrifuged at 27,000g were used. For very low levels the usual 10-min assay was extended to 20, 40, or 60 min as required.

Isolation of RNA

Livers from fetuses or newborns were homogenized in three volumes (w:v) of a buffer containing 0.25 M sucrose; 25 mM Tris-HCl, pH 7.4; 25 mM NaCl; and 5 mM $MgCl_2$. Homogenates were centrifuged at 300g, then 12,000g for 10 min, and finally at 27,000g for 20 min. Supernatants were taken and heparin added to 0.5 mg/ml. Polysomes and RNA-protein complexes were precipitated by the addition of $MgCl_2$ to 100 mM and incubation at 4°C for 1.5 hr as described by Palmiter [12]. The precipitate was collected by centrifugation at 27,000g for 30 min; suspended in 0.25 ml of 0.25 M EDTA, pH 7.4; and solubilized in 1% sodium dodecyl sulfate. This solution was brought to final concentrations of 10mM Tris-HCl, pH 7.4; 0.5% sodium dodecyl sulfate; and 0.5 M NaCl; and poly(A)-RNA was isolated by two passages through 1 ml oligo(dT)-cellulose columns as described by Krystosek et al [13]. The RNA was precipitated and stored under 2.5 vol ethanol.

Total cellular RNA was isolated from aliquots of the homogenates by the phenol-chloroform extraction described by Palmiter [12]. The RNA was precipitated and collected by centrifugation and washed three times with 3 M sodium acetate, pH 5.0, then reprecipitated and stored under ethanol.

Cell-Free Protein Synthesis and Immunochemical Analysis

Poly(A)-RNA was translated in nuclease-treated lysates of rabbit reticulocytes prepared essentially as described by Pelham and Jackson [14].Optimal concentrations of Mg^{2+} and K^+ were determined for all lysate preparations used, as was the concentration of added poly(A)-RNA giving a linear response in incorporation of radioactive leucine into protein. In standard translation assays, 100 μl reaction volumes, containing 50 μCi [^{3}H]-leucine (40–50 mCi/nmol, Amersham, England) were used, to which 1.0 μg poly(A)-RNA was added. After incubation at 30°C for 1.5 to 4 hr (again optimized for each lysate preparation), followed by centrifugation for 45 min at 200,000g, a small aliquot was removed for determination of total protein synthesis by the method of Mans and Novelli [15]. Aliquots of 50 μl were then used for specific immunoprecipitation with antibody prepared against highly purified aminotransferase, followed by analysis of the immunoprecipitates by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and quantitation as previously described [16]. Total cellular RNA was analyzed in the same manner, except that 10–20 μg RNA was added to the lysate reaction.

Polyacrylamide Slab Gel Electrophoresis

Slab gels containing 13% acrylamide, and 0.35% bis-acrylamide were prepared with 3% acrylamide stacking gels, following the procedure of Laemmli et al [17]. Samples were run through the stacking gel at 30 mA, then at 100 V through the

resolving gel. The gels were then soaked overnight in 10% acetic acid, 10% trichloroacetic acid, 30% methanol, and then treated with En^{3}Hance^{TM} for 1 hr. After precipitation of the fluor in water for 1 hr, the gels were dried under vacuum and exposed to Kodak RP X-Omat film at −70°C for 2–3 days.

Sucrose Gradient Analysis

Five to 20% sucrose gradients containing 20 mM sodium acetate, pH 5.0, 5 mM EDTA, and 0.5% sodium dodecyl sulfate, were prepared in 5-ml polyallomer tubes. Fetal or adult poly(A)-RNA (250 μg) was dissolved in 0.2 ml sterile H_2O, heated for 3 min at 80°C, and layered onto the tops of the gradients. The gradients were centrifuged at 40,000 rpm for 205 min in SW 50.1 rotor at 20°C. After centrifugation, the gradients were fractionated top to bottom and the absorbance at 254 nm was recorded.

RESULTS

Aminotransferase Expression in Fetal Liver

Enzyme activity in fetal liver. Others have reported that the low capacity of fetal livers to carry out transamination of tyrosine is actually due to another enzyme, probably aspartate aminotransferase (E.C. 2.6.1.1) as this enzyme can utilize tyrosine as an amino donor to some extent [18]. To resolve this question we subjected fetal and adult liver extracts to simultaneous partial purification followed by analysis of immunological inhibition of the partially purified enzymes. Cytosol fractions of livers from 18-day fetuses were pooled, yielding a preparation containing 0.78 tyrosine aminotransferase units per mg protein; specific activity of the comparable extract from the liver of an adult rat treated with hydrocortisone to induce the enzyme was 122 units per mg protein. After addition of the stabilizers pyridoxal phosphate and α-ketoglutarate both extracts were heated to 62°C and quickly cooled; recovery of the enzyme activity was essentially complete in both. The heated preparations were then each applied to columns of DEAE cellulose; these were washed with 0.1 M KCl and the enzyme then eluted with 0.4 M KCl, again without significant loss of activity from either preparation. Thus the apparent tyrosine aminotransferase activity of fetal liver is similar to the adult enzyme in heat stability and ion-exchange properties. After concentrating, the partially purified preparations were titrated with specific antibody prepared against homogeneous enzyme from adult livers, assaying for inhibition after a short incubation at 37°C (Fig. 1). Both preparations were inhibited about 90% by comparable amounts of antibody, and both exhibited the residual activity typical of the immune complexes of this enzyme [19]. It is apparent, then, that the low activity observed in fetal livers is authentic tyrosine aminotransferase. The basis of the discrepancy between these results and those of others is not known but may be related to procedures used in preparing extracts of fetal livers.

The tyrosine aminotransferase content of livers of fetuses did not vary much with gestational age from 18 to 21 days, ranging from 0.3 to an exceptional high of 4.6 units per mg protein. The mean of 30 specific-activity determinations of pools of livers of 180 18–21-day-old fetuses was 1.08 ± 0.20 (SE); this contrasts to the usual steady-state adult level of about 16 units per mg protein.

Functional mRNA in fetal liver. Poly(A)-RNA, prepared by oligo(dT)-cellulose chromatography following Mg^{2+}-precipitation of the 27,000g supernatant

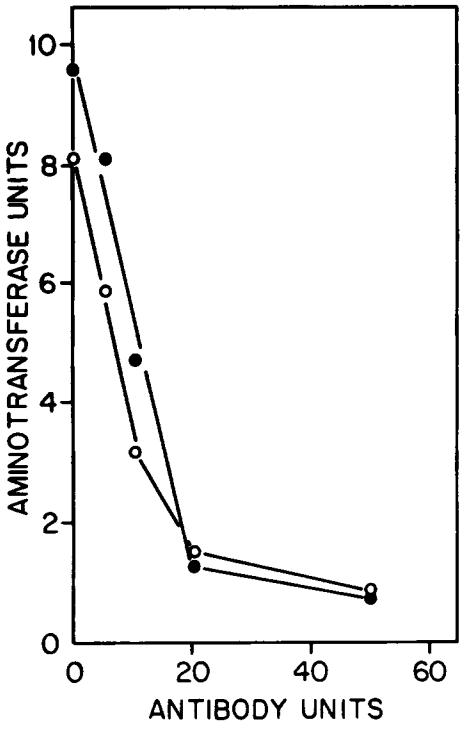


Fig. 1. Inhibition by antibody of tyrosine aminotransferase activity. The enzyme was partially purified from adult (○) and fetal (●) rat livers, then titrated with specific antiaminotransferase antibody as described in Experimental Procedures.

fractions of pooled fetal livers, proved to be highly efficient in stimulating synthesis of proteins in reticulocyte lysates, appreciably more so than comparable preparations from adult livers (Fig. 2). However, the immunoprecipitation assay for mRNA-directed synthesis of tyrosine aminotransferase, which readily detects this product in comparable translation assays of poly(A)-RNA from adult livers, was virtually negative when the translation products of poly(A)-RNA from 18-, 19-, 20-, or 21-day fetuses were analyzed in the usual way (see, for example, the analysis in Fig. 5). Thus it appears that the standard translation assay, using total cytoplasmic poly(A)-RNA without further selection, is not sufficiently sensitive to detect what could be anticipated as very low levels of functional aminotransferase mRNA in fetal livers. Aminotransferase mRNA was similarly undetectable when total fetal RNA, prepared by phenol-chloroform extraction of liver homogenates, was assayed by translation (not shown).

Major differences in the mRNA populations of fetal and adult livers may also contribute to difficulty in detecting aminotransferase mRNA in fetal liver, as suggested by the data presented in Figure 3. The partially hematopoietic function of fetal liver is reflected in the size distribution of fetal poly(A)-RNA fractionated on sucrose gradients, in that the bulk of the RNA sediments in the 9–10S region occupied by globin mRNA, in contrast to the much larger average size of adult liver poly(A)-

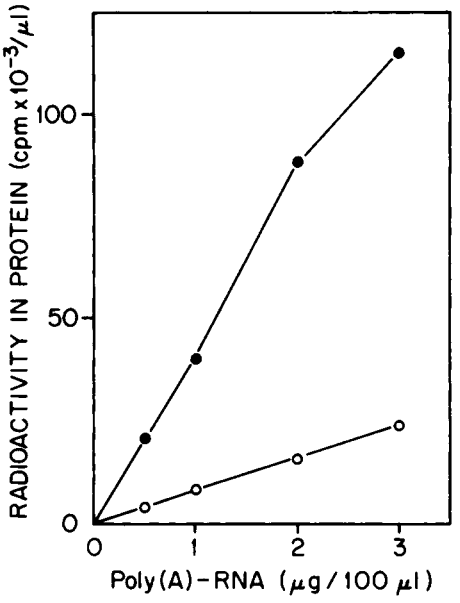
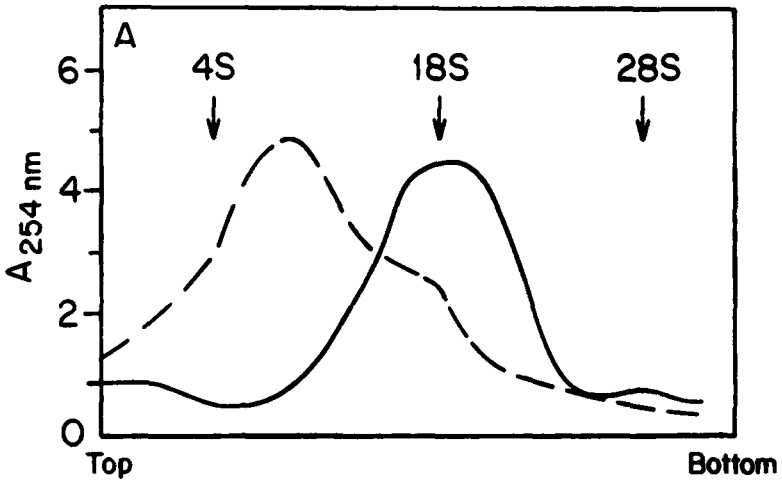


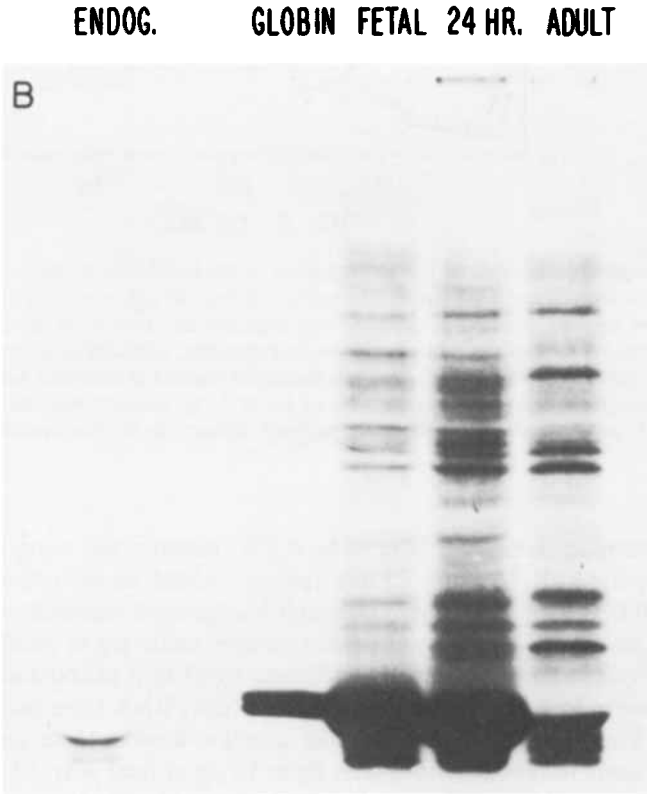
Fig. 2. Translation of poly(A)-RNAs in reticulocyte lysates. Increasing amounts of poly(A)-RNA isolated from livers of adult (○) and 18-day fetal (●) livers were added to the standard 100-μl assay mixture and the reaction allowed to proceed for 3 hr.

RNA (Fig. 3A). Electrophoresis of the translation products of poly(A)-RNA from fetal and neonatal rats demonstrates the preponderance of the 17,000-dalton subunits of globin which are absent in the products of translation of adult liver mRNA (Fig. 3B). As the smaller mRNAs of the fetal population are in high concentration and effectively translated, the contribution of very low levels of the large aminotransferase mRNA (c 22S, [20,21]) might be expected to be obscured.

In a further attempt to detect and quantitate functional aminotransferase mRNA in fetal livers, we selected the RNAs 18S and larger after centrifugation on sucrose gradients of poly(A)-RNA prepared from a large number of pooled fetal livers; a comparable preparation from adult liver was similarly treated to serve as control. The volume of the translation assay was also increased to 1.0 ml in order to assay tenfold more of the partially purified poly(A)-RNAs. With these modifications functional aminotransferase mRNA could be detected in poly(A)-RNA from fetal livers, as illustrated by the electrophoretic profiles of immunoprecipitates of translation products shown in Figure 4. With the usual correction for background radioactivity [16] we estimate the aminotransferase mRNA activity of the 18-day fetal preparation to be 0.019% of total mRNA activity of this selected population, and that of the similarly-

Fig. 3. Analyses of fetal and adult poly(A)-RNAs and their translation products. A) Sucrose gradient analysis. Poly(A)-RNAs isolated from adult (solid line) and 18 day fetal (dashed line) livers were centrifuged through 5 ml sucrose gradients as described in Experimental Procedures. The positions of cocentrifuged 18 and 28S ribosomal and 4S transfer RNAs from rat liver are indicated as markers. B) Electrophoretic analysis of the products of translation. Equivalent amounts of the radioactive protein





synthesized in reticulocyte lysates by poly(A)-RNAs from 18-day fetal, 24-hr neonatal, and adult livers were electrophoresed on a sodium dodecyl sulfate-containing polyacrylamide slab gel. ^{3}H-labeled rat globin and the soluble translation products of an endogenous reaction with no mRNA added are included for comparison. (Tyrosine aminotransferase subunits, constituting a very minor fraction of total translation products, cannot be detected in these gels.)

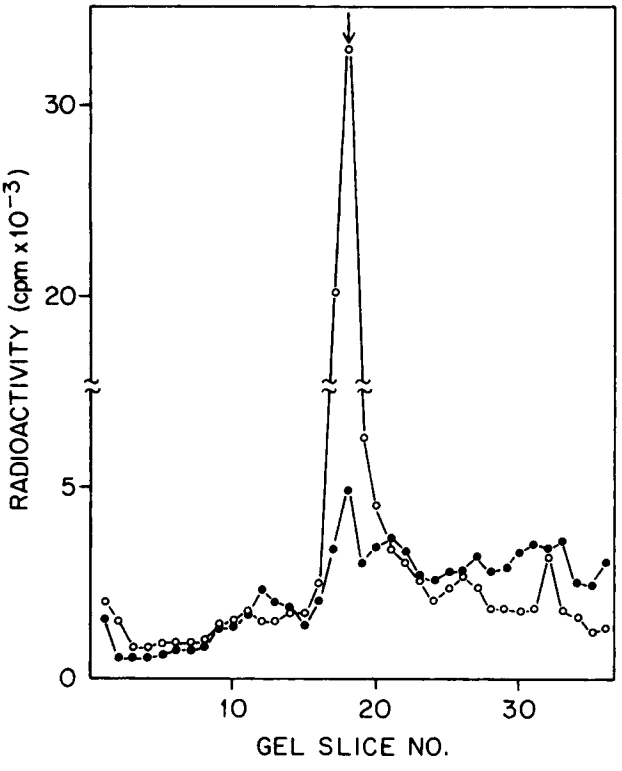


Fig. 4. Electrophoretic analyses of imunoprecipitates of the translation products of partially purified poly(A)-RNAs from fetal and adult livers. The poly(A)-RNAs (10 μg) from adult (○) and 18-day fetal (●) livers were partially purified by gradient centrifugation and assayed in the modified translation system as described in the text. Immunoprecipitates were purified, subjected to electrophoresis in sodium dodecyl sulfate-polyacrylamide gels, and the gels sliced and counted as described before [16]. Authentic tyrosine aminotransferase subunits migrate with an R_F of 0.295 (arrow); here the dye marker was in slices 60 and 61, and there were no discernable peaks of radioactivity beyond those shown.

treated preparation from adult liver to be 0.2%. Another run using these procedures to analyze poly(A)-RNA from 21-day fetuses yielded an aminotransferase mRNA activity of 0.020% of total; thus, although background radioactivity is high in the analyses of fetal mRNA activity the two estimates made are in good agreement. The pronounced enhancement in translational capacity of fetal mRNAs compared to adult mRNAs, seen before in the analyses of total poly(A)-RNA from each source (cf Fig. 2), was no longer apparent after the size selection used in these experiments. Total protein synthesis in reticulocyte lysates from 10 μg of fetal poly(A)-RNA selected as 18S or larger was 25 × 10^6 cpm; synthesis from 10 μg of selected poly(A)-RNA from adults was 22.5 × 10^6 cpm.

Effects of inducing hormones on expression in fetal liver. Tyrosine aminotransferase in livers of adult rats is inducible by glucocorticoids and by the protein hormones insulin and glucagon, the latter acting via the intracellular mediator cAMP; the induced acceleration of synthesis by each of the hormonal inducers has been shown to be associated with commensurate increases in mRNA activity [21–25].

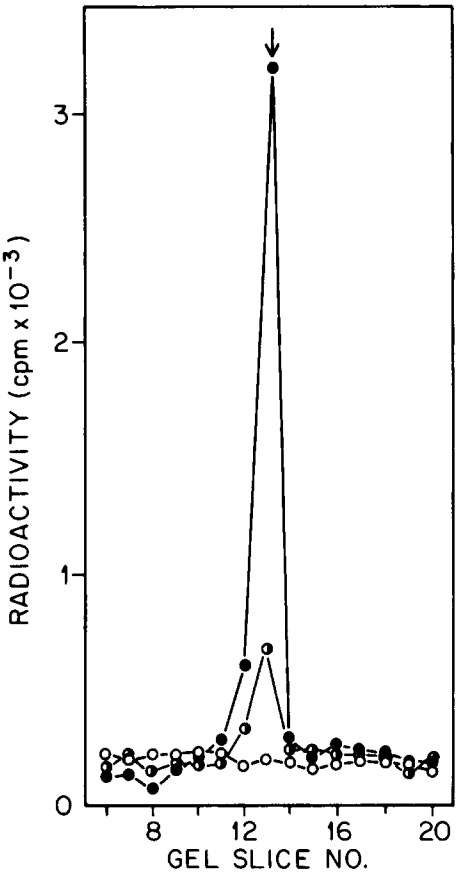


Fig. 5. Electrophoretic analyses of immunoprecipitates of the translation products of poly(A)-RNAs were isolated from livers of 18-day fetuses injected 5 hr previously with hydrocortisone (○), from 24-hr-old neonatal rats similarly treated with hydrocortisone (●), or from 24-hr-old neonatal rats that were untreated (◑). The standard translation assay was used, and immunoprecipitates analyzed as in the legend to Figure 4. The marker dye migrated to slice 44; regions of the gels not shown were essentially devoid of radioactivity.

Earlier work at the enzyme level has indicated that synthesis of the enzyme in fetal liver is not responsive to glucocorticoids [1]. The electrophoretic analyses of immunoprecipitates presented in Figure 5 show that the aminotransferase mRNA activity of unfractionated poly(A)-RNA from fetal liver remains undectable after glucocorticoid treatment, in accord with the failure of enzyme levels to change. Similar results have recently been reported by Ruiz-Bravo and Ernest [26]. By 24 hr after birth, after the differentiation event has occurred, the steroid-elicited response is readily observed in the translation assay. Insulin was without effect in fetal liver as observed previously [27].

The inability of fetal enzyme synthesis to respond to glucocorticoids or insulin does not extend to all the hormonal inducers; Greengard and others have reported that the low fetal aminotransferase activity can be temporarily elevated to a limited

TABLE I. Effects of Inducing Hormones on Tyrosine Aminotransferase mRNA and Enzyme Activities in Livers of Fetal and Neonatal Rats*

Treatment	mRNA activity (% of total)	Enzyme activity (U/mg protein)
Fetuses		
Untreated	ND	1.08 ± 0.20 (30)
Hydrocortisone (HC)	ND	1.01 ± 0.27 (7)
cAMP	0.015 ± 0.001 (16)	4.14 ± 0.54 (5)
HC + cAMP	0.016 ± 0.002 (7)	3.77 ± 0.28 (8)
Newborns		
Untreated	0.018 ± 0.003 (6)	15.5 ± 3.4 (4)
Hydrocortisone	0.098 ± 0.013 (5)	79.6 ± 12.3 (5)
cAMP	0.059 ± 0.006 (8)	48.2 ± 9.2 (3)
HC + cAMP	0.139 ± 0.015 (8)	116.2 ± 17.4 (3)

*Treatments were by direct intraperitoneal injection of 18- or 19-day fetuses in utero or of newborns, 24 hr after birth. Hydrocortisone treatment (HC, 5 mg/100 g) was for 5 hr, in both, and treatment with dibutyryi cyclic AMP (cAMP, 5 mg/100 g) was for 3 hr in fetuses or 1.5 hr in newborns, the times being chosen for peak response in each case. In combination experiments treatments were given in sequence to maintain these treatment times. Assays of mRNA and enzyme activities were by the standard procedures given in Experimental Procedures. Data are the means ± SE of the number of observations given in parentheses. ND, not detectable.

extent by treatment with glucagon or cAMP [3–5]. The data of Table I demonstrate that this effect of cAMP, as in the cAMP-mediated induction in adults, is associated with increased aminotransferase mRNA activity. This result has also been reported recently [26]. This stimulation by cAMP did not render synthesis of the enzyme responsive to steroid induction, as indicated by the failure of combined steroid plus nucleotide treatment to alter the nucleotide effect on either enzyme level or mRNA activity in the fetal livers. In contrast, the combined treatments elicited an essentially additive effect on both parameters in 24-hr-old neonates.

Postnatal Changes in Aminotransferase Expression

Functional mRNA. The first hours of postnatal life are associated with a rapid increase in functional aminotransferase mRNA, as illustrated in Figure 6. Unfractionated poly(A)-RNA from livers of rats taken immediately after birth contain barely detectable levels, at the limits of detection of the standard translation assay indicated by the dashed horizontal line representing the point below which no discernable peak is present in electrophoretic gels of immunoprecipitates. Within 3 hr significant levels of the mRNA have appeared and these continue to increase rapidly to a peak at 12 hr, at which time the enzyme product also reaches a maximum, as shown previously [1]. At this point the rapid turnover of the aminotransferase gene products becomes paramount and levels of both mRNA and enzyme fall in the anticipated sequence. By 24 hr both parameters have stabilized at the adult steady-state level. These analyses were repeated at several postnatal time points assaying total RNA rather than Mg^{2+}-precipitated poly(A)-RNA with results comparable to those shown.

Role in hormones in postnatal changes. The data presented show that release from the intrauterine environment triggers a rapid increase in functional aminotransferase mRNA and the simultaneous acquisition of the capacity to respond to glucocorticoids. In an earlier attempt to determine if glucocorticoids are required

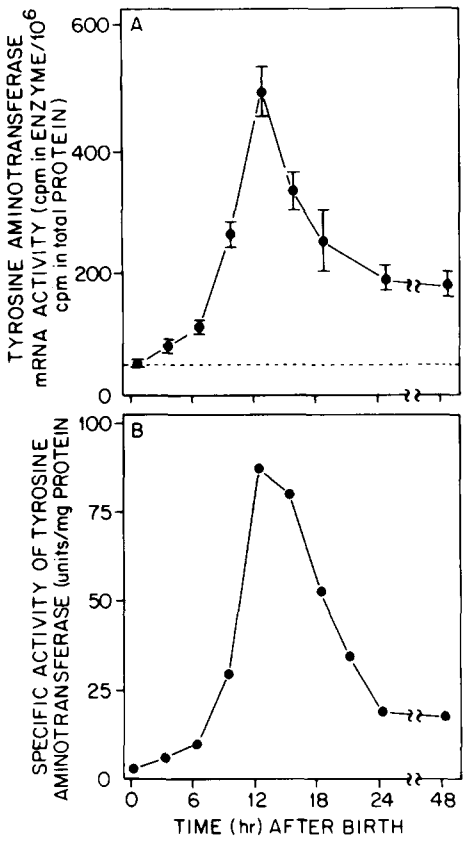


Fig. 6. Postnatal development of tyrosine aminotransferase. A) Functional aminotransferase mRNA. Poly(A)-RNAs were assayed in the standard translation assay described in Experimental Procedures. Data are the mean ± SE of multiple determinations made from two to five litters at each time point. The dashed line indicates the empirical limit of detection; all determinations made on fetal poly(A)-RNAs by this assay were below this limit. B) Tyrosine aminotransferase activity. Assays were made on 27,000g supernatant fractions of liver homogenates; each point represents the mean of multiple determinations from two or more litters at each time point.

for this event to occur, rats were adrenalectomized immediately after birth, resulting in abolishment of the marked overshoot of enzyme synthesis but not the smaller increase to the adult level [1]. However, this approach was not conclusive owing to the possibility of glucocorticoid release during surgery. Here we have used a more definitive technique, that of administering a potent antiglucocorticoid that blocks intracellular function of the steroid. The compound chosen, 5α-pregnen-3,20-dione, was shown before to inhibit completely the hydrocortisone-mediated induction of tyrosine aminotransferase in cultured hepatoma cells by competing for the glucocorticoid receptor [28]; it seems reasonable to assume that it acts similarly in the livers of newborns. Further, this steroid is present in the rat placenta throughout gestation and although in low concentration [29] might be considered as functioning in intrauterine repression. When large doses were administered immediately after birth and

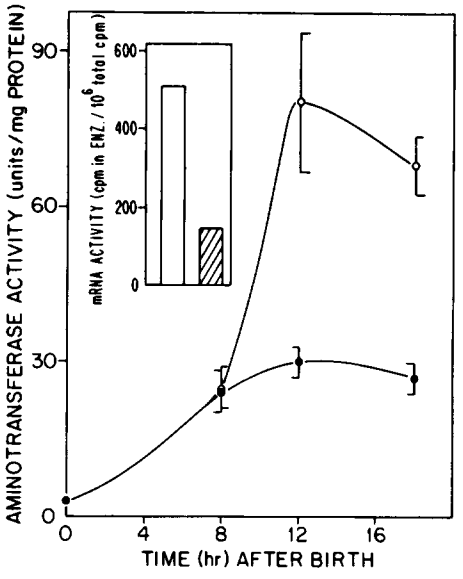


Fig 7. Effect of 5α-pregnen-3-20-dione on postnatal development of tyrosine aminotransferase. Newborns were untreated (○) or given 2.5 mg each of the steroid immediately after birth and again at 12 hr (●). Data are the mean ± SE of multiple determinations on individuals from two litters at each time point. Inset) Functional aminotransferase mRNA activities of poly(A)-RNAs isolated from livers of 12-hr-old newborns and measured in the standard translation assay. RNA from controls (open bar); RNA from animals treated at birth with the antiglucocorticoid (shaded bar).

again at 12 hr, the response was like that seen before following adrenalectomy, ie, the marked overshoot of enzyme was blocked but not the increase from low fetal levels to the adult level (Fig. 7). As documented in the insert, this effect of 5α-pregnen-3,20-dione was associated with a commensurate reduction in the postnatal production of functional aminotransferase mRNA. Thus it seems that induction by glucocorticoids is not requisite to the postnatal increase in expression. However, this shift elicits glucocorticoid responsiveness, and as the levels of these steroids are high immediately after birth [30], the postnatal change appears to be amplified by glucocorticoid induction resulting in the typical overshoot of production of functional mRNA and of enzyme.

Greengard [31] and Holt and Oliver [4] have demonstrated that treatment of newborns with glucose, in order to maintain the high glucose level typical of fetuses, can depress the postnatal increase in aminotransferase activity when given immediately after birth. We have repeated these experiments, administering glucose in multiple injections alone or together with insulin. The effects on enzyme levels of either treatment were virtually identical to that of the antiglucocorticoid seen in Figure 7; glucose, like the antiglucocorticoid, blocks the overshoot but has no effect on the increase from fetal to adult levels, as shown before [4,31]. Since treatment with glucose has previously been shown to inhibit aminotransferase induction by glucocorticoids [32,33] it seems possible that this effect on postnatal development could be attributed to an obscure antiglucocorticoid effect of the sugar. The alternative expla-

nation, implicating the glucagon-cAMP response to glucose and a cAMP-mediated increase in functional mRNA and enzyme contributing to the postnatal overshoot, cannot be excluded at this time. It is of interest that insulin is without effect on tyrosine aminotransferase synthesis throughout the prenatal and early postnatal periods, implying that some component of the induction response to this hormone has not yet developed.

DISCUSSION

The perinatal period in development of the rat is associated with major changes in structure and function of the liver. Largely a hematopoietic organ prior to 14 days of fetal life, the hepatic cell mass still contains 10% or more of hematopoietic cells during the period from 18 days to term; these persist for several days after parturition [34]. During this late phase of prenatal development hepatic function begins to predominate as the hepatocyte component both increases in quantity and undergoes differentiation-associated changes resulting in the typical enzymic complement of the liver cell. This differentiation is not complete until several weeks after birth, as exemplified by the slow appearance postnatally of liver enzymes such as tryptophan dioxygenase [35]. Tyrosine aminotransferase is one of several liver enzymes that increase dramatically just prior to or following birth in the course of hepatocyte differentiation.

Our results confirm and extend previous indications that expression of tyrosine aminotransferase is repressed prior to birth, full expression being attained shortly after release from the uterine environment [1–3]. It is clear, however, that prenatal repression does not completely silence this expression. Enzyme content in fetal liver is approximately 15-fold below that of the fully differentiated adult liver, and the catalytic activity measured is shown here to be authentic tyrosine aminotransferase. Activity of aminotransferase mRNA in fetal liver is more difficult to detect and quantitate, owing both to inherent insensitivity of the translation assay and to what appears to be a masking of hepatocyte mRNA input by preferential translation of the large amount of globin mRNA contributed by hematopoietic cells. When the latter mRNA was largely removed aminotransferase mRNA could be detected and was estimated to be present at a level about tenfold below that of a similarly-treated adult liver. The complication of hematopoietic cell input into these measurements makes precise comparisons unfeasible, but nonetheless it can be stated that fetal hepatocytes in late gestation are capable of expressing tyrosine aminotransferase, the level of expression being roughly 10- to 15-fold less than that of adult hepatocytes in terms of both functional mRNA and enzyme gene products.

This low but hardly negligible level of expression remains entirely refractory to the primary hormonal control mechanism regulating synthesis of this enzyme in the fully differentiated hepatocyte, that of induction by glucocorticoids. Neither enzyme nor functional mRNA levels of fetal liver are changed by direct administration of hydrocortisone, although the requisite receptor system is present [36] and glucocorticoids are able to elicit other responses in fetal liver [37]. Insulin is similarly without effect in fetal liver and throughout the perinatal period. However, the low fetal level of aminotransferase expression can be augmented by the third regulator of synthesis of this enzyme; both functional mRNA and enzyme are temporarily increased by administration of cyclic AMP, although to a limited extent. This response does not

render aminotransferase expression sensitive to glucocorticoid control, a result we find to be inconsistent with suggestions that implicate inductions by cyclic AMP [38] or by the combined actions of this nucleotide with glucocorticoids [26] in the activation of gene expression occurring after birth.

The limitation on expression in fetal liver is removed within the first few hr after birth, allowing production and accumulation of both functional mRNA and enzyme gene products; simultaneously expression gains the capacity to respond to glucocorticoid control. Treatment with a potent antiglucocorticoid reveals that the postnatal burst of expression can be dissociated into two components, one the fundamental shift from fetal to adult levels occurring over the first 12 postnatal hr, and the second a marked overshoot in enzyme and mRNA seen between 9 and 18 hr. That this overshoot is blocked by a glucocorticoid antagonist as well as by adrenalectomy [1] suggests that it can be attributed to an amplification of expression owing to induction by endogenous glucocorticoids, which are markedly elevated during this interval [30]. But the more fundamental shift in expression proceeds smoothly in the presence of the steroid antagonist, as well as in newborns given large amounts of glucose that would presumably lower circulating glucagon and thereby intrahepatic cyclic AMP. Thus we find no evidence for a necessary role of any of the inducing hormones in the differentiation event occurring immediately after birth, wherein expression of tyrosine aminotransferase transits from a low level refractory to regulation by steroids to a level 10- to 15-fold higher and responsive to this regulation. Once this event has occurred expression can be, and clearly is, modified by the hormonal regulators.

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