at distant sites. Near the implantation site of threshold doses of atropine, 66,000 to 88,000 dpm (disintegrations per minute) per milligram of tissue (dry weight) were recorded as compared to 1 to 6 dpm/mg of blood (dry weight) from the general circulation.

Small quantities of atropine, applied in crystalline form (*3*), seem to produce an effective spread which appears to be limited to a sphere of 1.0 to 1.8 mm in diameter. The quantity of the drug, within the limits of our dosage, appeared to increase the intensity of the activity within this sphere but not to significantly increase its diameter. Electrophysiological observations (*10*) confirm this finding. Microelectrode recordings obtained from cells more than approximately 1.5 mm from the tip of the drug implant do not, typically, react to even fairly large doses of the drug. We emphasize that our observations apply only to small quantities of atropine and perhaps closely related chemical substances which are permitted to dissolve in the tissue fluids, by a technique which does not induce mechanical forces due to the injection of liquids under pressure.

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8. ^{3}H-Atropine, specific activity 434 mc/mmole, was generally labeled by an exchange procedure and purified by sublimation and crystallization. Its radiochemical purity was determined by thin-layer chromatography (Amersham/Searle, Des Plaines, Illinois). The chemical nature of the radioactive material at the time of tissue excision was not determined.
9. Silver grain counts in areas remote from the implantation site gave up to 50 to 100 silver grains per 625,000 μm^2 in autoradiograms obtained between 5 and 12 minutes after the implantation. Considering that the medium range of β-particles from tritium is 1 μm and about 20 disintegrations statistically result in 1 silver grain [A. W. Rogers, *Techniques of Autoradiography* (Elsevier, Amsterdam, 1967)], it was calculated that 1 mg (dry weight) of brain contained 4.9×10^{-6} μc of radioactivity for 50 randomly distributed silver grains. This is equivalent to 3.3×10^{-6} μg of atropine. The radioactivity at the implantation site proper ranged from 0.02 to 0.03 μg of ^{3}H-atropine per milligram (dry weight), calculated from 10- to 12-mg samples of tissue containing the implantation core.
10. E. Kent and S. P. Grossman, unpublished observations.
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Hypobaric Hypoxia: Effects on Early Development of Tryptophan Oxygenase in Neonatal Rats

Abstract. *Despite a reduction in liver and body weights of neonatal rats born and reared at a simulated altitude of 5790 meters (oxygen pressure, 76.36 millimeters of mercury), the hepatic enzyme tryptophan oxygenase develops prematurely in these stressed animals as compared to controls reared at sea level. Also, the specific activities remain distinctly elevated through the first 9 days of age; thus, the competence for premature synthesis of tryptophan oxygenase is confirmed in neonatal rats.*

Premature development of enzymes has been effected by the administration of chemical stimuli either directly to the fetus or by way of the maternal circulation. Intraperitoneal injection of thyroxin into fetal rats increases the activities of liver glucose-6-phosphatase and reduced nicotinamide-adenine dinucleotide phosphate : cytochrome c oxidoreductase (*1*). Similarly, the fetal intraperitoneal injection of dibutyryl cyclic adenosine monophosphate (AMP) causes the premature induction of both tyrosine aminotransferase and glucose-6-phosphatase (*2*); injections *in utero* of glucagon into fetal rats cause an increase in tyrosine aminotransferase activity (*3*). L-Tyrosine fed to pregnant rabbits increased significantly the capacity of the liver of newborns to oxidize tyrosine as soon as 2 hours after birth (*4*).

In newborns, other means have been utilized to produce alterations in normal developmental patterns. For example, development of hepatic glucokinase in weanling rats can be induced or repressed by the glucose content of the diet (*5*); and simulated altitude conditions affect the activity as well as the isozyme patterns of lactate dehydrogenase in neonatal rats (*6*). Also, exposure to hypoxia for 12 and 24 hours increases the activity of glycolytic enzymes in newborn rabbits (*7*).

In normal rats, appreciable tryptophan oxygenase activity does not appear until day 12 after birth, while animals treated with L-tryptophan demonstrate hepatic tryptophan oxygenase activity as early as day 3 (*8*). In addition, injection of hydrocortisone produces significant tryptophan oxygenase activity in 7-day-old rats (*9*). Because exposure of adult animals to hypobaric hypoxia results in the induction of tryptophan oxygenase (E.C. 1.13.1.12, L-tryptophan : oxygen oxidoreductase) in the liver (*10*), we sought to determine whether this stress would similarly enhance the development of this enzyme in neonatal rats.

Sprague-Dawley rats (Charles River Breeding Laboratories) at the same stage of gestation were placed in separate cages with adequate nesting material and free access to food and water. All animals were housed at 21° ± 2°C in rooms having a 12-hour light (0600 to 1800 hour) period followed by a 12-hour dark (1800 to 0600 hour) period. At least 4 days before scheduled parturition, the experimental animals were brought to a simulated altitude of 5790 m (oxygen pressure, 76.36 mm-Hg) in closed Plexiglas chambers partially evacuated by a vacuum pump. Barometric pressure was controlled by an adjustable air-inlet valve. The animals remained at this simulated altitude through the remainder of pregnancy and birth, and throughout the postpartum period, with return to sea level only to remove neonatal animals. Control animals maintained at sea level were housed in the same room under the same cage conditions. Neonatal rats were weighed and killed; the livers were immediately excised, weighed, and frozen on Dry Ice.

Tryptophan oxygenase activity was assayed with slight modification of the methods of Knox and Auerbach (*11*). Whole homogenates were prepared in 0.14*M* KCl containing 2.5 m*M* NaOH. Methemoglobin (0.5 mg) and L-ascorbate (10 μM) were added to the incubation medium, and the reactions proceeded for 1 hour at 37°C under an atmosphere of O_2 and CO_2 (95 : 5) for maximum, consistent enzymatic ac-

Table 1. Mean weight ± S.E. of bodies and livers of neonatal rats reared at sea level and at a simulated altitude of 5790 m. The figures in parentheses refer to the number of animals sampled at each time interval.

Age (days)	Body weight (g)		Liver weight (g)	
	Sea level	Altitude	Sea level	Altitude
0*	5.97 ± 0.49(2)	5.62 ± 0.12(3)	0.34 ± .03	0.28 ± .03
1	6.77 ± 0.28(4)	6.23 ± 0.23(5)	0.33 ± .01	0.25 ± .01
2	8.15 ± 0.30(6)	6.29 ± 0.51(6)	0.30 ± .01	0.23 ± .02
5	11.74 ± 1.18(4)	8.55 ± 1.00(9)	0.38 ± .05	0.31 ± .02
6	14.19 ± 0.34(3)	9.74 ± 0.44(3)	0.49 ± .01	0.31 ± .01
9	21.72 ± 1.82(4)	10.64 ± 2.09(4)	0.65 ± .04	0.30 ± .04

*Time 0 rats were selected 4 to 6 hours after birth.

tivity. To avoid the effects of circadian periodicity on enzyme activity (*12*), all animals were killed between 0800 and 0830 hours.

The hypoxic conditions produced by simulated altitude (5790 m) had a detrimental effect on both the viability and the growth of fetal and neonatal rats. Of the 11 pregnant rats at that altitude, three probably resorbed the fetuses, one delivered but killed the offspring, and seven produced litters having reduced numbers of living newborns as compared to the five pregnant controls at sea level. Both body and liver weights for newborns reared at the simulated altitude were appreciably lower than those of the control animals through 9 days of age (Table 1), despite the reduced competition for food among the former group. Although growth was severely retarded, the development of hepatic tryptophan oxygenase was greatly stimulated in these rats (Fig. 1). Thus, at each interval studied, the specific activity of this enzyme in hypoxic rats was higher than in the controls. On days 1 and 2 the newborns reared at the simulated altitude had enzyme activities up to five times greater than controls. Thereafter, the specific activities remain elevated, although the total activities in the liver are comparable in the two groups. To determine whether enzyme activity is altered by exposing neonatal animals reared at sea level to a hypoxic environment, 9-day-old rats were brought to altitude for 3 days in a separate experiment. These animals also demonstrated a reduction in body and liver weights, with a 50 percent concurrent increase in specific activity (1.07 as opposed to 1.5 μmole of kynurenine formed per hour per gram).

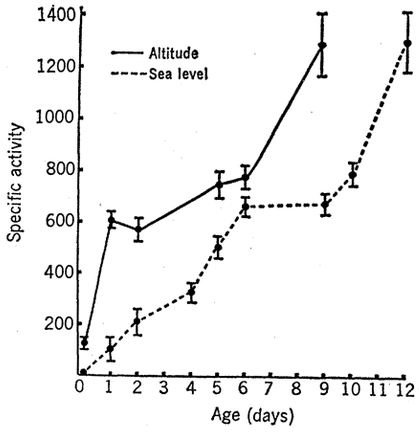

Fig. 1. Effects of hypobaric hypoxia (5790 m, simulated altitude) on the early development of hepatic tryptophan oxygenase in neonatal rats. Solid line represents values for animals reared at high altitude, broken line for sea level controls. Specific activity units are millimicromoles of kynurenine formed per hour per gram (wet weight). Values are means ± S.E. for activities of non-littermates; for the number of individual assays see Table 1.

Although hypoxia reduces the growth rate of neonatal rats, this environmental stress increases the activity of tryptophan oxygenase, probably by stimulated protein synthesis. Growth in general is retarded by exposure to acute hypoxia (*13*); in fact, maintenance of growth rate in young animals is one of the suggested criteria of altitude acclimatization. However, a general decrease in anabolism cannot be interpreted as repression of all protein synthesis. In the developmental period, the appearance and activity of an enzyme are regulated by controls specific for the particular protein.

In the neonatal rat, however, this tivity of hepatic tryptophan oxygenase can be regulated by several factors including glucocorticoids (*14*). Induction of tryptophan oxygenase as a result of hypoxic stress may represent a hypophyseal-adrenal response resulting in increased glucocorticoid secretion, which in turn effects a *de novo* synthesis of this enzyme.

In the neonatal rat, however, this mechanism is associated with the matter of competency. For example, a single injection of glucagon or epinephrine in the terminal fetus induces increased tyrosine aminotransferase activity (*2*), but if the injection is made more than 3 days before scheduled birth, no response is elicited; however, the younger fetus will respond to the administration of dibutyryl cyclic AMP, an indication that the earlier fetuses are unable to respond to glucagon or epinephrine with the production of cyclic AMP. Evidently certain genic constituents are inactive in the early fetuses rendering them incompetent to react to the administered stimuli. Significantly, tryptophan oxygenase can be induced prematurely by the administration of tryptophan and hydrocortisone, respectively, thus demonstrating the competency of neonatal rats for synthesis of hepatic tryptophan oxygenase prior to the time of normal development (*8*, *9*). Although the specific activity of tryptophan oxygenase in our study is relatively low, these experiments confirm the competency for synthesis of this enzyme even in the first day after birth. Moreover, throughout the period of early development the specific activity of hepatic tryptophan oxygenase of rats reared at high altitude was significantly elevated as compared to sea level controls of the same age. Hence, although the overall body growth rate and liver weight are both repressed by hypobaric hypoxia, the premature development of a particular protein is demonstrated.

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