Brain Research, 54 (1973) 397–402

The effect of prenatal malnutrition on dimensions of cerebral cortex

GEOFFREY M. CLARK*, STEPHEN ZAMENHOF, EDITH VAN MARTHENS, LUDMILA GRAUEL AND LAWRENCE KRUGER

U.C.L.A. School of Medicine, Departments of Microbiology and Immunology, Biological Chemistry, and Anatomy, Brain Research Institute and Mental Retardation Center, Los Angeles, Calif. 90024 (U.S.A.)

(Accepted February 6th, 1973)

Previous work from this laboratory[9,12] has demonstrated that maternal dietary protein restriction during pregnancy results in a reduction in cerebral cell number (as measured by DNA content) of the offspring at birth. This reduction also can be caused by maternal dietary calorie (glucose intake) restriction alone, with normal protein nutrition[10,11], an effect presumably related to the role of glucose as the main energy source for the fetus. However, cerebral cell number, although intimately involved in brain performance[4,7], is obviously unrelated to other factors of brain function such as the extent of the neuronal dendritic tree[3,4]. Cortical thickness and cortical cross sectional area, on the other hand, should reflect both the cell number and the development of cellular arborization.

In another paper (Clark and Zamenhof, in preparation) it has been demonstrated that for neonatal rat, the rostral cortical thickness is significantly correlated with cerebral weight, the parameter that is known to be significantly decreased by prenatal malnutrition[8–12]. The present investigation is concerned with the problem of whether cortical thickness is also reduced by prenatal calorie restriction. Similar studies, but with reference to postnatal general malnutrition only, were reported by Bass[1]; general prenatal and postnatal malnutrition has been reviewed in an unpublished M.A. thesis by Siassi[6].

Albino rats used in our work were Sprague–Dawley derived: they have now been bred in our laboratory for 28 generations. Virgin females 3 months old and weighing 200–260 g were mated. The control animals were fed a pelleted diet containing 20.5% protein (Wayne Mousebreeder Block, Allied Mills, Chicago, Ill.).

The experimental animals were maintained on a calorie restricted diet but with protein and vitamin content approximately the same as in the diet for the control group[10,11]. In this respect the diet differed from those in reported experiments, in which total food intake was reduced and protein and vitamin restrictions were produced as well as calorie restriction.

The restriction was imposed from the 10th to 20th day of pregnancy, since re-

* Post-doctorate trainee, USPHS Training Grant HD-00345 to Mental Retardation Center, U.C.L.A.

striction in this period yielded the most marked reduction in cerebral weight, protein and DNA content, following protein deprivation[12]. Before the start of calorie restriction on day 10 of pregnancy, the diet of restricted and control animals was the same (*ad libitum*, Wayne Mousebreeder Block). During the next 10 days the control diet remained unchanged whereas the experimental animals were maintained on the following diet: casein, 20.5%; vegetable oil, 2.56%; Salt Mix, U.S.A. XIV, 4%; Alphacel* 72.9%; and vitamin fortification mix. The experimental diet contained 1.39 kcal/g compared to 3.30 kcal/g in the control diet; the protein and vitamin contents were comparable. The average food intake for the control and experimental groups was the same. The animals left for postnatal study were nursed the same way in both the control and experimental group.

The first group (newborns) was weighed and decapitated within 6 h after delivery**. A second group was nursed by their own mothers and decapitated at the age of 10 days. Littermates were randomly assigned for chemical or histological study.

Cerebral hemispheres, without cerebellum, olfactory bulbs and brain stem, were removed immediately after decapitation, and weighed; they were then frozen and subsequently used for analysis. DNA was determined by a modification of the diphenylamine colorimetric method[13], and protein was determined by a modification of the Lowry colorimetric method[5] (newborns only).

After dissection and weighing, the brains of subjects to be studied histologically were placed immediately in 10% formalin and fixed for a minimum of 10 days. Serial 50 μm frozen coronal sections were cut, mounted and stained with cresyl-violet for quantitative study.

Stained sections at the rostral and caudal poles of the corpus callosum were selected and their projections ($\times$ 47) traced for subsequent measurements. All tracings and measurements were made in a 'blind' fashion, without knowing to which group the slide or drawing belonged. Measurements of cortical thickness were made from the upper limit of layer 2 to the white matter, to restrict measurement to the neuronal layers and to avoid errors due to tearing or thickening at the pial border.

The procedure for measurement on the traced sections was as follows: (1) establishing the width of the hemisphere at its widest point (layer 1 excluded); (2) multiplying that figure by 1/3, 2/3, and 9/10; (3) marking the points (a, b, c, d) on the surface of layer 2 corresponding to 1/3, 2/3, and 9/10 of the width of the hemisphere from the midline; (4) drawing lines from these points (a, b, and c) and from the widest point (d), perpendicular to the white matter; (5) measuring the length of these lines up to the white matter, to the nearest 0.1 mm (Fig. 1 and Table I).

On the tracing of the rostral section an additional line was drawn perpendicular to the midline and tangential to the dorsal surface of the anterior commissure, extending laterally to the surface of layer 2 (line V.L., Fig. 1). This line served as the ventral limit for measurements of cross sectional cortical area. The area of cortex dorsal to

* The possible effects of high levels of Alphacel in the diet are now being investigated.

** Experimental litters were delivered by cesarean section on day 22 of pregnancy to avoid neonatophagy. Control litters were born on or before day 22.

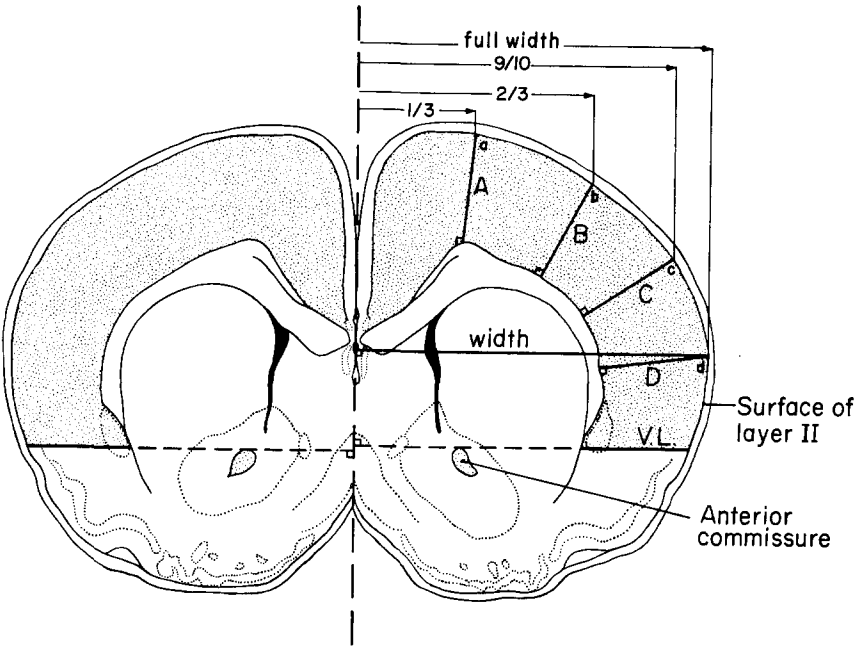

Fig. 1. Illustration of measurements on the rostral cerebral section.

this line seen in the rostral section (shaded in Fig. 1) was measured by tracing the outline with a planimeter. Three planimeter measurements were made and averaged for each area estimated. Measurements of area were limited to rostral coronal sections due to lack of appropriate landmarks or criteria for reproducible area measurements in caudal sections. Measurements from both hemispheres were averaged for analysis except in cases where the outer surface of layer 2 was damaged and only a unilateral measurement at a given location was possible. In this instance, unilateral measure was taken as the best estimate. Frozen sections were employed in an attempt to minimize shrinkage. They were blotted dry (not warmed) on the slide before staining, dehydration and covering.

Compression due to gravity during fixation was minimized by placing the brains with caudal surface down; coronal sections were cut at right angles to the direction of possible compression and therefore the sections were not likely to be deformed by it. Knife compression could not be avoided but all brains were cut in the same direction (dorsal to ventral surface); thus, each group should be affected equally. All comparisons were between group means using Student's *t* test. All probability figures shown are for a one-tailed test.

It can be seen (Table I) that maternal calorie restriction resulted in a significant reduction of all measured parameters of the offspring: body weight, cerebral weight, cerebral DNA, cerebral protein, cerebral widths and cortical thickness at all positions of rostral and caudal sections, and cortical area of rostral sections. The reduction in cerebral DNA was most conservative (compare refs. 10, 11), in keeping with the reported

TABLE I

THE EFFECT OF MATERNAL CALORIE RESTRICTION* ON NEONATAL PARAMETERS IN RATS

	Weight (g)		*Content*		*Dimensions of cortex (mean ± S.D.)***		
	Body	*Cerebrum*	*DNA (μg)*	*Protein (mg)*	*Rostral*		
					Thickness (μm) at positions		
					A	*B*	*C*
E*	4.07±0.71	0.12±0.02	533±26	7.03±1.26	630±112	648±119	603±102
n_E**	20	20	16	16	20	20	20
C	6.11±0.30	0.16±0.005	566±37	8.7 ±0.68	748± 52	783± 26	737± 18
n_C	8	8	20	20	8	8	8
$\bar{x}\Delta$	—33.4	—24.4	—5.8	—18.9	—15.7	—17.2	—18.2
P	< 0.0005	< 0.0005	< 0.005	< 0.0005	< 0.001	< 0.0005	< 0.0005

* Experimental group (E), on calorie restricted diet, 10–20th day of pregnancy. Control group (C), on normal diet.
** n_E, n_C, number of neonatal animals in experimental and control groups, respectively. $\bar{x}\Delta$, mean difference
*** One hemisphere (mean of both hemispheres).

tendency of the organism to maintain this neonatal parameter nearly constant. The reduction in protein content followed the reduction in brain weight.

If one assumes the same specific gravity of experimental and control brains, then the reductions in cerebral weights of experimental animals also represent the reductions in cerebral volume. If treated as a sphere and if such reductions were uniform in all directions, the reduction in one dimension would be the cube root of the reduction in volume. For the data in Table I ($\Delta = 24.4\%$ or -0.244), the reduction in one dimension would be $1 - \sqrt[3]{0.756} = 0.09$ or 9%, approximating the average reduction in cerebral widths of rostral sections (8.7%). In contrast, the average reduction in rostral cortical thicknesses is 16.2% or almost double the expected figure. The reduced rostral cortical area (100% — 21.2% = 78.8%) is close to the figure (76.5%) obtained from multiplication of reduced cortical thickness (100% — 16.2% = 83.8%) by reduced cerebral (cortical) width (100% — 8.7% = 91.3%). Essentially similar but less pronounced are the reductions in caudal sections where the cortex is uniformly thinner.

A small sample of animals (4 experimentals and 19 controls) was nursed (the same way for both groups) and sacrificed at day 10. The reductions in all parameters were similar to those at birth presented in Table I, but less pronounced. They were significant for the following parameters: brain weight, $\bar{X}\Delta = -10.0\%$, $P < 0.01$; anterior cortical thickness, A, $\bar{X}\Delta = -6.0\%$, $P < 0.005$; B, $\bar{X}\Delta = -7.5\%$, < 0.005; anterior cortical area, $\bar{X}\Delta = -7.7\%$, $P < 0.005$.

The results of this study (Table I) confirm our previous reports that prenatal maternal calorie malnutrition results in a significant reduction in body weight, cerebral weight, cerebral DNA, and cerebral protein of the neonatal offspring, and demonstrate

			Caudal				
	Width (mm)	*Area (sq.mm)*	*Thickness (μm) at positions*				*Width (mm)*
D			*A*	*B*	*C*	*D*	
600±98	2.93±0.19	2.77±0.73	496±77	510±63	486±51	396±69	3.53±0.31
20	20	20	15	15	15	15	15
694±25	3.21±0.09	3.51±0.27	580±38	595±17	588±27	442±62	3.74±0.06
8	8	8	8	8	8	8	8
−13.5	−8.7	−21.2	−14.5	−14.4	−17.4	−10.3	−5.7
< 0.0005	< 0.0005	< 0.0005	< 0.005	< 0.0005	< 0.0005	0.1 > P > 0.05	< 0.01

See text for details.
between E and C, in % of C. *P*, probability.

that cortical dimensions (cortical or cerebral width, cortical thickness and area) in neonatal animals also undergo significant reductions. The reductions in cortical thickness at 10 days of age have also been demonstrated for other kinds of prenatal[6] and postnatal[1] malnutrition.

Our results indicate that cortical thickness, especially in rostral sections, is reduced by almost double what would be expected from the reduction in the weight of the cerebrum as a whole (including diencephalon). Thus, there is an indication that the cortex itself is more affected by such malnutrition than the cerebrum as a whole. At birth, the most affected is cortical thickness at position C (Fig. 1). This is true for both rostral and caudal sections; in general, however, the reductions in rostral sections are more pronounced, and also appear to be statistically more reliable (Clark and Zamenhof, in preparation).

It is of interest that the above significant reductions, reflecting on the volume occupied by cortical cells, are accompanied by significant decreases in one of the factors that must enter into this parameter, namely the number of neonatal cerebral cells (DNA); the other factor, the volume occupied by an average neonatal perikaryon, has not been determined.

At 10 days, the cortical thickness is already close to the adult value[2]. Our sample of 10-day-old animals is too small to draw definite conclusions concerning this age, but the preliminary figures (see above) suggest that the reductions, at least in some rostral cortical thicknesses, are still demonstrable at this age, albeit they are less pronounced than at birth. A gradual disappearance of differences in cortical thickness after 10 days of age was also observed by Bass *et al.*[1] for postnatal malnutrition of a different kind.

In conclusion, not only previously reported cerebral parameters of the offspring (weight, DNA, protein) but also cortical dimensions (thickness at several positions, width, area) are significantly reduced when pregnant rats are maintained on calorie deficient diets. The reduction in cortical thickness is approximately double that expected from the reduction in cerebral weight, which suggests that the cortex itself is more affected by such malnutrition than the cerebrum as a whole. All these decreases are more pronounced at birth than at 10 days or than in later development.

This research was supported by USPHS Grants HD-04612, HD-05615, HD-00345, HD-05394 and NS-05685. Computing assistance was obtained from the Health Sciences Computing Facility, U.C.L.A., sponsored by NIH Special Research Resources Grant RR-3.

The authors thank Mrs. Oladunni Arigbede and Mrs. Jane Chen for expert histological preparations.

1 BASS, N. H., NETSKY, M. G., AND YOUNG, E., Effect of neonatal malnutrition on developing cerebrum, *Arch. Neurol.(Chic.)*, 23 (1970) 289–302.
2 CALEY, D. W., AND MAXWELL, D. S., Ultrastructure of the developing cerebral cortex in the rat. In M. B. STERMAN, D. J. MCGINTY AND A. M. ADINOLFI (Eds.), *Brain Development and Behavior*, Academic Press, New York, 1971, pp. 89–107.
3 CLENDINNEN, B. G., AND EAYRS, J. T., The anatomical and physiological effects of prenatally administered somatotrophin on cerebral development in rats, *J. Endocr.*, 22 (1961) 183–193.
4 HOLLOWAY, R. L., JR., The evolution of the primate brain: Some aspects of quantitative relations, *Brain Research*, 7 (1968) 121–172.
5 LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J., Protein measurement with the Folin phenol reagent, *J. biol. Chem.*, 193 (1951) 265–275.
6 SIASSI, F., *Nature of Cellular Recovery of Central Nervous System in Malnourished Newborn Rats*, M.A. Thesis, U.C.L.A., Los Angeles, Calif., 1971.
7 WELKER, W. I., JOHNSON, J. I., AND PUBOLS, JR., B. H., Some morphological and physiological characteristics of the somatic sensory system in raccoons, *Amer. Zool.*, 4 (1964) 75–94.
8 WINICK, M., Malnutrition and brain development, *J. Pediat.*, 74 (1969) 667–679.
9 ZAMENHOF, S., VAN MARTHENS, E., AND MARGOLIS, F. L., DNA (cell number) and protein in neonatal brain: Alteration by maternal dietary protein restriction, *Science*, 160 (1968) 322–323.
10 ZAMENHOF, S., VAN MARTHENS, E., AND GRAUEL, L., Prenatal cerebral development in nutritionally restricted animals: Rehabilitation by treatment with growth hormone, *Science*, 174 (1971) 954–955.
11 ZAMENHOF, S., VAN MARTHENS, E., AND GRAUEL, L., DNA (cell number) in neonatal brain: Alteration by maternal dietary calorie restriction. *Nutr. Rep. Int.*, 4 (1971) 269–274.
12 ZAMENHOF, S., VAN MARTHENS, E., AND GRAUEL, L., DNA (cell number) and protein in neonatal rat brain: Alteration by timing of maternal dietary protein restriction, *J. Nutr.*, 101 (1971) 1265–1269.
13 ZAMENHOF, S., GRAUEL, L., VAN MARTHENS, E., AND STILLINGER, R. A., Quantitative determination of DNA in preserved brains and brain sections. *J. Neurochem.*, 19 (1972) 61–68.