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The Effect of Development and Hydrocortisone on Tryptophan Oxygenase, Formamidase, and Tyrosine Aminotransferase in the Livers of Young Rats*

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ABSTRACT: Tryptophan oxygenase, formamidase, and tyrosine aminotransferase activities were determined by optimal assays in livers of rats from late fetal through weaning age. Assay of the total tryptophan oxygenase activity in the neonatal rat required the addition of EDTA but not formamidase, plus a prior incubation, to activate it fully. Activity of formamidase was present before but increased markedly at the time of birth, that of tyrosine aminotransferase appeared just after birth, and that of tryptophan oxygenase 14–17 days after birth. Administered hydrocortisone had no significant effect on formamidase activity at any age studied; it induced tyrosine aminotransferase as soon as the birth-connected rise subsided, and it induced the appearance of tryptophan oxygenase activity shortly before the basal enzyme was detectable. Tryptophan oxygenase and tyrosine aminotransferase activities increased in response to the stress of weaning in the 21-day-old rat, although they were unresponsive to the same stress at an earlier age.

The purpose of this investigation was to determine the ages at which tryptophan oxygenase (EC 1.13.1.12, L-tryptophan:oxygen oxidoreductase), formamidase (EC 3.5.1.9, aryl-formylamine amidohydrolase), and tyrosine aminotransferase (EC 2.6.1.5, L-tyrosine: 2-oxoglutarate aminotransferase) activities appeared in the livers of young rats, and the effects of hydrocortisone on these enzymes at an early age. In previous studies of these activities in young rats, the tryptophan oxygenase did not appear until the 12th (Auerbach and Waisman, 1959) to 15th (Nemeth, 1959) day after birth, formamidase was present in fetuses (Nemeth, 1961), and tyrosine aminotransferase appeared suddenly a few hours after birth (Auerbach and Waisman, 1959; Sereni *et al.*, 1959). However, Spiegel and Spiegel (1964a) reported that tryptophan oxygenase could be demonstrated under certain conditions in the livers of neonatal rats, and it was of interest to test this with the sensitive new assay system for the enzyme that ensures accurate determination of the total enzyme activity present in soluble liver extracts (Knox *et al.*, 1966a; Knox and Piras, 1966). Slight modifications of the several assays made it possible to determine all three enzyme activities in single livers from perinatal rats for comparison with each other and with the same three enzymes in the developing chick (Knox and Eppenberger, 1966). The results demonstrate that each enzyme behaves uniquely and differently in the rat than in the chick.

Materials

Animals. NEDH inbred, male rats were used, except for the fetal rats (20–40 mm long, *viz.*, day 19–22 of gestation (Altman and Dittmer, 1962)) and 1-day-old

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rats whose sex could not be determined accurately. Minimal age designations are given by which "newborn" rats are 0–24-hr old, "1-day-old" rats are 24–48-hr old, etc. Several litters, each divided between control and experimental groups, were used at each age. Both the control and experimental young rats were placed in a separate cage from the mother 6 hr prior to use. Hydrocortisone-treated rats received intraperitoneal injections of 2.5 mg of hydrocortisone 21-phosphate (hydrocortisone phosphate, Merke Sharp and Dohme, West Point, Pa.)/100 g of body weight. Preliminary experiments in 14- and 21-day-old rats, as well as in adult rats of the same strain and sex (Knox *et al.*, 1966b), demonstrated that this dosage was in excess of that required to obtain a maximal increase in tryptophan oxygenase activity 5 hr after injection. Rats were sacrificed by decapitation 5 hr after injection and/or 6 hr after separation from the mother, always at approximately the same hour of the day (1–2 PM).

Chemicals. *N*-Formyl-L-kynurenine was prepared by the method of Auerbach and Knox (1957). The methemoglobin used was commercial twice-crystallized horse hemoglobin (Nutritional Biochemicals). The sulfonated sodium salt of bathocuproine (Diehl and Smith, 1958) was obtained from G. Frederick Smith Chemical Co., Columbus, Ohio.

Methods

Preparation of Liver Enzymes. Body weights were measured, then the livers were removed quickly and weighed or the volume was determined by displacement. Livers from three fetal rats were pooled, but livers from animals of all other ages were treated individually. Using a Potter-Elvehjem Teflon homogenizer, 25% liver homogenate was prepared in 0.14 M KCl–0.02 M sodium phosphate buffer (pH 7.0). The supernatant obtained from a 27,000 rpm (approximately 69,000*g*) centrifugation of the homogenate for 1 hr in a Spinco Model L type 30 rotor was used for assay of all three enzymes. Preparation of supernatants from individual livers of 7-day-old and younger animals required the use of Kolmer-Brown tubes, a microhomogenizer and 2-ml capacity centrifuge tubes (Beckman part no. 303369) in custom-made nylon adapters for the type 30 rotor.

Enzyme Assays. Tryptophan oxygenase activity was assayed by the method of Knox *et al.* (1966a; Knox and Piras, 1966) by which all of the enzyme present was converted to the active (reduced holoenzyme) form. It was modified by the addition to the assay of neutralized Na_2EDTA (0.05 M final concentration). The 25% liver supernatant fraction was incubated for 30 min at 37° immediately before the assay with an equal volume of the homogenizing medium containing 0.8 mg of methemoglobin/ml, 8 mM L-tryptophan, and 0.06 M freshly neutralized ascorbate. The 3.0-ml assay mixture consisted of additions at 25° to a 1-cm cuvet in this order: 0.7 ml of 0.2 M sodium phosphate buffer (pH 7.0), 0.2 ml of 0.05 M tryptophan, 0.6 ml of distilled water, 1.0 ml of 0.15 M neutralized disodium EDTA, 0.4 ml of the incubated (12.5%) liver fraction, and 0.1 ml of freshly neutralized 0.3 M ascorbate. After mixing, it was placed in the sample chamber of a Gilford Model 2000 automatic spectrophotometer equilibrated at 25° and the initial change in absorbance at 360 mμ was plotted against time using a water blank.

In view of the very low apparent activities in livers of fetal and young rats, we determined the blank increase in absorbance that was not definitely referable to kynurenine formation by the enzyme. Tryptophan was required for most of the increase in absorbance. In the absence of both enzyme and EDTA the increase in absorbance was less than 0.01 unit/assay (equivalent to less than 0.2 unit/g of liver with the standard additions). With only enzyme omitted, the blank averaged 0.03 unit/assay (equivalent to 0.6 unit/g of liver). Kynurenine was recognized as a product by its absorption curve in metaphosphoric acid filtrates (Knox, 1955a) only in reaction mixtures with apparent activities above 0.05 unit/assay (equivalent to 1.0 unit/g). Preparations from adult rats to which excess antiserum against tryptophan oxygenase was added to inhibit the enzyme (Knox and Piras, 1967) also showed an apparent activity of 0.05 unit/assay in the absence of EDTA. The average value observed with fetal to 1-day-old livers was 0.042 unit/assay (0.85 unit/g). It was apparent that no enzymic significance could be attributed to apparent activities less than 0.03, and definite enzymic activity only to more than 0.05 unit/assay (equivalent to 1.0 unit/g). This value has not been subtracted from the results of the tryptophan oxygenase assays reported here.

Tyrosine aminotransferase activity was assayed by the enol–borate method of Lin *et al.* (1958). Additions to the 3.5-ml reaction mixture were: 2.0 ml of tyrosine–borate buffer (0.006 M L-tyrosine in 1.0 M sodium borate buffer, pH 7.8), 0.40 ml of 0.2 M α-ketoglutarate, 0.20 ml of 0.025 M diethyl dithiocarbamate (sodium salt), 0.10 ml of pyridoxal phosphate (300 μg/ml), 0.25 ml of phenylpyruvate tautomerase (EC 5.3.2.1) (activity 2*k*/ml (Constantsas and Knox, 1966)), 0.50 ml of distilled water, and 0.05 ml of 25% liver supernatant. After mixing, the initial change in absorbance at 310 mμ was measured. Blank values without liver or without α-ketoglutarate were equivalent to less than 2 units/g of liver and were subtracted.

The formamidase method of Knox (1955b) was modified by the incubation and EDTA addition was used for the tryptophan oxygenase assay. A 0.05-ml aliquot of fresh rat liver supernatant was incubated as described for assay of tryptophan oxygenase activity, with tryptophan omitted. Additions to the 3.0-ml assay mixture were: 0.30 ml of 0.01 M *N*-formylkynurenine dissolved in 0.20 M sodium phosphate buffer (pH 7.0), 0.40 ml of 0.20 M sodium phosphate buffer (pH 7.0), 1.15 ml of distilled water, 1.00 ml of 0.15 M neutralized Na_2EDTA, 0.05 ml of the incubated liver fraction, and 0.10 ml of freshly neutralized 0.30 M ascorbic acid. After mixing, the initial change in absorbance at 360

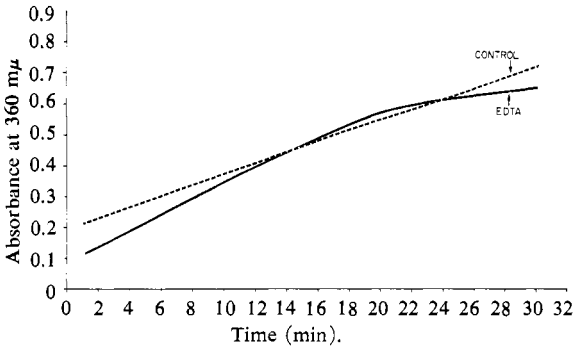

FIGURE 1: Effect of EDTA on assay for tryptophan oxygenase activity in young rat liver supernatants. Assays are shown with and without 0.04 M EDTA added to the liver supernatant from a 21-day-old hydrocortisone-injected rat. The activities were 1.04 and 0.73 units/assay, or 20.9 and 14.6 units/g, respectively, with and without EDTA.

TABLE I: Effect of Addition of EDTA during the Assay of Liver Supernatants from Young Rats for Tryptophan Oxygenase Activity.[a]

	Tryptophan Oxygenase Act.		
Age (days)	No EDTA (units/g)	Plus EDTA (%)	Optimal EDTA (M)
Newborn	0.6	154	0.063
1	0.4	211	0.038
7	1.2	159	0.050
10	2.3	157	0.050
14	4.8	125	0.050
21	13.5	151	0.050

[a] Na_2EDTA (0.15 M, pH 7.0) was added to the assay mixtures in place of an equal volume of distilled water. The final concentration of EDTA was varied from none to 0.075 M, and the results with the optimal EDTA concentration are recorded as per cent of the activity without EDTA. The rats of different ages were treated with hydrocortisone.

mμ was measured as in the tryptophan oxygenase assay. The formamidase activity of enzyme preparations measured under these conditions used for the tryptophan oxygenase assay was the same as with the unmodified method of Knox (1955b). Blanks without enzyme were equivalent to 0.5 unit/g of liver and were not subtracted.

The dependence of activity on enzyme concentration was established for each of the three assays used. Activities are expressed as units (micromoles of product formed per hour at 25°) per gram of wet liver.

Results

Optimal Conditions for Assay of Tryptophan Oxygenase in Neonatal Rats. Additions of 0.025 M EDTA and formamidase to the assay were tried, which permitted Spiegel and Spiegel (1964a) to demonstrate activity in livers of 1-day-old rats. The results of the addition of various concentrations of EDTA are summarized in Table I. EDTA increased the apparent tryptophan oxygenase activity about 50% when added to the assay. The maximum effect was obtained with 0.05 M EDTA. The effect was no different if the addition was made during the prior incubation period. EDTA did not change the requirements of the incubation or assay. The addition of EDTA increased the initial rate of the reaction but shortened its linear period, as shown in Figure 1. Additional EDTA shortened the linear period even more. The increase in the activity occurred in extracts from livers up to 3 weeks of age, a period during which the absolute activity increased at least tenfold. EDTA under the same conditions had no effect on the activity of extracts from livers of adult rats.

The contention that chelation of copper, present in relatively high concentration in newborn rat liver, accounted for the activation of tryptophan oxygenase by EDTA (Spiegel and Spiegel, 1964a) was tested with the more specific chelator of copper-sulfonated bathocuproine (Table II). Low concentrations of this reagent inhibited tryptophan oxygenase to the same extent, whether added to the prior incubation or to the assay. In the presence of concentrations of bathocuproine that were partially inhibitory, EDTA did not have its usual activating effect. This result is consistent with the binding of inhibitory copper by the bathocuproine, even though very low concentrations of it did not regularly activate as did EDTA. The activation may be lost because the neonatal rat liver enzyme, like that from *Pseudomonas*, is inhibited by bathocuproine, an effect that has been attributed to the participation of copper in the enzyme reaction (Maeno and Feigelson, 1965).

Although it has long been agreed that formamidase activity is in excess over tryptophan oxygenase activity in adult and fetal rat liver (Knox, 1951; Nemeth, 1961), Spiegel and Spiegel (1964a) claimed that formamidase activity was rate limiting, and that its addition increased the conversion of tryptophan to kynurenine during the assay of liver homogenates from newborn rats. Table III summarizes the results obtained upon addition of two different purified formamidase preparations to tryptophan oxygenase assays of liver supernatants from young rats of various ages. The assay mixtures contained additional formamidase equivalent to that present in an assay of adult liver. Addition of the extra formamidase was not required to measure all the apparent tryptophan oxygenase activity present. Actual measurements of endogenous formamidase activity in liver supernatants derived from rats of various ages and assayed under the conditions used

TABLE II: Effect of Bathocuproine on the Tryptophan Oxygenase Assay.[a]

Bathocuproine		EDTA in Assay[b]	
In Preliminary Incubn (M)	In Assay (M)	None	0.05 M
None	None	100	140
None	2.5×10^{-2}	0	
None	2.5×10^{-3}	57	
None	2.5×10^{-4}	76	
4.8×10^{-4}	6.4×10^{-5}	46	49
2.4×10^{-4}	3.2×10^{-5}	74	70
None	2.5×10^{-5}	97	
2.4×10^{-5}	3.2×10^{-6}	111	94
2.4×10^{-6}	3.2×10^{-7}	94	100

[a] Liver supernatant fractions from 14-day-old rats treated with hydrocortisone were assayed with and without EDTA after addition of bathocuproine to the preliminary incubation or the assay mixture. The average activity from two pools of liver is expressed as per cent of untreated enzyme in the standard assay (7.2 units/g). [b] Per cent of control activity.

in the tryptophan oxygenase assay are presented below. They showed that the absolute formamidase activity was already high at birth, and that its relative excess over the tryptophan oxygenase activity was very large, and greater in young than in adult rats.

The Effect of Age on the Basal and Hydrocortisone-Induced Activities. The liver and body weights of the rats examined at the various ages are shown in Figure 2. Hydrocortisone treatment caused no significant difference in these parameters. The ratio of the weight of the liver to the total body weight was high (0.062–0.066) in the 19–22-day-old fetal rats, decreased to a minimum of 0.026 in the 10-day-old animal, and then rose again to 0.033–0.037 in rats at 21 days and older. The enzyme activities, expressed here per gram of liver, show the same changes plus the effects of the concurrent changes in relative sizes, if expressed per total liver or per unit body weight. The activities measured in untreated rats are called "basal enzyme," and those (when elevated) in rats treated with hydrocortisone the "induced enzyme."

TABLE III: The Effect of Addition of Purified Formamidase during the Assay of Liver Supernatants from Young Rats for Tryptophan Oxygenase Activity.[a]

	Act. (units/g)	
Age (days)	Control	Plus Formamidase
Newborn	0.8	0.5
1	0.6	0
7	1.7	1.9
10	1.8	1.4
14	3.6	3.7
21	24.0	25.2

[a] Purified formamidase (11.6 μmoles/hr at 25°) was added to the assay mixtures containing 0.05 M EDTA. Rats were treated with hydrocortisone.

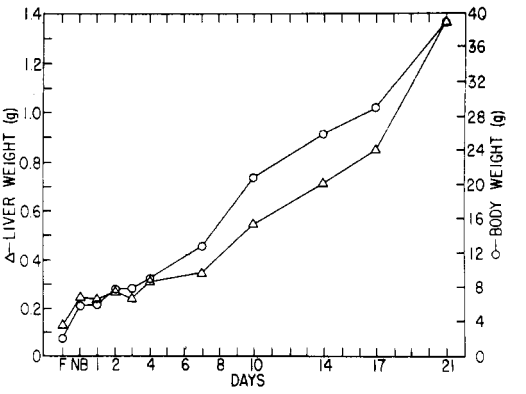

FIGURE 2: The liver (—Δ—) and body (—o—) weights of rats at the ages studied. Each point represents 12–30 rats, made up of equal numbers untreated and treated with hydrocortisone. The standard error of the mean was less than 5% of each value.

Formamidase activity in the livers of untreated young rats and those receiving hydrocortisone at various ages is shown in Figure 3. Hydrocortisone administration had no significant effect on the basal level of formamidase activity at any age. A relatively small activity of formamidase was present in the 19–22-day-old fetal rats, and there was an immediate fivefold increase in activity after birth which was thereafter maintained. This change preceded any marked changes in liver or body weights.

The formamidase activity was in equally large excess over the apparent tryptophan oxygenase activity of fetal rats (50/0.85 = 59-fold excess) and in untreated 21-day-old rats (310/5.5 = 57-fold excess). This excess was greater than in older rats in which the tryptophan oxygenase was raised by hydrocortisone treatment. These direct assays confirmed the conclusion drawn from the additions of extra formamidase (Table III) that formamidase activity did not limit the assay of tryptophan oxygenase activity in young rats.

The basal and hydrocortisone-induced tryptophan oxygenase activities are plotted against the age of rats in Figure 4. The apparent activity in the livers of uninjected rats through 14 days of age averaged 0.9 unit/g, which was slightly above the blank value without enzyme (equivalent to 0.6 unit/g) but less than the activity of 1.0 unit/g at which definite tryptophan oxygenase activity could clearly be recognized by this assay. Thus we could not confirm the presence of sig-

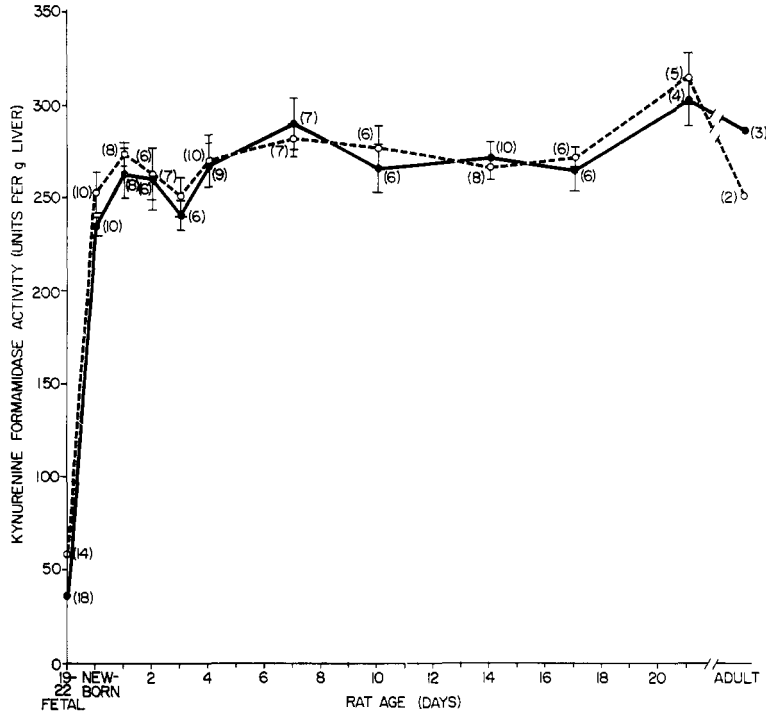

FIGURE 3: The change in formamidase activity with age in young rats' liver. Average values from untreated (---O---) and hydrocortisone-treated (—●—) rats in the numbers shown in parentheses are given with plus and minus standard error of the mean for each point indicated by brackets. The adult rats are pregnant females.

nificant activity in the first days after birth (Spiegel and Spiegel, 1964a). After day 7, significant tryptophan oxygenase activity appeared in the livers in response to hydrocortisone. The induced activity following hydrocortisone in the 10-day-old rats was 2.7 ± 0.3, three times the apparent basal level of 0.9 ± 0.1 (units per gram plus and minus standard error of the mean for nine rats in each group). This difference was highly significant ($p < 0.01$). The difference was equally significant statistically on days 4 and 7, but the absolute magnitudes were less. The appearance of the induced enzyme on day 7 or earlier thus preceded the appearance of significant basal enzyme activity after day 14. At 21 days of age the basal activity (of rats not stressed by separation from their litter) and the increase due to hydrocortisone administration were both comparable to those observed in adult rats.

TABLE IV: The Tryptophan Oxygenase Activity of 21-Day-Old Rats Separated from Their Litters.[a]

Treatment	Tryptophan Oxygenase (units/g ± SEM)
Unseparated	5.5 ± 0.6 (6)[b]
Separated	8.9 ± 0.9 (11)[b]
Unseparated + hydrocortisone	20.0 (2)
Separated + hydrocortisone	19.4 ± 0.9 (10)

[a] Rats (21-day-old) were separated from their litter approximately 6 hr prior to sacrifice, or not removed from their litters ("unseparated"). The litters were divided among the groups. The tryptophan oxygenase activity is expressed as the mean plus and minus the standard error of the mean, for the number of rats given in parentheses. [b] The difference between these two groups was significant at the level of $p = 0.02$.

Untreated 21-day-old rats and possibly 17-day-old rats had higher basal tryptophan oxygenase activities than their younger counterparts. As usual, they had been separated from their litters 6 hr prior to sacrifice. The tryptophan oxygenase activities found in livers of untreated and hydrocortisone-treated rats that had not been handled or separated from the litter are compared in Table IV to the activities obtained from those that were separated. There was no difference between the separated and unseparated rats that were treated with hydrocortisone. The untreated rats that were separated had significantly more activity than the unseparated rats. This increase in tryptophan oxygenase activity with separation from the litter may indicate that a significant adrenocortical secretion accompanies this separation in rats of normal weaning age, but not at ages earlier than 17 days.

Basal and hydrocortisone-induced tyrosine aminotransferase activities are plotted against rat age in Figure 5. Basal tyrosine aminotransferase activity

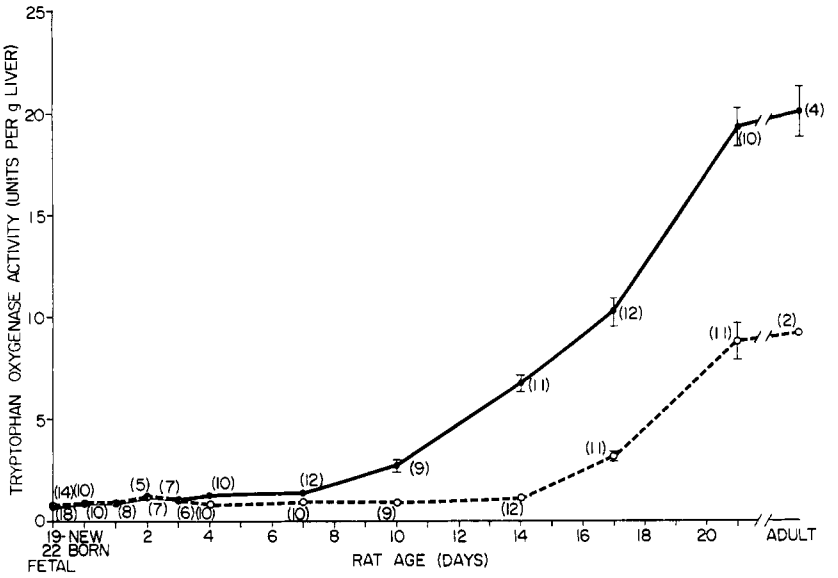

FIGURE 4: The effect of age on tryptophan oxygenase activity in livers of young rats. Values from untreated (---o---) and hydrocortisone-treated (—●—) rats are presented as described in Figure 3. The effect of removal of the 21-day-old rats from their mother is apparent in their elevated basal level. The increase with hydrocortisone on day 10 was highly significant ($p < 0.01$).

appeared at birth, increased markedly in the first 48 postnatal hr, and then decreased until 7 days of age. At 17 days of age it rose again, perhaps in consequence of the separation from the litter that elevated the tryptophan oxygenase in these same rats. The adrenal glands apparently participate in the initial rise immediately after birth (Sereni *et al.*, 1959; Greengard *et al.*, 1963), and this probably explains why an additional effect with hydrocortisone could not be obtained until 72 hr after birth. After this, a large hydrocortisone effect was apparent throughout the youth of the animal, with values occurring around 14–21 days of age that were greater than in adult rats.

Discussion

Quantitative assay of an enzyme by its activity can affirm its presence above some critical amount, but not its absence below that amount. The formamidase assay used here detected as little as 0.002 of the adult level. Considerably more than this was present in the livers at the earliest ages examined. The assay of tryptophan oxygenase and tyrosine aminotransferase detected as little as 0.15 and 0.025 of the adult basal levels of the enzymes, respectively. There was less than these detectable amounts, perhaps none, of the latter two enzymes present at the earlier ages. In studies like these of the developmental formation of enzymes, the enzyme is said to "appear" when amounts detectable by the assay used are first formed. More sensitive assays will be needed to determine if the enzymes are already present but in amounts below detection with the assays used.

We could not confirm the contention of Spiegel and Spiegel (1964a) that tryptophan oxygenase activity was detectable in newborn rats, even if copper were removed by EDTA and if formamidase were added. In agreement with previous workers we found that formamidase did not limit kynurenine formation at any age in various species (Knox, 1951; Knox and Mehler, 1951; Knox, 1955a; Nemeth and Nachmias, 1958; Nemeth, 1961; Spiegel and Spiegel, 1964b), although in young rats with enzyme the addition of 0.05 M EDTA did increase the activity somewhat. Even with this modification and the improved assay system now used (Knox *et al.*, 1966a; Knox and Piras, 1966) there was no significant tryptophan oxygenase activity in the newborn rats. Until 14 days of age the apparent activity in untreated rats was below the limit (1 unit/g) at which the absorbancy changes definitely reflected significant kynurenine formation. The lack of significant tryptophan oxygenase activity in the newborn rat reported here is in agreement with most earlier studies (Auerbach and Waisman, 1959; Nemeth, 1959; Auricchio *et al.*, 1960; Nemeth, 1961; Ginoulhiac *et al.*, 1962; Greengard and Feigelson, 1963; Schapiro *et al.*, 1966). Nevertheless, it is reasonable to suppose that a little enzyme might be present some time before 14 days of age because the apparent activity then shows an additional characteristic of the enzyme. This is its increase after hydrocortisone treatment, which was demonstrable after the fourth day. Previously, Goldstein and Knox (1963) obtained a significant hydrocortisone effect in the 14-day-old but not in the 7-day-old rat.

A detectable amount of tyrosine aminotransferase, which is relatively less than the detectable amount of tryptophan oxygenase, was absent in the fetal liver

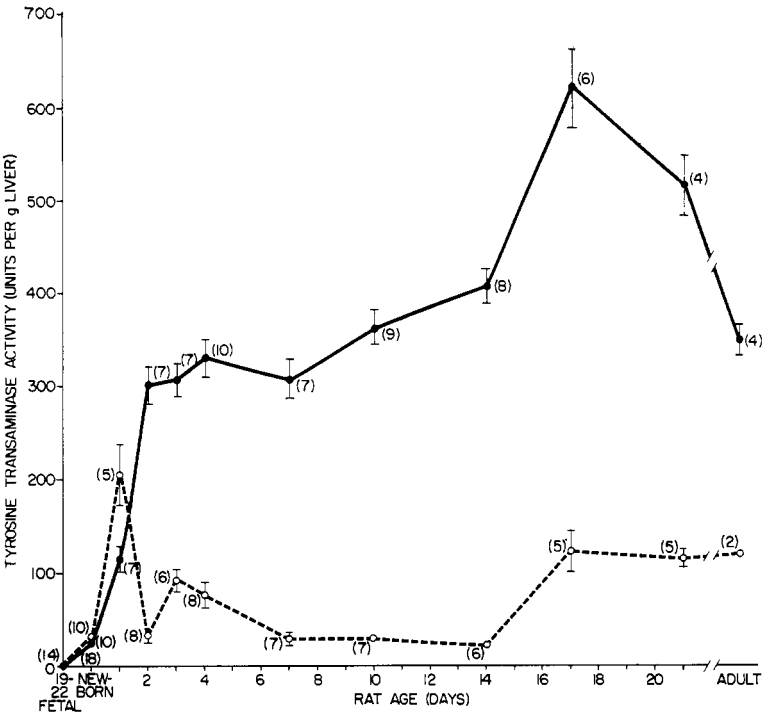

FIGURE 5: The effect of age on tyrosine aminotransferase activity in young rats. Values for untreated (---o---) and hydrocortisone-treated (—●—) rats are given.

and rose dramatically after birth as previously shown (Kretchmer and McNamara, 1956; Auerbach and Waisman, 1959; Sereni *et al.*, 1959; Schapiro *et al.*, 1965, 1966). Maximal levels were reported to be reached on day 1 (Auerbach and Waisman, 1959) or after 12 hr (Sereni *et al.*, 1959); our results show that the elevation persists for 48 hr. Presumably, the newborn rat liver is maximally stimulated by glucocorticoids, so there was no added response to hydrocortisone treatment at that time. From the age of 2 days, tyrosine aminotransferase responded to hydrocortisone as markedly as it did in adult rats. Thus, the failure of tryptophan oxygenase to be induced during the first few days of life is clearly not due to the failure of the hydrocortisone to reach the liver. The absence of detectable amounts of both tryptophan oxygenase and tyrosine aminotransferase in fetal liver is also unlikely to be due to the lack of hydrocortisone, since glucocorticoids can cross the rat placenta (Moog, 1959; Jost, 1966; Milkovic and Milkovic, 1966), and furthermore, the administration of hydrocortisone directly to fetal rats does not cause the enzymes to appear (Sereni *et al.*, 1959; Nemeth, 1961; Litwack and Nemeth, 1965; Greengard and Dewey, 1967). Other inducers may uniquely govern the developmental formation of each of these enzymes. For tyrosine aminotransferase, glucagon may be one of these factors, since it induced this enzyme in fetal rats (Greengard and Dewey, 1967).

The elevations of the basal levels of tryptophan oxygenase and tyrosine aminotransferase activities in 17–21-day-old rats that were separated from their litters (Figures 4 and 5 and Table IV) were apparently inductions by stimulated secretions of the adrenal cortex. A doubling of plasma corticosterone concentration has been observed in young rats separated in this way (Levine, 1967). The absence of such elevations at earlier ages, when the enzymes can nevertheless respond to administered hydrocortisone, suggests a lower responsiveness to stress of the pituitary–adrenocortical system at the younger ages. Other studies have revealed such a period of unresponsiveness (Moog, 1959; Jost, 1966; Milkovic and Milkovic, 1966; Levine and Mullins, 1966). Schapiro *et al.* (1966) also found a lower response of tryptophan oxygenase to mechanical stress in 15-day-old rats than in 21-day-old and adult rats.

Formamidase differed from the other two enzymes studied by being definitely present in the rat fetus prior to birth. At birth there was an explosive increase in the activity to adult levels, occurring somewhat earlier than the rise in tyrosine aminotransferase. Nemeth (1961) noted the existence of formamidase in the 21-day-old fetal rat liver, but the dramatic rise in the activity of this enzyme at birth has not previously been reported. Knox and Eppenberger (1966) found a similar pronounced increase in this enzyme in the livers of chicks at hatching, which has been confirmed (Wagner and Payne, 1967).

The three enzymes studied here each showed a unique pattern of development with time. The time of appearance of liver tryptophan oxygenase activity

has been related to the maturity of fetuses at the time of birth in different mammals (Nemeth, 1959; Careddu *et al.*, 1961; Nemeth, 1961; Vassella and Hellström, 1962). The same gradual development of maturity cannot be responsible for the totally different patterns of development of the formamidase and tyrosine aminotransferase occurring in the same rat livers. These three enzymes had still other developmental patterns in the chick liver (Knox and Eppenberger, 1966). Both tryptophan oxygenase and tyrosine aminotransferase were present long before hatching, but only the latter was inducible by hydrocortisone. Only the formamidase in the hatching chick behaved like its counterpart in the newborn rat. In both species it was unresponsive to inducers but developed dramatically during hatching and birth. It would seem reasonable to suppose that these varied developmental changes are due to a variety of rapid metabolic alterations that are specific for each particular enzyme, in each tissue, and in each species.

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