LASTING BIOLOGICAL EFFECTS OF EARLY ENVIRONMENTAL INFLUENCES

VIII. Effects of Neonatal Infection, Perinatal Malnutrition, and Crowding on Catecholamine Metabolism of Brain

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Physiological and environmental stresses have been shown to alter the catecholamine metabolism of brain. For example, electric shocks (1) and cold exposure (2) significantly reduce the brain content of norepinephrine. In mice, psychosocial stimulation leads to a marked increase in the catecholamine content and in the enzymatic activities of phenylethanolamine-*N*-methyl transferase (PNMT), monoamine oxidase, and tyrosine hydroxylase. In contrast, isolation decreased PNMT and tyrosine hydroxylase activities but not the concentration of catecholamines (3). Pharmacologically induced increases in nerve activity (4) and various types of stress (5, 6) all lead to an increase in the activity of enzymes involved in catecholamine synthesis.

The catecholamine content of the adrenergic neuron may remain constant despite marked fluctuations in the competing processes of biosynthesis and utilization of catecholamine. Measurement of the rate of conversion of radioactive precursors into specific catecholamine, or the rate of catecholamine turnover, therefore constitutes more reliable index of catecholamine metabolism than do tissue levels.

In previous publications we have described the lasting biological effects of neonatal infection and early undernutrition on the physical development and brain metabolism of specific pathogen-free mice. These stresses decrease the biosynthesis of brain protein and ribonucleic acid (7), alter cyclic adenosine 3′,5′-monophosphate (cyclic AMP)[1] metabolism, and impair binding ability of cyclic AMP to synaptosomes (8); the synaptosomes may play a role in catecholamine storage and release, synaptic function, and processes mediated by neurohormones (9, 10).

The present paper reports (*a*) the effects of neonatal infection, perinatal malnutrition, and crowding on the metabolic turnover of brain catecholamine, and (*b*) the hormonal regulation on the brain level of catecholamine. The metabolic turnover of catecholamine was estimated from the incorporation of tyrosine-^{14}C into brain tissue and the catabolic activities of norepinephrine-^{3}H at various times after intracisternal administration. A study was also made of the effect of maternal treatment with growth hormone on the brain enzymatic activity and on the catecholamine contents of the offspring.

[1] *Abbreviation used in this paper:* cyclic AMP, cyclic adenosine 3′,5′-monophosphate.

Materials and Methods

Enterovirus and Experimental Animals.—The origin of the mouse enterovirus preparation used in the present study has been described in an earlier paper (11).

Specific pathogen-free mice of the COBS strain (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were used in all experiments.

The malnourished group consisted of the progeny of mothers fed a diet containing 20% wheat gluten as the sole protein source. The dams were placed on the gluten diet from day 14 of pregnancy to day 21 after delivery (7). After weaning all animals were transferred to D & G pellets (Dietrich & Gambrill, Inc., Frederick, Md.). Control animals were kept on D & G diet throughout the duration of the experiment.

The crowded group consisted of the progeny of mothers housed in 7 × 11½ × 5 inch cages, four dams per cage during gestation and lactation. During lactation four dams nursed

TABLE I

Outline of Experiments for the Study of the Effects of Crowding

Housing conditions	Gestation period (mother/cage)	Lactation period (young/mother)	Age		
			3 wk	1 month (young/cage)	> 2 months
Controls	1	8/1	8	5	5
Crowded	4	32/4	32 → 16, 16	16	16
Crowded shift back to noncrowded conditions	4	32/4	32 → 16, 16	16 → 5, 5, 5	5

The day after birth, all pups born to four mothers housed under crowded conditions were pooled and then reallocated to the lactating mothers in such a manner that the crowded group contained 32 young nursed by four mothers. In order to reduce the overcrowded condition at weaning, the crowded groups were divided into subgroups with 16 pups per cage. At 1 month of age the crowded mice were placed under noncrowded conditions, five mice per cage.

32 young per cage. After weaning 16 young were caged together. At 1 month of age some of the crowded group were redistributed in groups of five mice per cage. Thus, there were three experimental groups exposed to different housing conditions (Table I).

Administration of Tyrosine-^{14}C and Norepinephrine-^{3}H and Measurement of Radioactivity in Brain Tissue—Mice, lightly anesthetized with ether, received an intracisternal injection of 0.05 μCi of DL-tyrosine-(3-^{14}C) (specific activity 40 mCi/mmole; Schwarz Bio Research, Inc., Orangeburg, N.Y.) and 0.02 μCi of DL-norepinephrine-(7-^{3}H) (specific activity 3.0 Ci/mmole; Schwarz Bio Research, Inc.) in a volume of 0.1 ml. The animals were killed by neck dislocation ½, 1, 2, 4, or 8 hr after injection, and the brains were immediately removed and chilled in cracked ice. The brains were homogenized in 4 ml of ice-cold 0.9% sodium chloride solution with a Teflon grinder. Portions of the homogenates were precipitated with 10% trichloroacetic acid in an ice bath for 10 min. After centrifugation a 0.2 ml portion of the supernatant fluid was transferred to a vial, mixed with a small amount of solubilizer (Bio-Solv, formula BBS-3; Beckman Instruments, Inc., Fullerton, Calif.) and 5 ml of scintillation fluid containing 7% Liquifluor (New England Nuclear Corp., Boston, Mass.) and 93% toluene.

All samples were counted with a Packard scintillation spectrometer, model 3003 (Packard Instrument Co., Inc., Downers Grove, Ill.). The radioactivity of incorporated ^{14}C and ^{3}H was calculated according to the discriminator-ratio method (12).

Assay of Norepinephrine and Dopamine in Brain Tissue.—The catecholamines in brain homogenate were extracted with 15% 1 N formic acid plus 85% acetone (v/v) and separated from their precursors and other interfering substances by ion exchange chromatography of Amberlite CG 50 columns. The resin was eluted with 1 N hydrochloric acid. Catecholamines were oxidized to form fluorescent trihydroxyindole derivatives. The fluorescent compound was measured in alkaline solution (13) with a fluorometer (model 111, printed circuit type; G. K. Turner Associates, Palo Alto, Calif.).

Maternal Treatment with Growth Hormone.—Experimental animals received subcutaneously 0.2 IU of growth hormone (Mann Research Laboratories, Inc., New York) in 0.1 ml of 0.9% sodium chloride solution daily, from day 14 of pregnancy to day 14 after delivery. In groups not receiving growth hormone, 0.9% sodium chloride was injected in the same volume and at the same time. Body weight gain was measured daily during these periods.

Assay of Tyrosine Hydroxylase Activity.—For the determination of tyrosine hydroxylase activity, L-tyrosine-(3,5-^{3}H) (specific activity 52 Ci/mmole; Schwarz Bio Research, Inc.) was used as substrate and the amount of water-^{3}H produced during 3-hydroxylation was measured (14). Brain homogenates were mixed with the incubation medium and incubated in a water bath at 37°C for 30 min. The mixtures were centrifuged at 0°C and supernatants were transferred quantitatively to a small column of cation exchange resin, Dowex AG 50W-× 8 (200–400 mesh, hydrogen form, 0.5 × 4 cm; Bio-Rad Laboratories, Richmond, Calif.), which was placed on another column of anion exchange resin, Dowex AG1-×8 (200–400 mesh, hydroxide form, 0.5 × 4 cm; Bio-Rad Laboratories). Columns were then washed three times with 1-ml portions of distilled water. Aliquots (0.2 ml) of the combined effluents were transferred to counting vials, mixed with a small amount of solubilizer and 5 ml of scintillation fluid. Radioactivity was counted with a Packard scintillation spectrometer, model 3003.

RESULTS

Metabolic Turnover of Tyrosine-^{14}C and Norepinephrine-^{3}H.—Figs. 1 and 2 show the incorporation of tyrosine-^{14}C into the brain tissue of mice having experienced neonatal infection, perinatal malnutrition, or crowding. In all cases the incorporation of tyrosine-^{14}C into the brain tissue was rapid and reached a maximum 30 min after injection. After 1 hr there was a slow but progressive fall in the incorporation.

In mice neonatally infected with enterovirus, the amino acid incorporation was significantly decreased at 2 and 4 hr. In contrast, the progeny of malnourished mothers showed significantly higher incorporation at 1 hr after injection. Total or specific incorporation of tyrosine-^{14}C into brain tissue was lower in 2-month old mice than in 3-month old mice.

Animals raised under crowded conditions showed higher amino acid incorporation than the control group at 1 and 8 hr after injection. There was no significant change in amino acid incorporation when the crowded animals were shifted back to noncrowded conditions.

Figs. 3 and 4 show the catabolic activity of norepinephrine-^{3}H in brain tissue of mice subjected to neonatal infection, perinatal malnutrition, or crowding. In 3-month old mice maximum radioactivity in the brain was observed 1 hr

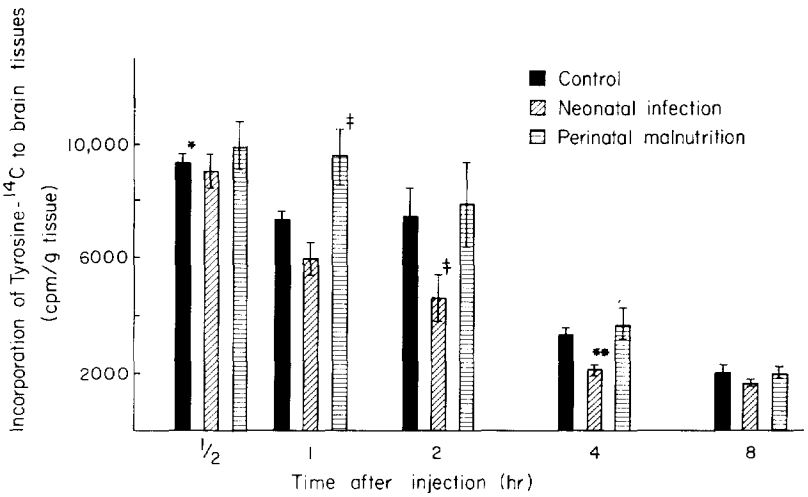


FIG. 1. Metabolic turnover of tyrosine-^{14}C in brain of mice exposed to neonatal infection and perinatal malnutrition. Altogether 12 experimental (6 in each group) and 5 control males, 3 months of age, received 0.05 μCi of tyrosine-^{14}C and 0.02 μCi of norepinephrine-^{3}H in a volume of 0.1 ml by the intracisternal route. (*), mean of respective group $\pm$ SE; (‡), $P < 0.05$; (**), $P < 0.01$.

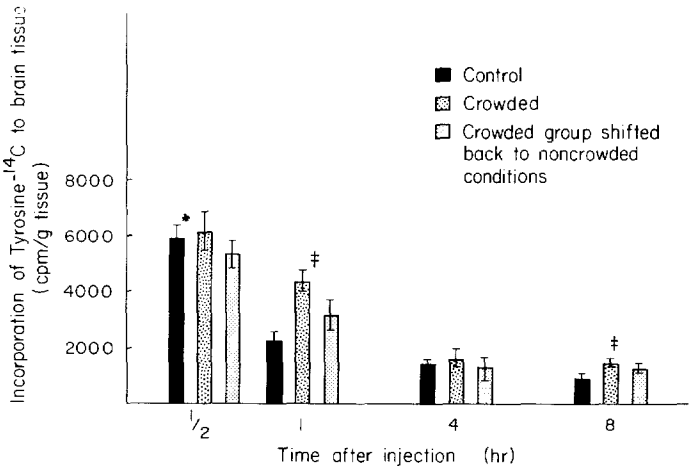


FIG. 2. Metabolic turnover of tyrosine-^{14}C in brain of crowded mice. Altogether 6 crowded, 5 crowded group shifted back to noncrowded conditions, and 6 control males, 2 months of age, were used. Doses of 0.05 μCi of tyrosine-^{14}C and 0.02 μCi of norepinephrine-^{3}H in a volume of 0.1 ml were administered by the intracisternal route. (*), Means of respective group $\pm$ SE; (‡), $P < 0.05$.

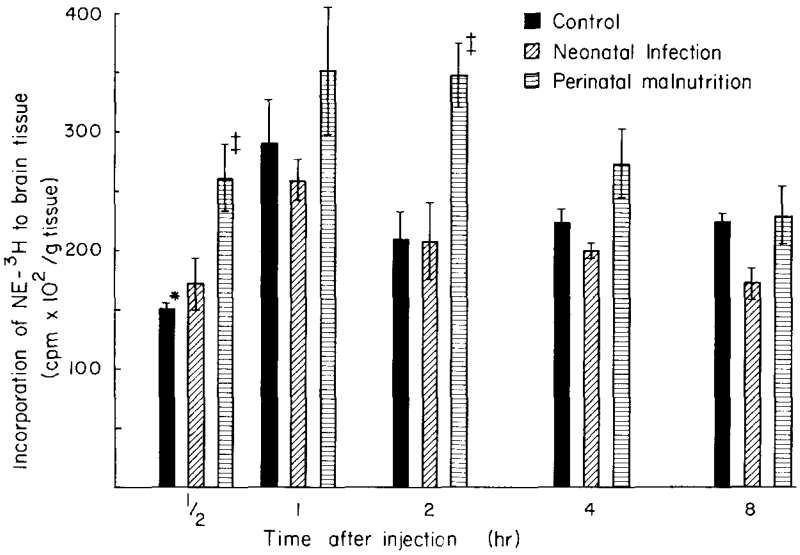


FIG. 3. Metabolic turnover of norepinephrine-^{3}H in brain of mice exposed to neonatal infection and perinatal malnutrition. The experimental conditions used were the same as described in Fig. 1.

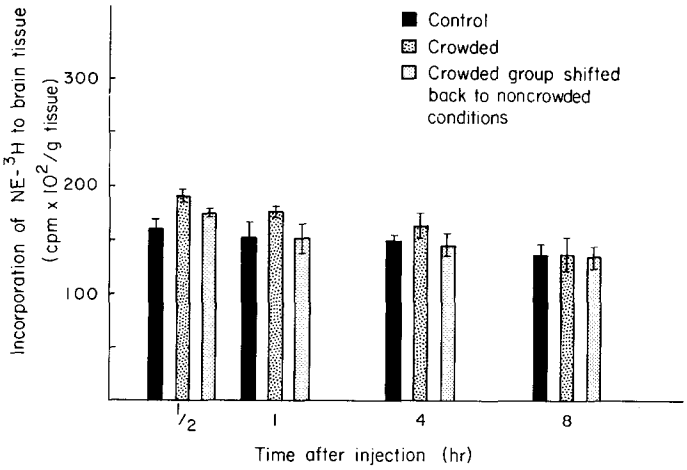


FIG. 4. Metabolic turnover of norepinephrine-^{3}H in brain of crowded mice. The experimental conditions used were the same as described in Fig. 2.

after injection of norepinephrine-^{3}H. There was no decline in total radioactivity in the brain from 2 to 8 hr after injection. There was no difference in catabolic activity of norepinephrine-^{3}H in brain, expressed in injected total radioactivity, between infected, crowded, and control groups. In progeny of malnourished

TABLE II

Brain Catecholamine Contents in Mice Exposed to Neonatal Infection, Perinatal Malnutrition, and Crowding

	3-month old males			2-month old males		
	Control	Infected	20% Gluten	Control	Crowded	Crowded shifted to noncrowded
Body weight, grams	42.6	38.8	32.5	38.4	33.0	34.8
Brain wet weight, milligrams	551	503	491	544	464	515
Catecholamine contents						
Dopamine						
μg/brain	0.375 ± 0.020*	0.329 ± 0.033	0.250 ± 0.020§	0.235 ± 0.035	0.197 ± 0.024	0.216 ± 0.024
(μg/g wet wt)	(0.702)	(0.660)	(0.517)‡	(0.429)	(0.412)	(0.416)
Norepinephrine						
μg/brain	0.215 ± 0.028	0.218 ± 0.037	0.138 ± 0.022§	0.187 ± 0.020	0.163 ± 0.010	0.165 ± 0.014
(μg/g wet wt)	(0.390)	(0.386)	(0.240)§	(0.344)	(0.340)	(0.318)

Each group consisted of eight males, 2 or 3 months of age. Catecholamine content is expressed per whole brain rather than per gram of protein or per gram of brain. The retardation of myelin development in the brain of malnourished animals contributed substantially to the brain's low weight. Since dopamine and norepinephrine were found only in catecholamine-containing neurons, calculations in terms of protein weight or brain weight might obscure significant effects caused by malnutrition in these neurons.

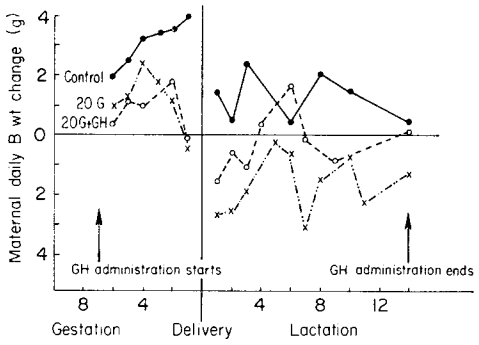
* Mean of respective group ± SE.

‡ $P < 0.05$.

§ $P < 0.01$.

mothers the total radioactivity from metabolic activity of norepinephrine-^{3}H was significantly higher than in the control group at ½ and 2 hr after injection.

Catecholamine Contents of Brain.—Table II shows the catecholamine contents of brain in mice exposed to neonatal infection, perinatal malnutrition, and crowding. As in the case of neonatal infection and perinatal malnutrition, one of the gross effects of crowding was inhibition of body growth. Pups de-



	Control	20% Gluten	20% Gln+GH
Average fetal wt. at birth (g)	1.68±0.03	1.14±0.03 ΔC -32%	1.53±0.04 ΔC -9% Δ20G +34%
Mean change of maternal B wt during first 14 days of lactation (g)	+6.4 (6)	-7.6 (5)	-1.1 (5)

FIG. 5. Effect of maternal treatment with growth hormone during gestation and lactation on reproductive performance. Groups of five pregnant mice fed the 20% gluten diet, five hormone-treated animals, and six controls were used. The experimental groups were fed 20% gluten diet from day 14 of pregnancy to day 21 after delivery. In the hormone-treated group on the 20% gluten diet, a dose of 0.2 IU growth hormone was administered subcutaneously daily, from 14 days of pregnancy to 14 days after delivery. In other groups 0.9% sodium chloride was injected instead of hormone. (ΔC), Per cent difference from control group; (Δ20G), per cent difference from 20% gluten group; (*), mean of respective group ± SE (10 males in each group).

livered by mothers from crowded conditions weighed 1.49 g at birth; in contrast, pups from control mothers weighed 1.69 g. At 2 months of age the body weight and wet weight of the brain were smaller in the crowded group than in the control group; but relative to the body weight, there was no difference in brain weight between these two groups. Shifting the crowded group back to noncrowded housing conditions improved physical growth.

The contents of the brain in norepinephrine and dopamine, expressed by either total or per unit weight basis, were significantly depressed in mice raised by malnourished mothers. However, there was no significant difference in catecholamine contents between infected, crowded, and control groups.

Effects of Maternal Treatment with Growth Hormone on the Reproductive Performance of Mother and on the Enzymatic Activity and Catecholamine Contents of Offspring.—Fig. 5 shows the effect of maternal treatment with growth hormone on the body weight of mother and offspring. The maternal body weight of controls increased by 2–4 g daily during the last week of pregnancy, and by 0.4–2.4 g daily during the 1st 2 wk of lactation. In malnourished pregnant

TABLE III

Effect of Maternal Treatment with Growth Hormone on Brain Enzymatic Activity and Catecholamine Contents of Offspring

	Control	20% Gluten	
		Nontreated	Growth hormone-treated
Body weight, grams	34.4	17.7	23.8
Brain wet weight, milligrams	526 ± 7.0*	438 ± 6.8‡	483 ± 9.0
Tyrosine hydroxylase activity			
Picomoles/brain	3.33 ± 0.21	1.35 ± 0.17‡	3.17 ± 0.27
Picomoles/g protein	598 ± 35.4	279 ± 31.5‡	633 ± 44.0
Catecholamine contents			
Dopamine			
μg/brain	0.228 ± 0.008	0.163 ± 0.004‡	0.204 ± 0.009
(μg/g wet wt)	(0.434)	(0.386)‡	(0.423)
Norepinephrine			
μg/brain	0.090 ± 0.005	0.067 ± 0.002‡	0.082 ± 0.002
(μg/g wet wt)	(0.181)	(0.154)‡	(0.172)

Groups of 14 experimental (7 in each group) and 9 control males, 5 wk of age, were used. Experimental conditions for maternal treatment with growth hormone were the same as described in Fig. 1. The determinations of tyrosine hydroxylase activity and brain contents of dopamine and norepinephrine were processed as in Materials and Methods. Enzymatic activity was expressed as picomoles of tyrosine converted per 30 min per brain.

* Mean of respective group ± SE.

‡ $P < 0.01$.

females the daily weight gain was much decreased and malnourished mothers lost 0.2–3.1 g daily during the period of lactation.

Maternal treatment with growth hormone did not improve weight gain of malnourished pregnant mice during gestation, but helped to retain weight during lactation. There was some weight depression in progeny from hormone-treated mothers at birth and at 5 wk of age, but it was significantly less than in the nontreated malnourished group.

Table III shows the effects of maternal treatment with growth hormone on brain enzymatic activity and catecholamine contents of offspring. Whereas enzymatic activity of brain tyrosine hydroxylase was significantly lower in

progeny of malnourished mothers, this was corrected by treatment with growth hormone. Similarly, the contents of brain norepinephrine and dopamine in malnourished group was restored to normal by treatment of the mother with growth hormone.

DISCUSSION

In these experiments neonatal infection, perinatal malnutrition, and crowding were found to alter the biosynthesis of catecholamine from precursor tyrosine; the catabolic turnover of norepinephrine was abnormal in the malnourished group. The brain content of norepinephrine and dopamine was decreased only in the progeny of malnourished mothers. Since the dynamic processes of metabolic turnover appear to be more responsive to physiological or environmental influences than are the tissue levels, the measurements of biosynthesis and catabolic activity are more sensitive indicators of alterations in catecholamine metabolism.

When sympathetic nerve activity is increased, there is a concomitant increase in the rate of synthesis and in the catabolism of catecholamines. Exposure of animals to either higher or lower environmental temperatures results in an increase in turnover rate of brain norepinephrine (15). The present study has revealed that perinatal malnutrition has an effect similar to that of increased activity of sympathetic neurons in that both the biosynthesis of catecholamine and the catabolic activity are changed. During the period of ½ to 6 hr after intraventricular injection of norepinephrine-^{3}H, the labeled catecholamine was metabolized in such a way that 41–61% norepinephrine, 11–15% normetanephrine, 2.5–4.0% deaminated catechols, and 22–35% *O*-methylated deaminated metabolites were found in the brain (16). Present findings do not establish whether the high level of radioactivity in brains of malnourished group is due to increased accumulation of metabolites produced from the catabolic activity of norepinephrine-^{3}H or to the slow metabolic turnover or utilization of catecholamine.

Brain tissue levels of catecholamine may be regulated at many sites. These include the sensitivity of receptor, control mechanism in release and reuptake system, and the relative rates of biosynthesis and degradation of enzymes involved in catecholamine metabolism (17). The brains of rats raised from mothers fed a low protein diet contained less norepinephrine and dopamine than brains of adequately fed littermates, but the tyrosine hydroxylase activity was increased in the brains of the former group (18). Low levels of brain norepinephrine were also found in rats that had been malnourished postnatally by giving 16 or more pups to one lactating mother (19). Our own results show that perinatal malnutrition causes low levels of brain catecholamines and depression of enzymatic activity of tyrosine hydroxylase. Thus, two different methods of maternal dietary deprivation, namely poor quality protein (wheat gluten) and quantitative restriction of diet, had different metabolic effects.

Rats, placed in an enriched environment at weaning and maintained in it for about 80 days, exhibited greater weight and thickness of cortex, increased acetylcholinesterase and cholinesterase activities than animals in an impoverished environment (20, 21). In the present study, crowding affected body weight and brain weight, but not the metabolic activity of norepinephrine and the brain levels of catecholamine. In fact, crowded animals incorporated tyrosine into the brain more actively than noncrowded animals.

Tyrosine hydroxylase, the enzyme which converts tyrosine to dihydroxyphenylalanine (DOPA), occurs only in catecholamine-containing neurons, and is the rate-limiting factor in catecholamine biosynthesis (22). The impaired enzymatic acitivity of tyrosine hydroxylase in progeny of malnourished mothers might be one of the factors responsible for their low levels of brain catecholamine.

It has been reported that the progeny of underfed mothers have smaller pituitaries, are deficient in growth hormone, and respond with increased weight gain to administration of this hormone (23). Administration of both growth hormone and insulin to neonatally infected or malnourished mice partially corrects body weight depression and restores the biosynthesis of brain protein and ribonucleic acid to normal levels (7). When bovine growth hormone is administered to pregnant rats with restricted caloric intake during pregnancy, it improves the body weight, cerebral weight, and brain levels of DNA and protein of the offspring at birth. This may be achieved in part through the mobilization of maternal nutrient reserves (24). In the present study, treatment of malnourished mice with growth hormone during gestation and lactation prevented almost completely the loss of weight by the mother during lactation and resulted in greater body weight and brain weight in the offspring. If growth hormone does not cross the placenta (25), its effects on the offspring might be indirect and result from improvement of food utilization by the mother, reduction of catabolism, or increase in placental transport of nutrient to the fetus. Whatever the explanation, maternal treatment with growth hormone also restored to normal the levels of brain catecholamine in the malnourished group and increased the enzymatic activity of brain tyrosine hydroxylase.

SUMMARY

The effects of neonatal infection, perinatal malnutrition, and crowding on the metabolism of brain catecholamine were studied in specific pathogen-free mice. Metabolic turnover of catecholamine was determined by measuring the incorporation of precursor tyrosine-^{14}C into brain tissue, catabolic activity of norepinephrine-^{3}H at various times after intracisternal injection, and tissue levels of dopamine and norepinephrine.

The rate of tyrosine incorporation was decreased by neonatal infection but was increased by perinatal malnutrition and crowding. There was no differ-

ence in catabolic activity of norepinephrine between infected, crowded, and control groups. In the malnourished group, however, the total radioactivity from norepinephrine was significantly higher than in the control group ½ and 2 hr after injection.

The brain contents of dopamine and norepinephrine were depressed in the malnourished group. There was no significant difference in catecholamine levels between infected, crowded, and control groups.

In the malnourished group, treatment of the mothers with growth hormone prevented almost completely weight loss during lactation, and also resulted in higher fetal weight. Hormone treatment restored to normal the levels of brain catecholamine and the enzymatic activity of brain tyrosine hydroxylase in progeny of malnourished mothers.

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