Perinatal Undernutrition: Accumulation of Catecholamines in Rat Brain

Abstract. *Brains of rats undernourished from midgestation and killed at weaning contained 25 percent less norepinephrine than brains of adequately fed littermates. Perinatal undernutrition also suppressed the accumulation of brain dopamine. Paradoxically, the activity of tyrosine hydroxylase, the enzyme thought to be rate-limiting in catecholamine biosynthesis, was significantly increased in brains from undernourished animals.*

It has been suggested that inadequate nutrition early in life can modify the biochemical composition of the brain and certain behavioral functions (*1*); some of these modifications may be irreversible. Using brain DNA content as an index of cell number, Winick and his collaborators have shown that perinatal malnutrition decreases the number of cells in the brains of rats (*2*) and children (*3*). This decrease could reflect changes in the number of neurons; however, it might also be limited to neuroglia, which normally represent a majority of the cells in the brain. In order to study the effects of early undernutrition on a specific property of brain neurons, we have measured substances found exclusively within those cells (*4*) in the brains of rats experimentally malnourished from birth to weaning. Our data indicates that 24 days after birth, such brains contain 25 to 30 percent less of the neurotransmitter norepinephrine than those of control animals do. Amounts of dopamine in the brain are also depressed, but the activity of the catecholamine-synthesizing enzyme tyrosine hydroxylase (*5*) is significantly elevated.

Timed pregnant rats obtained from the Charles River Laboratories at approximately 14 days of pregnancy were caged individually and housed in a temperature-controlled room illuminated from 9 a.m. to 9 p.m. by Vita-Lite bulbs (220 to 440 lu/m^2). Animals were given free access to isocaloric diets, prepared as ½-inch pellets, and water. Half of the pregnant rats consumed food containing 8 percent protein: half were fed 24 percent protein (*6*). Thus, two kinds of offspring were produced at birth, and there were four experimental groups after "cross-fostering" (Fig. 1).

The overall effect of nursing from a mother that consumed an 8 percent protein diet was inhibition of body growth (Table 1). Pups nourished by control mothers grew at a rate of 3.5 g/day whether they were born to control (C) or deprived (D) mothers; in contrast, pups fed by deprived mothers gained only 0.6 to 1.0 g/day. It has been demonstrated that the low-protein diet consumed by the mother decreases the quantity of milk produced but does not alter its composition (*7*).

The nursing pups were decapitated 12 and 24 days after birth, and their brains were weighed and homogenized in 8 ml of 0.4*N* perchloric acid. Samples of the 10,000*g* supernatant fluid were analyzed for norepinephrine and dopamine by means of fluorimetric assays (*8*). The content of brain norepinephrine was significantly depressed in rats suckling deprived mothers at 24 days of age (Table 1). Prenatal deprivation did not depress amounts of norepinephrine in the brain among animals suckling control mothers (D-C group); however, it did magnify the effect of postnatal malnutrition in the D-D group. Amounts of dopamine in the brain were also lower 24 days after birth in rats nursed by deprived mothers (Table 1).

One effect of undernutrition on the developing brain is the retardation of the accumulation of myelin (*9*). The relative lack of this substance in the brain of the undernourished rat contributes substantially to the brain's low weight. Hence, expressing the concentration of a neurotransmitter substance such as norepinephrine, which is found in only a fraction of brain neurons,

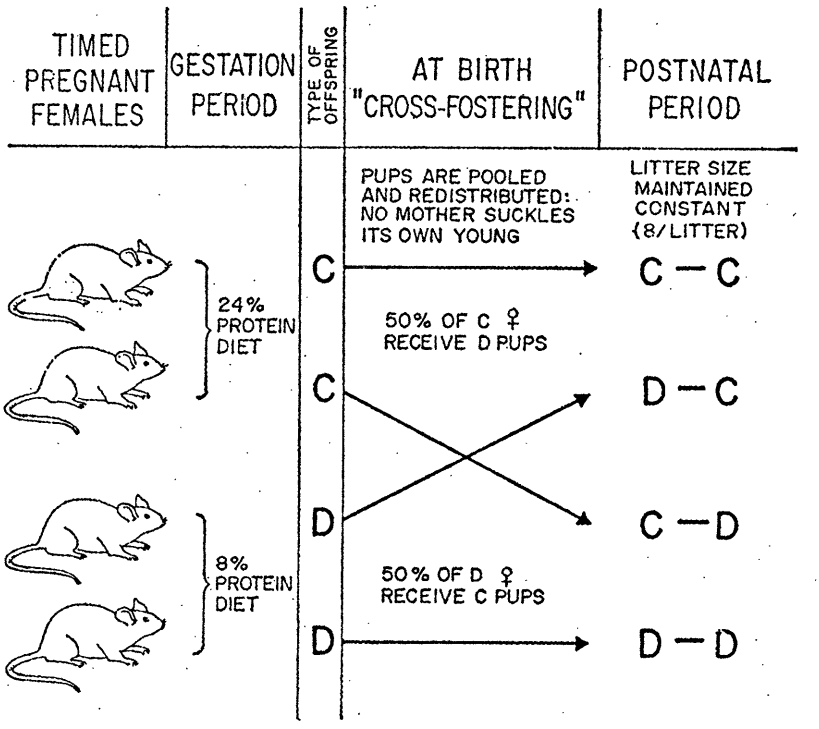

Fig. 1. Diagram of method used to produce nutritionally deprived and well-nourished littermates. The day after birth, all pups born to mothers eating 24 percent protein (control) were pooled, as were animals born to mothers consuming 8 percent protein (deprived). The pups were then redistributed to the lactating mothers, so that all litters contained eight young and no mother nursed her own pups. Postpartum, each mother continued to consume the same diet as during pregnancy. Four experimental groups of young were generated: (i) pups born to control mothers and nursed by control mothers (C-C); (ii) pups born to control mothers and nursed by deprived mothers (C-D); (iii) pups born to deprived mothers and nursed by control mothers (D-C); and (iv) pups born to and nursed by deprived mothers (D-D). Adapted from Chow *et al.* and Miller (*23*).

Table 1. Amounts of norepinephrine and dopamine 12 and 24 days after birth. Each value for dopamine represents that for eight to ten brains.

Experimental group	Number	Weight (g)		Amount (μg/brain)	
		Brain	Body	Norepinephrine	Dopamine
			12 days		
C-C	8	1.30	38.5	.353 ± .029	
D-C	8	1.23	35.6	.366 ± .021	
C-D	4	1.01	12.5	.341 ± .010	
D-D	4	0.95	11.9	.311 ± .039	
			24 days		
C-C	23	1.63	79.5	.397 ± .023	.510 ± .008
D-C	10	1.61	77.3	.368 ± .010	.500 ± .005
C-D	10	1.36	30.8	.342 ± .018	.410 ± .005*
D-D	26	1.12	17.3	.287 ± .012*	

* Differs from C-C group, $P < .01$; values and standard error of the mean are given.

per unit weight of brain, might obscure functionally significant changes caused by malnutrition in central noradrenergic neurons. Similarly, changes in the number of brain cells caused by perinatal undernutrition (*2*) might occur among cell types that do not contain norepinephrine (such as oligodendrocytes, or neurons whose cell bodies lie in the cerebral cortex). Therefore, expressing the norepinephrine content in brains of malnourished animals as the amount per cell might also obscure significant changes among noradrenergic neurons. For these reasons, we have expressed brain catecholamine content per whole organ rather than per gram of brain or per cell.

Tyrosine hydroxylase, the enzyme which converts tyrosine to dihydroxyphenylalanine (dopa), is located only in catecholamine-containing neurons (*5*). A partial purification of this enzyme was performed (*10*) on brains from C-C and D-D rats, and its activity was measured by a modification of the method of Nagatsu *et al.* (*11*). Paradoxically, the brains of malnourished rats which contained subnormal amounts of norepinephrine had significantly more tyrosine hydroxylase activity than those of littermate control animals (Table 2). The fact that the activity of tyrosine hydroxylase is increased in brains that are slightly decreased in size lends further support to the contention that use of tissue weight or total cell number for normalizing measurements of compounds in specialized cell populations in the brain can be misleading. Other enzymes not involved in catecholamine pathways (for example, 1,6-diphosphofructoaldolase, creatine phosphokinase, and isocitric dehydrogenase) show decreased activity per brain in undernourished rats at 21 days of age (*12*).

Brains from a few malnourished animals were assayed for dopa (*13*), an intracellular amino acid precursor of dopamine and norepinephrine that is normally indetectable in rat brain (*14*). We could not detect any dopa in control or deficient animals.

Unlike dopamine, norepinephrine, and tyrosine hydroxylase, brain tyrosine (the physiological precursor for norepinephrine) is not confined solely within catecholamine-producing cells; this amino acid is necessary for protein synthesis in all cells. At present, no method is available which allows the measurement of tyrosine levels within just those brain cells that convert the amino acid to catecholamines. We measured whole brain tyrosine concentrations in control (C-C) and deprived (D-D) groups of rats and found those concentrations to be similar (35.1 and 34.6 μg per gram of brain, respectively).

This observation does not rule out the possibility that biosynthesis of norepinephrine in the brain is impaired in undernourished weanlings, and that this impairment results from inadequacies in the amounts of tyrosine available for conversion to dopamine and norepinephrine. It is, of course, also possible that the depression in brain norepinephrine results not from impaired synthesis but from accelerated turnover of the catecholamines. That is, their biosynthetic rate may be normal, or even increased; however, a continued acceleration of their catabolism or release (or both) might cause low steady-state amounts of brain catecholamines.

Table 2. Tyrosine hydroxylase activity 24 days after birth. Activity is expressed as nanomoles of tyrosine converted per hour per brain and standard error of the mean. Tyrosine hydroxylase was measured by the method of Nagatsu *et al.* (*11*), with the following modifications: 3-iodotyrosine (0.6 mg) was added to the incubation mixture for a blank reading; alumina columns were used to separate radioactive tyrosine from radioactive dopa; the columns were eluted with 3 ml of 0.2*N* acetic acid.

Experimental group	Number	Tyrosine hydroxylase activity
C-C	26	8.8 ± 0.8
D-D	19	11.8 ± 0.9*

* Differs from C-C group, $P < .02$; D-C and C-D groups were not assayed.

Our results show that when rats are undernourished prior to weaning, the accumulation of brain norepinephrine and dopamine is impaired. These observations agree with those of Sereni *et al.* (*15*), who found low amounts of norepinephrine in the brains of 14-day-old rats that had been malnourished postnatally when 16 or more pups were placed with one lactating mother. Our data do not allow us to draw any conclusions about whether perinatal malnutrition causes a decrease in the number of noradrenergic neurons in the brain, or in the norepinephrine content per neuron. In the latter case only, this effect of malnutrition might be expected to be reversible by nutritional means. Preliminary observations on the uptake by brain of intracisternally administered [^{3}H]norepinephrine reveal no differences among the four experimental groups at 10 minutes or 3 hours after its administration. If uptake is indicative of the number of central noradrenergic neurons, these data suggest that malnutrition does not decrease the number of such neurons.

Using a similar method of nutritional deprivation, Levitsky and Barnes (*16*) reported that the behavior of rats nutritionally deprived early in life is abnormal when tested as adults. It is also well known that children severely undernourished early in life display deficits in both mental ability and psychological development that persist after nutritional rehabilitation (*17*). Catecholamine-containing cells in the brain have been implicated in a variety of visceral and neuroendocrine functions (*18*). Moreover, these neurons may also participate in learning (*19*), the control of mood (*20*), and motor functions (*21*).

Tyrosine must ultimately be supplied by the diet, either as such or as phenylalanine; tyrosine could become the lim-

iting factor in the synthesis of dopamine and norepinephrine. Rats are born with only 15 percent and 30 percent of the normal adult amounts of brain dopamine and brain norepinephrine, respectively (*22*). A deficiency in the tyrosine available for the biosynthesis of catecholamines might alter the mechanisms that are responsible for the complex regulation of these neurotransmitters. It is possible that some of the physiological and behavioral sequellae of early protein-calorie malnutrition result from the changes in brain catecholamine metabolism described in this report.

WILLIAM J. SHOEMAKER
RICHARD J. WURTMAN
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139

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Scleroderma and the Subcutaneous Tissue

Abstract. *Our study revealed that as observed with both light and electron microscopy the most specific abnormality in scleroderma skin is the replacement of the subcutaneous tissue by markedly abnormal connective tissue.*

Systemic scleroderma is a disease of connective tissue which may affect the skin, lungs, kidneys, gastrointestinal tract, and heart. The disease may be fatal. The skin, the most common site of involvement, shows marked induration, ischemia, and sometimes gangrene. Despite these striking clinical manifestations, numerous histochemical, electron microscopic, and biochemical studies have failed to demonstrate the nature of the alteration in the connective tissue of the skin (*1*). On regular biopsies, the following features have been emphasized: (i) thickening of the dermis by fibrosis, (ii) homogeneous appearance of the connective tissue, and (iii) vascular changes. However, in many instances the dermal changes are minor and are not diagnostically significant. We now report evidence that the most striking pathology in scleroderma skin consists of the replacement of the subcutaneous fat by markedly abnormal connective tissue.

Ten patients (seven females and three males, 21 to 69 years old) who had had systemic scleroderma for 2 to 14 years were selected for this study. All patients had acrosclerosis and Raynaud's phenomenon, six had pulmonary fibrosis with impairment in pulmonary diffusion, and seven had dysphagia. Kidney involvement, as determined by biopsy, was noted in five cases. Skin biopsies were only performed on markedly indurated areas (dorsum of hand and forearm in eight patients; dorsum of finger in two). Specimens of normal skin for controls were obtained from similar areas. In order to include the entire subcutaneous tissue, the biopsies were taken down to the depth of the muscle fascia. Specimens for histochemistry were fixed in 10 percent buffered formalin and stained with the following stains: hematoxylin and eosin, periodic acid–Schiff reagent (PAS), Gomori's trichrome, aldehyde fuchsin, Verhoef's elastic tissue stain, and Alcian blue, *p*H 2.5. Seven cases were also studied by electron microscopy. Each biopsy was sectioned with a scalpel at three different levels, from the top to the bottom, so that four specimens, about 1 mm^3 each, could be studied independently. The specimens were fixed in 3 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer for 3 hours, washed several times with 12 percent sucrose in cacodylate buffer, treated with 2 percent osmium tetroxide, dried in graded alcohols, embedded in Spurr low-viscosity resin (*2*), and stained with uranyl acetate and lead citrate or phosphotungstic acid. The blocks were sec-

Fig. 1. Scleroderma of the forearm. Eccrine sweat glands (*ESG*) are located in the lower third of the dermis. Note the difference in connective tissue structure between the dermis and subcutaneous tissue area. Focal areas of panniculitis at the lower level (*P*) (hematoxylin and eosin, × 32).