Fetal Brain Growth: Selective Action by Growth Hormone

Abstract. *Growth hormone was administered to pregnant rats maintained under dietary control, and fetal and placental growth and nutrition were examined. Growth hormone had a selective action on brain growth that could not be attributed to nutrient mobilization but suggested a trophic factor which is unique to the brain.*

Offspring of rats given growth hormone during pregnancy have been shown to have larger brains as a result of elevated cellular content and, at maturity, to demonstrate superior learning ability (*1*, *2*). Zamenhof *et al.* (*3*) have reported increases in cerebral cellular content only when growth hormone is administered to malnourished dams. Progeny of rats maintained on a normal diet did not display any significant increase in brain DNA content when similarly treated. Zamenhof proposed that any increase in cellular content resulting from growth hormone treatment must therefore be due to the use of inferior animals and inadequate diet. Similarly he suggested that the reversal of fetal retardation produced by maternal malnutrition resulted from mobilization of maternal nutrients for fetal utilization by the exogenous growth hormone. We therefore decided to examine the effects of growth hormone on fetal and placental growth when administered under conditions of nutritional control. Maternal and fetal metabolism were examined to determine the mechanism of growth hormone action.

Pregnant rats of an inbred Wistar strain were used. Time of conception was determined by vaginal smears taken each morning. Females were 100 days old and weighed 200 g prior to mating. Since commercial rat pellets were found to have a low protein content (less than 10 percent), rats were maintained on a semisynthetic diet containing 23 percent protein, 56 percent carbohydrate, 11 percent fat, with a calculated caloric value of 4.1 kcal/g (*4*). This was equivalent to control diets used by Zamenhof *et al.* Each rat was given free access to the diet, and the daily food consumption after correction for spillage was recorded. Pregnant rats were allocated at random to control and experimental conditions. Experimental animals received daily subcutaneous injections of 100 μg of porcine growth hormone dissolved in 0.5 ml of normal saline from days 7 to 21 of gestation. Control animals received the same volume of vehicle alone. Porcine growth hormone, with an activity of 0.6 unit/mg, was obtained in pure crystalline form (Sigma) and stored under desiccation at 4°C. A double isotope labeling technique was used to simultaneously determine the cellular content of both brain and placenta. On day 16 of gestation each pregnant rat was injected intravenously with 10 μc of [methyl-^{14}C]thymidine (^{14}C-TdR). Since placental mitosis is maximal at this time, the incorporation of ^{14}C-TdR into placental DNA provides an index of cellular content. On day 20 of gestation, each pregnant rat was injected intravenously with 1 mc of [methyl-^{3}H]-thymidine (^{3}H-TdR) to determine the proliferation of cortical neurons. The incorporation of ^{3}H-TdR given at this time into brain DNA has been shown by fine-resolution autoradiography to provide a selective measure of neuronal proliferation in the supragranular layers of the cerebral cortex (*2*). On day 21, each pregnant rat was killed by decapitation, plasma was collected, and fetuses and placentas were removed. Plasma was pooled from fetuses within each litter; after DNA was extracted (*5*) from individual brains and placentas, the radioactivity in each fraction was determined. Maternal plasma insulin (*6*), glucose (*7*), protein (*8*), and α-amino acid nitrogen (*9*) concentrations were measured. Fetal glucose (*7*) concentrations were also determined.

As we showed in earlier findings (*2*), growth hormone increased both brain weight and cerebral neuron content (Table 1). Lack of significant increases in fetal body weight suggests that growth hormone has a selective action on brain growth. The effect could not be attributed to maternal nutrient alterations (Table 2). Growth hormone administration had no significant effect on maternal food consumption or litter size. In spite of significant difference in weight gain during gestation, protein metabolism—as determined by plasma α-amino acid nitrogen and serum protein concentrations—was unaltered. Maternal glucose concentrations were

Table 1. The effect of maternal growth hormone administration on fetal growth. The results are expressed as means ± standard deviations. The numbers in parentheses refer to the number of animals in each group; dpm, disintegrations per minute.

Test	Body weight (g)	Brain weight* (mg)	Uptake of ^{3}H-TdR into brain DNA* (dpm)	Uptake of ^{14}C-TdR into placental DNA (dpm)
Control (60)	2.506 ± 0.375	88 ± 23	27724 ± 9676	3045 ± 1064
Growth hormone (69)	2.653 ± 0.652	122 ± 57	42586 ± 17289	4740 ± 2660
		Analysis		
t	1.5261	4.2123	5.6244	3.6382
	$P > .05$	$P < .001$	$P < .001$	$P < .001$
Percent change		39	54	57

* Cerebral hemispheres without olfactory lobes and cerebellum.

Table 2. The effect of growth hormone administration on pregnant rats. The results are expressed as means ± standard deviations. The numbers in parentheses refer to the number of pregnant females in each group.

Item	Control (6)	Growth hormone (6)	t	P	Change (%)
Weight gain during gestation (g)	41.8 ± 7.6	60.8 ± 16.4	2.3494	< .025	45
Daily food consumption (g)	11.4 ± 0.9	12.3 ± 2.5	0.7565	> .05	
Litter size	10.3 ± 2.2	11.6 ± 1.1	1.2060	> .05	
Plasma insulin concentration (μunit/ml)	22.3 ± 9.7	22.5 ± 7.8	0.0448	> .05	
Plasma glucose concentration (mg/100 ml)	82.1 ± 8.4	90.5 ± 5.0	1.8914	< .05	10
Plasma α-amino acid nitrogen concentration (mg/100 ml)	9.5 ± 1.7	9.9 ± 1.4	0.4130	> .05	
Serum protein concentration (g/100 ml)	5.3 ± 0.8	4.5 ± 0.4	1.5740	> .05	

found to be elevated; however, these were within the normal range and far below those associated with hyperglycemia (*10*). Accordingly, maternal insulin and fetal glucose concentrations (control, 16.7 ± 12.5 mg/100 ml; growth hormone treated, 20.5 ± 19.5 mg/100 ml; $t = 0.3395$, $P > .05$) were unaffected by the growth hormone administration. Explanation for the increase in maternal weight gain is unclear. Whereas the protein anabolic effect of growth hormone and metabolic adjustments during pregnancy would suggest increased protein deposition, our findings do not indicate general mobilization of the protein source for transfer to the fetus. In view of the large increase in brain growth, it is likely that metabolic alterations would be evident in concentration estimations; however, further studies of metabolic turnover may be required.

Although maternal nutrition appears largely unaffected, placental growth was stimulated as measured by the incorporation of ^{14}C-TdR into placental DNA (Table 1). Correlation of placental growth with elevated brain weight further supports placental regulation of fetal growth. Where this has been found to be retarded, as in malnutrition or intrauterine growth retardation (*11*), fetal growth is curtailed, an action attributed to impaired fetal nutrient supplies (*12*). Although our data provide the only evidence relating placental overgrowth, it cannot be incorporated into a similar nutritional hypothesis, which suggests that the role of the placenta needs reconsideration, perhaps in terms of production or transfer of trophic factors.

Contrary to the proposal of Zamenhof *et al.* (*3*), elevated cellular content was thus obtained in fetuses from well-nourished dams given growth hormone. This discrepancy cannot be attributed to maternal diet since both daily food intake and composition are comparable. However, Zamenhof *et al.* report mean litter numbers of 6 and 11 for control and growth hormone groups, respectively, whereas no change was observed in our study, and this may account for the inconsistency between their and our findings. The administration of growth hormone during pregnancy appears to produce a unique effect on brain growth. Similar specificity was observed by Zamenhof, Mosley, and Schuller (*1*). These results indicate more than stimulation during a vulnerable period, for other prenatal factors, such as malnutrition (*13*), have a generalized influence at birth, affecting body as well as brain development. Rather, the specificity of growth hormone action indicates a selective effect on growth of the brain. Pituitary growth hormone does not cross the placenta (*14*), and thus its action must be mediated by secondary changes. From our study, however, this is not via nutrient mobilization. Alternatively, a second messenger, such as somatomedin, or a similar trophic substance, perhaps of placental origin, may be able to directly influence fetal brain growth. Indeed the specificity of growth hormone action suggests the presence of a trophic substance which is unique to the brain.

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References

1. S. Zamenhof, J. Mosley, E. Schuller, *Science* **152**, 1396 (1966).
2. V. R. Sara and L. Lazarus, *Nature (Lond.)*, in press.
3. S. Zamenhof, E. van Marthens, L. Grauel, *Science* **174**, 954 (1971).
4. M. G. McCall, G. E. Newman, J. R. P. O'Brien, J. S. Valberg, L. J. Witts, *J. Nutr.* **16**, 297 (1962).
5. H. N. Munro and A. Fleck, *Methods Biochem. Anal.* **14**, 113 (1966).
6. C. N. Hales and P. J. Randle, *Biochem. J.* **88**, 137 (1963).
7. D. G. Cramp, *J. Clin. Pathol.* **20**, 910 (1967).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
9. E. G. Frame, J. A. Russell, A. E. Wilhelmi, *ibid.* **149**, 255 (1943).
10. L. Krulich and S. M. McCann, *Endocrinology* **78**, 759 (1966); E. Herrera, R. H. Knopp, N. Freinkel, *J. Clin. Invest.* **48**, 2260 (1969); S. Chernik and M. Novak, *Diabetes* **19**, 563 (1970).
11. M. Winick, *Am. J. Obstet. Gynecol.* **109**, 166 (1971); S. Zamenhof, E. van Marthens, L. Grauel, *Nutr. Rep. Int.* **4**, 269 (1971).
12. H. Bard, *Clin. Obstet. Gynecol.* **13**, 511 (1970).
13. F. J. Zeman and E. C. Stanbrough, *J. Nutr.* **99**, 274 (1969); M. Winick, *Pediatr. Clin. North Am.* **17**, 69 (1970).
14. Z. Laron, S. Mannheimer, S. Guttman, *Experientia* **22**, 831 (1966); Z. Laron, A. Pertzelan, S. Mannheimer, J. Goldman, S. Guttman, *Acta Endocrinol.* **53**, 687 (1966).

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Geniculate Neural Plasticity in Kittens after Exposure to Periodic Gratings

Abstract. *Kittens were exposed for 2 hours a day to a periodic vertical grating during the first 10 weeks after birth, and otherwise kept in darkness. The spatial frequency of the grating fell in the range of highest contrast sensitivity of normal cats. After the 10-week exposure period, cortical evoked potentials and lateral geniculate mass responses to alternating gratings showed a reduced amplitude for the spatial frequency of exposure. This reduction was independent of grating orientation. An analysis of orientational sensitivity of cortical units did not show any bias in favor of the vertical orientation.*

It has been demonstrated that if a kitten is brought up in a visual environment containing only bars of a given orientation, the visual cortex of the kitten develops only neurons subserving that orientation (*1, 2*). Cortical neurons in cats and in monkeys, besides being selective for the orientation of the visual stimulus, are specific for the spatial frequency of it (*3*). Thus we performed an experiment in which the only visual experience offered to a kitten, otherwise kept in darkness, was a grating of a given spatial frequency. We always used square-wave gratings with vertical bars and with spatial frequency corresponding to the peak of the contrast sensitivity curve of the cat. We found that cortical and geniculate neural responses to a grating of the same spatial frequency as that to which the animals were exposed were reduced as compared with the responses to other spatial frequencies. This reduction of cortical and geniculate responses was independent of the orientation of the grating. Moreover, an analysis of the orientational selectivity of cortical units did not show any bias in favor of the vertical orientation.

The kittens were kept from birth in a completely dark room. From the age of 2 to 3 weeks, they were exposed to a periodic grating for 2 to 3 hours a day, 6 days a week. The spatial frequency of the grating was 0.22 cycle/deg for some of the kittens and 0.45 cycle/deg for the others. These two spatial frequencies fall in the range of highest contrast sensitivity of the cat (*4, 5*). This routine was stopped when the kittens were 2½ or 3 months old. Seven kittens were exposed to gratings by being suspended in the middle of a cylinder 2 m in diameter, the walls of which were painted with periodic black and white vertical stripes. The kittens stood on the floor of a small plastic