

# The Effect of Severe Early Malnutrition on Cellular Growth of Human Brain

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## *Extract*

In ten 'normal' brains, obtained from well-nourished Chilean children who died accidentally, weight, protein, and DNA and RNA content were all normal when compared with those values derived from similar children in the United States. Table I demonstrates the values obtained in these children. In nine infants who died of severe malnutrition during the first year of life, there was a proportional reduction in weight, protein, and RNA and DNA content. The actual values for these determinations are given in table II. The number of cells was reduced but the weight or protein per cell was unchanged. Three infants who weighed less than 2,000 grams at birth (Infants 2, 3, and 4, table II) were the most severely affected. These data are similar to previous data in animals and demonstrate that in children, severe early malnutrition can result in curtailment of the normal increase in brain cellularity with increase in age.

## *Speculation*

At present there is growing concern that malnutrition early in life may retard normal development. Studies conducted in Africa, in South America, in Mexico, in Guatemala, and in our own country suggest that this is true. Retarded brain growth has also been suspected in malnourished children. The decreased head circumference often noted has been cited as evidence for retardation in brain growth. Although numerous chemical changes secondary to undernutrition have been shown in brain of animals, similar studies have not been available in human brain. This study demonstrates such changes and establishes that cell division is curtailed in human brain by severe early malnutrition. The data provide yet another link in the ever lengthening chain of evidence linking malnutrition to faulty brain growth and development.

## *Introduction*

Total content of DNA reflects cell number in any organ made up primarily of diploid cells [2]. Although some tetraploidy has been reported in brain [6], the overwhelming majority of both neurones and glia are diploid. Total brain DNA reflects the total number of brain cells, and the ratio of protein to DNA or that of

RNA to DNA reflects the average amount of protein or RNA per cell.

In rats, malnutrition at a time when brain cells are actively dividing curtails cell division and results in an ultimate reduction in total brain cell number [13]. This reduction in cell number will occur if the rats are malnourished from birth or if their mothers are malnourished during pregnancy [17], and will persist even

if the animals are later given an adequate diet. In contrast, 'overfeeding' rats from birth to weaning will increase the rate of cell division in brain [15]. This increase in the rate of cell division will occur even after a short period of malnutrition if rehabilitation is begun while cell division is still actively occurring [16]. It would appear that the state of nutrition from before birth to 17 days of age will influence the rate of cell division and the ultimate number of cells in rat brain.

Examination of a series of 'normal' human brains collected in the United States from therapeutic abortions and from children who died from accidents or poisonings has demonstrated that DNA content increases linearly until birth, more slowly until 6 months of age, and very little thereafter [11]. It seems probable, therefore, that if the response to malnutrition in human brain is analogous to the response in rat brain, the critical postnatal period during which cell division could be curtailed would be the first six months of life.

#### Materials and Methods

The studies described here were carried out in two groups of children in Santiago, Chile. In the first group, all ten children were well nourished and were within the normal height and weight curves for both Chilean [1] and American children [10]. They showed no clinical evidence of malnutrition and died acutely of accidents or poisonings. Two fetuses studied were the products of therapeutic abortions for psychiatric reasons.

In the second group, all the infants died during the first year of life and showed typical signs of severe third degree malnutrition. They were all well below the third percentile for height and weight and presented a clinical picture of severe infantile marasmus. In none of these nine cases was breast feeding practiced; the major source of food was a liquid made of flour and water.

Brains were removed within one hour of death and immediately frozen. These were homogenized *in toto* to a 20% suspension in distilled water. DNA, RNA, and protein were separated by a modification of the Schmidt-Thannhauser procedure [8]. Incorporation and recovery studies previously performed using rat brain showed that the methods used were effective for fractionation of these components of the central nervous system. DNA was determined by Burton's modification of the diphenylamine reaction [4]. When verified by direct ultraviolet spectrophotometry, there was agreement within 5% between the methods. RNA was determined by the orcinol reaction [5] and protein by the method of LOWRY *et al.* [7]. Complete details of these methods have been published elsewhere [11, 12, 14].

#### Results

In all cases, the normally nourished infant in Santiago showed weights and amounts of protein, RNA, and DNA content in brain comparable to that of the population previously studied in the United States (figs. 1-4). The brains of the nine infants who died of severe malnutrition all showed reduced weights and reduced quantities of protein, RNA, and DNA content (figs. 1-4). In three infants (Infants 2, 3, and 4, table II), DNA content was approximately 40% of that expected. These three infants all weighed less than 2,000 grams at birth, indicating that they were prematures or had suffered fetal growth retardation. Unfortunately,

Table I. Control population

Age	Weight	Protein	RNA	DNA
	g		mg	
15 weeks gestation	32	1.8	28.3	22
18 weeks gestation	54	2.4	47.6	48
36 weeks gestation	280	10.8	315	520
39 weeks gestation	214	14.2	402	580
1 3/4 months	312	19.8	501	706
2 months	408	22.3	620	790
5 3/4 months	570	29.0	732	880
6 1/2 months	602	31.8	924	910
8 1/2 months	720	40.0	1040	920
11 1/2 months	850	49.1	1306	910

Each set of values represents value for total brain of a single patient. Determinations were carried out in triplicate with less than 2% variation in all three samples.

Table II. Malnourished population

Age	Weight	Protein	RNA	DNA
	g		mg	
1/2 month	208	10.2	345	512
1 3/4 months	136.	7.4	210	380
3 1/2 months	105	7.6	200	300
5 1/2 months	132	7.4	160	315
6 months	400	15.8	560	700
6 3/4 months	420	18.2	595	720
7 months	386	15.6	510	680
9 months	505	20.3	660	690
11 months	557	19.0	605	510

Each set of values represents value for total brain of a single patient. Determinations were carried out in triplicate with less than 2% variation in all three samples.

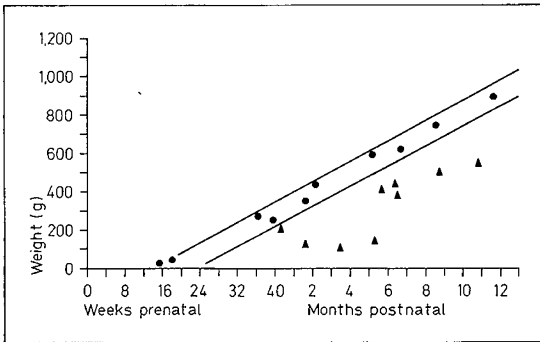
ly, gestational age records were not available to make this distinction. The reduction in weight (fig. 1), protein (fig. 2), and RNA (fig. 3) is roughly proportional to the reduction in DNA (fig. 4) and, thus, the ratios are normal, suggesting that the average protein or RNA content per cell is normal. If the data are plotted against weight instead of age, there is no reduction in DNA content in brain, and the reduction in cell number is proportional to the reduction in the weight of these children.

*Discussion*

These data demonstrate that the brains of well-nourished Chilean children who died accidentally contain

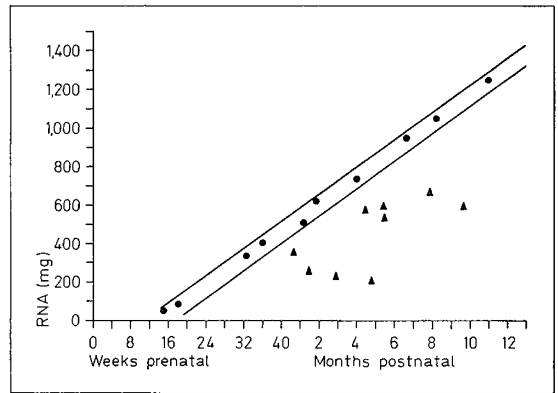
the same number of cells as the brains of children accidentally dying in the United States. In contrast, children who died of severe malnutrition (marasmus) during the first year of life showed a reduced DNA content in brain. The data also suggest that the younger the child when malnutrition strikes, the more marked the effects. The three children in this study who weighed less than 2,000 grams at birth had a 60 % reduction in DNA content in brain. These results imply either that the brain of a very small infant may be more sensitive to severe postnatal malnutrition or that intra-uterine malnutrition had already occurred in these three infants. Better prenatal case histories will have to be collected to separate these possibilities.

The retardation in brain growth in all nine children



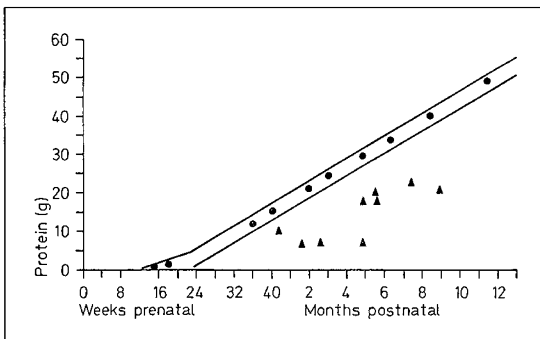
*Fig. 1.* Lines indicate normal range for US population [11].

- indicates normal Chilean children.
- ▲ indicates Chilean children who died of severe malnutrition during the first year of life.



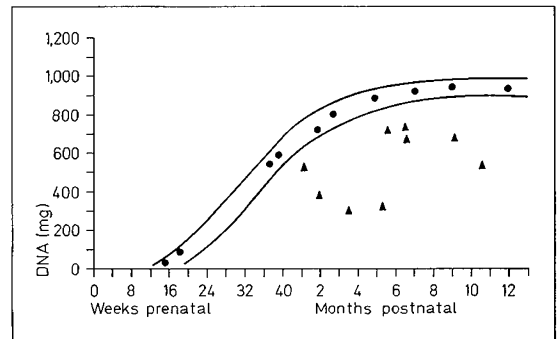
*Fig. 3.* Lines indicate normal range for US population [11].

- indicates normal Chilean children.
- ▲ indicates Chilean children who died of severe malnutrition during the first year of life.



*Fig. 2.* Lines indicate normal range for US population [11].

- indicates normal Chilean children.
- ▲ indicates Chilean children who died of severe malnutrition during the first year of life.



*Fig. 4.* Lines indicate normal range for US population [11].

- indicates normal Chilean children.
- ▲ indicates Chilean children who died of severe malnutrition during the first year of life.

studied can be entirely explained by the decreased number of brain cells. The protein/DNA ratio (protein per cell) remained unchanged. The retardation in brain growth, which has been inferred from measurements of head circumference [9] and demonstrated more directly by reduced brain weight [3], was a result of the curtailment of cell division. The brains of these children contained fewer cells than brains of well-nourished children of similar age.

#### Summary

Total brain weight and protein, RNA and DNA content were studied in children who died of severe malnutrition during the first year of life in Santiago, Chile. Brains of the well-nourished children were the same weight and contained the same quantities of DNA, RNA and protein as brains from a comparable population studied in the United States. In contrast, the brains of the infants who died of malnutrition were proportionally reduced in weight and in nucleic acid and protein content. These data indicate that severe early malnutrition retards cell division in human brain.

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18. This research was supported by the National Foundation Grant 1270, the Nutrition Foundation Grant 357, and the New York City Health Research Council Contract U 1769.
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