STUDY OF FACTORS INFLUENCING PRENATAL BRAIN DEVELOPMENT*

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Summary

This article attempts to assess some of the factors which may affect brain development, in either direction, deterioration or improvement.

The brain starts to develop very early, and its foundations and potential for further development are partially laid already at birth; thus, our considerations have also to refer to prenatal period. The evidence suggests that environmental, rather than genetic factors, are of primary importance in that period. Genetic factors affecting prenatal brain development are essentially unknown and do not lend themselves to manipulation; this also applies to the factors that determine gene derepression and re-repression and thus start and stop neuronal DNA synthesis and neuron proliferation. On the other hand there is some information concerning the effects of environmental factors and their manipulation. Of these, embryonal and fetal nutrition has been discussed first.

The maternal nutrient supply to the fetus (embryo) offers an easy target for interference with prenatal brain development: Protein (essential amino acids) deprivation or glucose deprivation results in an underdeveloped fetal brain. However, the full nutrient requirements of the fetus (embryo) are not well known, and a biochemical study of "unknown growth factors" may still yield some surprises. This also applies to the multitude of regulatory factors which control the complex flow of nutrient to the site of synthesis of components in the proliferating neuron (neuroblast). This control is not always for the benefit of the fetus.

Other studies presented here indicate the transfer of the effects of prenatal malnutrition in one generation, on brain development in the next generation; the mechanism involved is a non-mendelian, maternal inheritance. These studies reveal the existence of the long-range regulatory mechanisms which, generation after generation, cumulatively adjust the size of individuals and their organs (within genetic limits) to the nutritional opportunities confronting a given strain.

The deterioration of prenatal brain development in general may result in a typical mental retardation. More numerous, and therefore more important for the society are the borderline (subclinical) cases: the non-fulfillment of the genetic potential of the individual.

The *deterioration* of prenatal brain development by a *single* factor, e.g. deficiency of one nutrient, is biochemically easier to understand: this factor soon becomes the rate limiting step in the synthesis of components of the neuron (neuroblast). On the other hand, the *enhancement* of brain development may *a priori* appear impossible: the system as complex as this may consist of a multitude of closely overlapping checks so that if one factor is enhanced, the next one becomes the rate limiting step, and so on. Nevertheless, cases of enhanced brain development (admittedly rare), in genetically uniform strains, do occur naturally; possibly, many factors have changed in concert. Moreover, such enhancement can be produced at will in the laboratory, and the techniques to achieve it have been presented. Thus, the limits imposed on prenatal brain development are not completely precise and inviolable.

* an invited article

Introduction

This review will deal with some biochemical and biological factors affecting prenatal brain development, in particular in the direction of possible improvement of this development. The emphasis on prenatal development stems from the recognition that by the time of birth the foundations for the future brain have already been laid (reviews in[1-3]): In the rat cerebral neurons (neuroblasts) essentially end proliferation at that time; in primates the end of their proliferation occurs as early as the second trimester of pregnancy (rhesus monkey[4], human[5]). Thus, the final number of neurons, one of the parameters limiting the potential of the cerebrum, is an outcome of factors active in this early period of development. In addition, future development (differentiation) must depend on the integrity of neurons at birth. These factors can be broadly divided into two categories:

Genetic and Environmental. The relative contribution of each of these factors for the final outcome is essentially unknown. According to one estimate[6], at birth (i.e. when neuron proliferation is terminated) the contribution of the genetic factor to neonatal weight is only 38%, and of environmental factor (intrauterine environment), as much as 62%. For such reasons, and also because at present the genetic factors are unknown and do not lend themselves to manipulations, it appears more fruitful to concentrate on the environmental factors. This review will present evidence that such factors alone allow to change early brain development in either direction (depression or enhancement). The review is divided into three parts: General Scope, which presents an overview without going into details; Specific Experimental Results, to support it; and Summary and Conclusions.

General Scope

Maternal Nutrients

Fig. 1 shows a schematic representation of the flow of nutrients to the site of synthesis of neuronal (neuroblast) components of the embryo or fetus.

Of all the nutrients taken in by the pregnant female, only essential components have been listed. In addition to these now obvious components, the need for "unknown growth factors"[7] cannot yet be excluded.

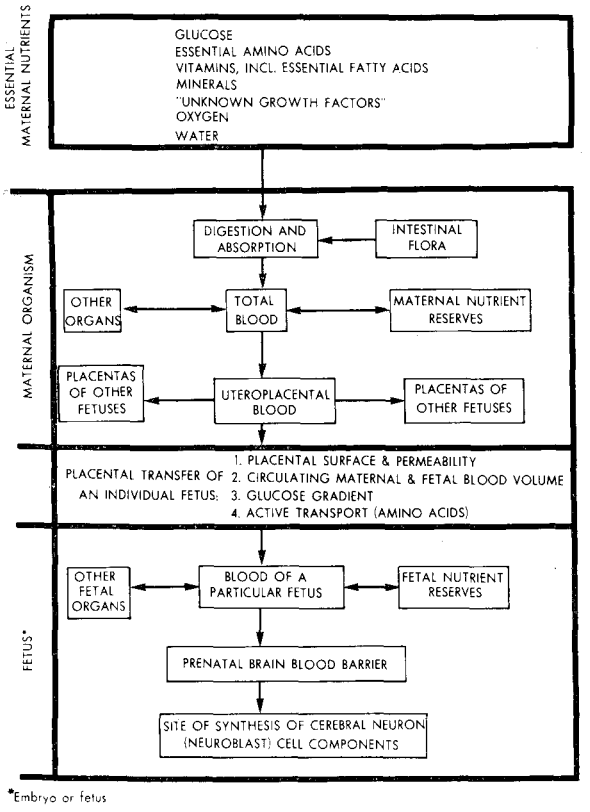

Fig. 1. Schematic representation of the flow of essential nutrients to the site of synthesis of neuronal (neuroblast) cell components.

In particular, in our hands a maternal diet containing the known components failed to meet fully the requirements of the growing rat fetus[8].

Glucose is the main energy source for the fetus, and maternal glucose ("calorie") deficiency results in offspring that have at birth less total cerebral DNA (cell number) and protein, less protein per cerebral cell and a smaller cerebral cortex[9-12]. Similar results follow the restrictions of protein[13-16] or omission from the maternal diet of one of the essential amino acids[8].

Fetal deficiency of vitamins and minerals is more difficult to produce because of the small requirements and the sufficient storage of these nutrients in the maternal organism; also, vitamins may be produced by intestinal flora. Nevertheless, the effect of mineral deficiency has been demonstrated.[17] On the other hand, considerable oxygen needs and lack of storage lead to easier demonstrable deleterious effects on fetal

brain development at low oxygen tension (or high altitudes)[18].

Flow of nutrients to the site of synthesis of fetal neuronal cell components

Even the simplified schematic flow of nutrients to the fetal neurons (neuroblasts) (Fig. 1) is still very complex, and illustrates the multitude of potential regulatory sites on which such neuronal proliferation depends.

Maternal dietary nutrients, digested, absorbed and ultimately available in the blood, can be supplemented by substances (such as non-essential amino acids) manufactured in maternal organs, primarily liver, and by vitamins manufactured by the intestinal flora; the amounts of nutrients may be further decreased or increased by the participation of maternal nutrient reserves. In particular, one of the hormones mobilizing these reserves is the pituitary growth hormone. The blood level of this hormone is known to increase during fasting and during pregnancy; we have demonstrated[10] that growth hormone, administered to the nutritionally-deprived mother, prevents the deficiencies in cerebral DNA and protein in the offspring.

It is now well recognized that the fetus is not a parasite which extracts from maternal organism all it needs; rather, the fetuses may be sacrificed to save the mother. This is particularly well demonstrated in the rat: Maternal dietary protein deprivation around the time of implantation results in resorption of fetuses around day 10, even though by day 15, the protein content of all fetuses and their supporting tissues would constitute only an insignificant fraction of the total protein intake of the pregnant female[14]. This failure was traced to faulty implantation and placentation (review in[14]).

It is obvious that proper placental transfer of nutrients to the fetus is a key requirement for the normal fetal development. In the above described case, the lack of proper placentation, caused by the consequences of regulatory action of the lack of proteins, precluded subsequent transfer of nutrients even if they would become available[14]. In general, maternal malnutrition is one of the conditions that cause placental underdevelopment[14, 19]. Thus, lack of nutrients may have a twofold effect: deficiency of transfer to the fetus on top of deficiency of substances to be transferred. This deficiency of transfer due to placental underdevelopment might also affect the passage of "nutrients" present in abundance, such as oxygen and water, as well as the removal of waste material from the fetus.

As mentioned above, glucose is the main energy source for the fetus. The factor determining the passage of glucose through placenta appears to be the concentration gradient (although the carrier is probably also involved). Glucose concentrations in maternal and fetal blood are, in turn, hormonally regulated. Thus it is possible, for example, to increase glucose transport to the fetus by introducing more insulin to the fetus which lowers fetal blood glucose level and thus increases the gradient[20]. On the other hand, the passage of amino acids through placenta involves active transport: the concentration of amino acids in the fetal blood is higher than in the maternal (review in[21]).

From the foregoing it is clear that placental development and placental transfer are factors of paramount importance for fetal brain development. The problem of quantification in the placenta is a difficult one because of the number of factors involved. The permeability of the placenta, the exchange surface, and the maternal blood flow to the placenta are all involved, to a not well defined degree. Taking as placental parameters placental weight, placental DNA (cell number) and placental protein we were able to establish significant correlations between these parameters and neonatal brain DNA (cell number) (rabbits[22]) or head circumference (humans[23]).

Competition between fetuses

It is well known that in humans, birth weight of singletons is higher than that of twins, and the latter, in turn, is higher than that of triplets and quadruplets. However, as shown by McKeown and Record[24], these differences appear only after 26 weeks of gestation. Until that time there are enough nutrients and enough space even for each of quadruplets to be the same size as singletons. As mentioned before, in humans the number of cerebral neurons is final after 20 weeks[5]. Thus, during evolution an efficient controlling mechanism has been developed to prevent restriction of size in multiple births, in the period in which neurons still proliferate. This should result in the same number of neurons in multiple as in single births, but in humans this subject has not been investigated. In rats, neurons proliferate until birth, and

there indeed we were able to demonstrate that the amount of neonatal cerebral DNA (cell number) is the same regardless of litter size[25]. Thus, the controlling mechanism achieves the constancy of this important parameter, the neonatal brain cell number. However, in our laboratory, this situation was changed surgically by reducing the number of the fetuses that developed. Each of the newborns in this experimental group had significantly more brain DNA and brain cells[25-27]. Presumably, this was because the controlling mechanism was set to support the growth of the full number of fetuses, but fewer actually developed; thus, each had more nutrients.

The above considerations of intrauterine competition may be an example of intrauterine interorganismic regulation. The organisms of different genomes (fetuses) regulate the growth of each other while confined to the common limited source of nutrients.

Regulation of synthesis of fetal neuronal cell components

The utilization of the nutrients that have ultimately reached the site of synthesis of cerebral neuron (neuroblast) cell components depends of course, on the derepression of genes for proper enzymes; in the brain in which neuron proliferation ends early, this derepression must be followed by a re-repression. Should, for instance, the synthesis of neuronal cell components (especially DNA) terminate earlier, or proceed slower during the proliferation period (before birth), the resulting brain will contain lower final number of neurons. Needless to say, very little is known about the factors that regulate gene derepression and re-repression. However, the activity of enzymatic systems involved in DNA synthesis was studied by MARGOLIS[28] in our laboratory. He first determined the DNA content of cerebral hemispheres, optic lobes, cerebellum, and remainder, in chicken brains from the eleventh day of embryonic life to 6 weeks after hatch. Each region showed a characteristic pattern of variation during development. The cerebellum showed the most rapid and the optic lobes the least rapid rate of DNA increase during the period studied. The nature of the DNA-polymerase activity in soluble extracts from these brain regions seemed to be similar to the properties reported for this enzyme activity in other vertebrate tissues. At nearly every age, the level of enzyme activity was highest in extracts from the cerebellum.

The particulate fraction from brain homogenates decreased the DNA-polymerase activity observed in soluble brain extracts. The data obtained indicate that this inhibition was the result of dephosphorylation of the deoxynucleoside triphosphate substrates by an ATPase-like enzyme in the brain particulate fraction whose activity increases during ontogeny. This activity, then, may contribute to the determination of final DNA level of the particular part of the brain.

Specific Experimental Results

This article will summarize some of our data that led to the general view outlined in the preceding section. The data refer mostly to DNA and protein content of the neonatal cerebrum for the following reasons:

Normal neuron and glia cells at birth are essentially diploid and the amount of DNA per diploid cell of a given species is constant[29,30]. While there have been reports of polyploid neurons, they concern cerebellar Purkinje cells[31,32], Betz cells of the motor cortex[33], or large pyramidal cells in the hippocampus of the mature brain[34], but not cells in the neonatal cerebral cortex. Thus, determination of neonatal brain DNA is a convenient and objective quantitative method for determination of total neonatal brain cell numbers. Such determinations[30,35] have been used throughout our work.

From the DNA values per brain, the actual total number of brain cells (neurons and glia) could be

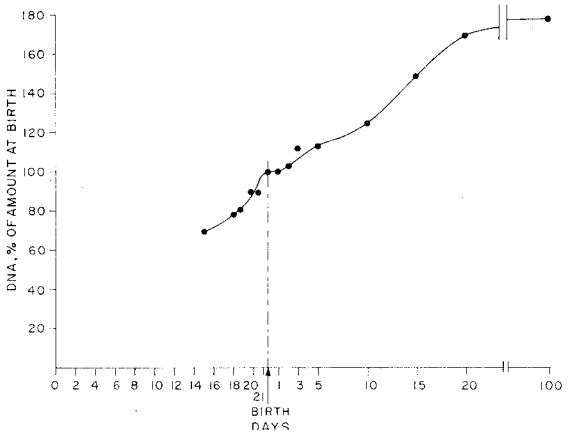

Fig. 2. DNA synthesis in developing rat brain (cerebral hemispheres). Abscissa: embryonal (fetal) age before birth and age after birth. Ordinate: DNA, in % of the amount at birth (From[1]).

calculated by dividing by a (constant) DNA content per cell (6×10^{-6} μg for the rat which was the animal mostly used in this work). The total amount of brain DNA in the developing rat reaches a transient plateau at birth (Figure 2). At the plateau this amount (that is, also total brain cell number) is one of the most constant parameters of the developing organism. This amount cannot be changed by any of the numerous factors that can change neonatal body weight or neonatal brain weight. Brain weight, the parameter most often reported in the literature, represents a composite of many factors [such as amount of water (88 per cent in the neonatal rat), lipid, protein, etc.] and, for individual animals, is not correlated with the amount of brain DNA (0.35 per cent in the neonatal rat).

The significance of the amount of neonatal cerebral protein for the future brain development is not known that exactly. One may only surmise that this amount will determine future dendritic and axonal development in cerebral neurons, the myelination and the production of biogenic amines; prenatal malnutrition which results in neonatal deficiency of protein content per brain cell[13], also leads to smaller cortical thickness and area[11], (see below).

Prenatal protein deficiency

In our first work on protein deprivation (ZAMENHOF et al.[13]), we maintained the experimental group (Table 1) on a low (8%) protein diet. The control group was maintained on an adequate (27%) protein diet 1 month prior to mating and throughout pregnancy. The caloric difference in the two diets was adjusted by the addition of starch. This restriction was made to still permit full-term gestation and a normal number of offspring. As a result of this protein deprivation, the offspring in the experimental group had considerably lower body and cerebral weights. The reduction in cerebral DNA was more conservative but still highly significant (Table 1). These results have been corroborated by WINICK[36], and by ZEMAN and STANBROUGH[37]. Since the change in DNA content was in neonatal animals, the neuron number was most likely to be affected. Such a deficiency is permanent since the cells destined to become neurons do not multiply after birth.

Table 1

The Effect of Maternal Dietary Protein Restriction on Weight and Content of Brain of Newborns[1]

Number of animals					Brain content of offspring[3]	
			Offspring weights (gm)			
Diet[2]	Mothers	Offspring	Body	Brain[3]	DNA (μg)	Protein (mg)
Control	4	32	6.38±0.4	0.181±0.014	546±22	9.29±0.43
Exper.	4	31	4.46±0.22	0.139±0.081	491±29	7.45±0.57
Decrease[4]			30%	23	10	19.8
			$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$

1 From Zamenhof et al.[13]
2 Diets: control, full, containing 27% protein; Exper. restricted, containing 8% protein.
3 Cerebral hemispheres, without cerebellum and olfactory lobes; ± SD.
4 Decrease in weights between 27% and 8% protein groups.

The amount of protein per cerebral cell was also 10% lower. Thus, the cellular deficiency was not only quantitative but also qualitative. This protein deficiency may affect the development of the cellular arborization, myelin formation, and the synthesis of biogenic amines during postnatal neuron differentiation.

In what part of pregnancy is the maternal malnutrition most harmful to the fetus? To get some information on this subject we have timed the maternal dietary protein restriction (ZAMENHOF et al.,[14]). Pregnant rats were fed a protein-free diet during five periods of pregnancy (days 0-10, 10-15, 13-18, 15-20, or 10-20) and normal diet during the

Table 2

The effect of Protein-Free Diet on the Offspring[1]

Period of protein deprivation[2]	Mothers		Offspring[3]		Offspring[1]			
	No. mated	No. littered	No. living per liter	% Still-born	Body weight g	Cerebrum Weight g	DNA μg	Protein mg
None (controls)	27	26	10.0	2	6.2±0.5[4]	0.1718±0.017	585±28	9.33±1.14
0–10	13	5	10.6	7	5.9±0.5[6]	0.1671±0.0182	552±38[5]	8.50±1.40[5]
10–15	6	6	8.7	7	5.8±0.5[5]	0.1561±0.0108[5]	541±34[5]	8.06±0.83[5]
13–18	5	4	9.3	18	5.4±0.6[5]	0.1620±0.0167[6]	587±34	7.69±0.54[5]
15–20	5	3	10.0	9	5.4±0.8[5]	0.1521±0.0180[5]	543±23[5]	8.13±0.67[5]
10–20	10	10	9.0	8	4.6±0.9[5]	0.1497±0.0198[5]	522±37[5]	8.10±0.91[5]

[1] From Zamenhof et al.[14]
[2] Days after mating.
[3] Neonatal examination.
[4] Each value represents the mean ± SD.
[5] Significant at $p<0.001$ level.
[6] Significant at $0.01>p>0.001$ level.

remaining time. In the case of the days 0-10 group, 62% of the females failed to litter. There was no significant change in the amount of food intake of pregnant animals in any of the groups; yet in all cases there were significant decreases in body weights, cerebral weights, cerebral DNA (cell number), and cerebral protein of the neonatal animals (Table 2) even though, until day 15, the total protein increment of all the embryos and their supporting tissue constituted only 1.3% of the average maternal protein intake.

Thus, the observed effects are unlikely to be due to an actual deficiency of amino acids per se as required for embryonal protein synthesis. This is especially true for the period before implantation (days 0-6). Work in other laboratories (review in ZAMENHOF et al.,[14]) demonstrated that the disturbance can be traced to deficient placental development caused by a deficiency in estrogen and progesterone[38-43], caused, in turn, by a deficiency in maternal pituitary gonadotropic hormones[44], especially prolactin[41,45]. The latter deficiency might presumably be triggered by a change in amino acid balance (or serum proteins) acting in the pituitary and/or the hypothalamus[46] that produces pituitary hormone-releasing factors. After day 11, when the placenta starts to assume the hormonal functions of the maternal pituitary, the deficiency in placental development may further contribute to the overall effect. It is of interest that WINICK[19] also demonstrated the adverse effects of maternal protein restriction on placental development as early as day 13.

Our data on the 16- and 20-day cesarean following deprivation from days 10-15, also indicate deficient placental development. This deficiency persists to term (20-day cesarean).

After day 15, the total protein increments of the fetus and its supporting tissue cease to be insignificant. Brain underdevelopment due to protein deprivation after this time may be due to a direct deficiency of amino acids required for protein synthesis. The adverse effect on the development of the placenta may also continue. The most pronounced effects were obtained by protein deprivation from days 10-20. This, indeed, seems to indicate the cumulative effect of both these mechanisms. Thus, at least in the rat, the pregnancy operates on a very tight schedule. There is no second chance; a growth phase missed or slowed down by malnutrition even for a short time cannot be rehabilitated by subsequent normal feeding. But the mechanisms involved are, as can be seen, quite complex.

In the course of the above experiments we have obtained evidence that the normally nourished female offspring (F_1) of mothers (F_0) malnourished during pregnancy, will produce offspring (F_2) that still have significantly lower brain parameters (ZAMENHOF et al.,[15,16], Table 3). This transfer of deficiencies to next (F_2) generation is only through F_1 females, but

Table 3

The Effect of Restriction of Maternal (F_0) Dietary Protein on Newborns in Second Generation (F_2)[1]

Group[2]	Number of F_1 ♀	Number of F_2	Item[3]	F_2 (neonatal) Weights (gm) Body	Weights (gm) Cerebral Hemispheres	Cerebral content DNA (μg)	Cerebral content Protein (mg)
B	10	63	V	5.7 ±0.52	0.1578±0.0146	565±34.6	8.29±0.782
			Δ	−7	−7	−6	−5
			p	<0.001	<0.001	<0.001	0.01>p>0.001
C	8	59	V	5.9 ±0.45	0.1587±0.0159	555±32.3	8.19±1.090
			Δ	−3	−7	−7	−6
			p	0.05>p>0.02	<0.001	<0.001	0.01>p>0.001
Control	14	74	V	6.1±0.52	0.1702±0.0128	598±32.6	8.75±0.805

[1] From Zamenhof et al.[15]

[2] F_0 on 8% protein diet 1 month prior to mating and throughout pregnancy. Group B, F_1 nursed by their own F_0 mothers but changed at birth to the normal protein diet; Group C, F_1 nursed by control F_0 mothers on normal (20.5%) diet. After weaning, all F_1 females maintained on normal diet and mated to normal males.

[3] V, Average value, ± SD; Δ, difference between experimental and control, in percentage of control; p, probability.

not through F_1 males; clearly, it is not a Mendelian inheritance. Several possible explanations have to be considered. Poor lactation of F_0 nursing mothers was not the cause: the effects on the brain in F_2 in group C (Table 3) were essentially the same although the nursing mothers were never protein-restricted.

Of other possible explanations of the effect on brain in F_2 animals, one may consider the following: Due to protein restriction of F_0 mothers before delivery, the F_1 offspring are born handicapped, not only with regard to the brain (ZAMENHOF et al.[13]) but also in other respects. HALL and ZEMAN[47] have reported that the offspring of rats similarly protein-restricted during pregnancy suffer from retardation of kidney development and altered kidney function. LEE and CHOW[48] have reported that the restricted progeny showed reduced feed efficiency and low nitrogen balance; they excreted more amino acids than the controls. Thus, each progeny (F_1) may indeed suffer from cryptic malnutrition, even when postnatally given full access to normal food. As a result, the progeny (F_2) of F_1 had a cerebral cell deficiency (Table 3) in accordance with our original findings (ZAMENHOF et al.[13]).

Another possibility is that the F_1 organs affected were endocrine glands. STEPHAN et al.[49] have recently shown that similar F_1 animals had smaller pituitaries containing lower concentrations of growth hormone. Deficiencies of this and possibly other maternal (F_1) hormones may well affect fetal brain development of the F_2 offspring.

Prenatal deficiency of essential amino acids

A protein-free diet containing a complete chemically defined mixture of L-amino acids (AA) or this mixture deprived of one of the essential amino acids, tryptophan, lysine or methionine, respectively, was fed to pregnant rats[8]. The feeding period was 0-21 or 10-21 days of pregnancy. At birth the following newborn parameters were measured: body weight, cerebral weight, cerebral DNA (cell number) and cerebral protein, as well as placental weight, placental DNA and placental protein. As compared with normal (pelleted) stock diet, AA diet gave small decreases that were significant for body weight and cerebral parameters, though not significant for placental parameters; thus, it still remains uncertain whether our present knowledge of nutritional factors for optimal fetal development is sufficient to devise a faultless synthetic diet for pregnancy. Omission of tryptophan, lysine or methionine, respectively, from AA diet, resulted in offspring significantly inferior to AA diet offspring in all parameters; the deficiencies were essentially similar to those produced in our previous study by total protein

Table 4

The Effect of Maternal Treatment with Bovine Growth Hormone on Neonatal Offspring[1]

			Body		Placenta		Cerebrum Total		DNA		Protein	
Group[2]	Mothers	Offspring	Weight (gm)	Δ (%)	Weight (gm)	Δ (%)	Weight (gm)	Δ (%)	Content (μg)	Δ (%)	Content (mg)	Δ (%)
R	11	97	4.0±0.8		0.395±0.078		0.131±0.015		539±35		7.06±0.84	
ΔC				−33[3]		−24[3]		−19[3]		−11[3]		−17[3]
R+GH	8	58	4.9±0.5		0.478±0.090		0.159±0.012		588±39		8.18±0.93	
ΔC				−18[3]		− 8		− 2		− 3		− 4
ΔR				+23[3]		+21[3]		+21[3]		+ 9[3]		+16[3]
C+GH	5	55	6.2±0.6		0.516±0.105		0.176±0.018		616±28		9.40±0.62	
ΔC				+ 3		+ 1		+ 8[3]		+ 1		+10[3]
C	10	59	6.0±0.4		0.520±0.071		0.162±0.013		607±35		8.51±0.66	

[1] From Zamenhof et al.[10]

[2] R, Restricted diet (one-third of the number of calories of the control diet) administered from day 10 to day 20 of pregnancy; C, control diet (pelleted food ad libitum); GH, bovine growth hormone administered 3 mg/day intravenously, concomitantly with restricted diet; Δ, % difference, ΔC, % difference from group C; ΔR, % difference from group R. S.D. follows each weight.

[3] $p<0.001$.

deprivation[8,14]. Thus, omission of single essential amino acids during pregnancy may be as harmful as total absence of dietary protein.

Prenatal calorie deficiency

In the above experiments (Tables 1-3) the maternal dietary restriction was in protein only, while caloric intake was kept normal. However, since glucose is the main energy source for the fetus, it was of interest to see whether restriction of caloric intake alone (with normal protein intake) will also affect prenatal brain development (ZAMENHOF et al.,[9,10]). The results are represented in Table 4. It can be seen that such restriction of caloric intake to one-third of normal, even during the second half of pregnancy only, resulted in highly significant decreases in neonatal body weight, placental weight, neonatal cerebral weight, cerebral DNA (cell number), and cerebral protein.

Cerebral cell number, although intimately involved in brain performance[50,51], is obviously unrelated to other factors of brain function such as the extent of the neuronal dendritic tree[50,52]. Cortical thickness and cortical cross sectional area, on the other hand, should reflect both the cell number and the development of cellular arborization. We were, therefore, concerned with the problem whether cortical thickness and area are also reduced by prenatal caloric restriction[11]. The restriction was imposed from the 10th to 20th day of pregnancy. The experimental diet contained 1.39 Kcal/g compared to 3.330 Kcal/g in the control diet.

At birth, at 10 days of age and adulthood, the brains were dissected out; on some, cerebral DNA (cell number) and cerebral protein were determined as

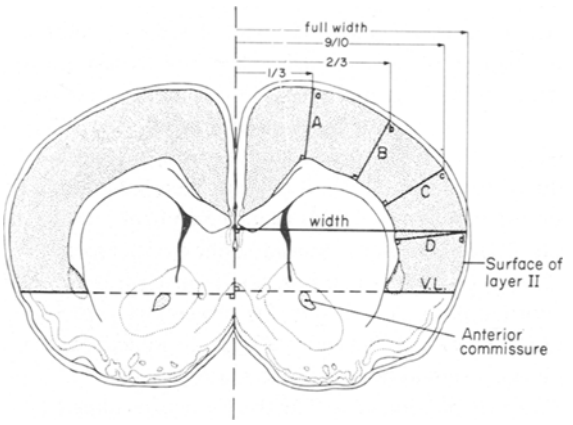

Fig. 3. Illustration of measurements on the rostral cerebral section. A-D: cortex thicknesses measured; stippled field-area measured (From[11]).

Table 5

The Effect of Maternal Calorie Restriction[1] on Neonatal Parameters in Rats

	Weight (g)		Content		Dimensions of cortex (mean ± S.D.)										
	Body	Cerebrum	DNA (μg)	Protein (mg)	Rostral						Caudal				
					Thickness (μm) at positions				Width[3] (mm)	Area[3] (sq. mm)	Thickness (μm) at positions				Width[3] (mm)
					A	B	C	D			A	B	C	D	
E[1]	4.07±0.71	0.12±0.02	533±26	7.03±1.26	630±112	648±119	603±102	600±98	2.93±0.19	2.77±0.73	496±77	510±63	486±51	396±69	3.53±0.31
n_e[2]	20	20	16	16	20	20	20	20	20	20	15	15	15	15	15
C	6.11±0.30	0.16±0.005	566±37	8.7 ±0.68	748±52	783 ±26	737± 18	694±25	3.21±0.09	3.51±0.27	580±38	595±17	588±27	442±62	3.74±0.06
n_c[2]	8	8	20	20	8	8	8	8	8	8	8	8	8	8	8
x̄Δ	−33.4	−24.4	−5.8	−18.9	−15.7	−17.2	−18.2	−13.5	−8.7	−21.2	−14.5	−14.4	−17.4	−10.3	−5.7
P	<0.0005	<0.0005	<0.005	<0.0005	<0.001	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.005	<0.0005	<0.0005	01> P>0.05	<0.01

[1] Experimental group (E), on calorie restricted diet, 10-20th day of pregnancy. Control group (C), on normal diet. From[11].
[2] n_e, n_c, number of neonatal animals in experimental and control groups, respectively. x̄Δ, mean difference between E and C, in % of C. P, probability.
[3] One hemisphere (mean of both hemispheres).

described above. Other brains, 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 (X 47) traced for subsequent measurements (Fig. 3).

The results for newborns are represented in Table 5. It can be seen that not only cerebral parameters of the offspring (weight, DNA, protein) but also cortical dimensions [thickness at several positions (A-D), 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. It was also found that all these decreases were more pronounced at birth than at 10 days or than in later development.

Release from Maternal Nutrient Storage

As can be further seen from Table 4, the decreases in cerebral parameters did not occur if mothers on the restricted diet were treated concomitantly with bovine pituitary growth hormone (ZAMENHOF et al.[10]). The improvements with growth hormone, as compared with the restricted animals, were statistically highly significant.

Growth hormone is unlikely to cross the pla-

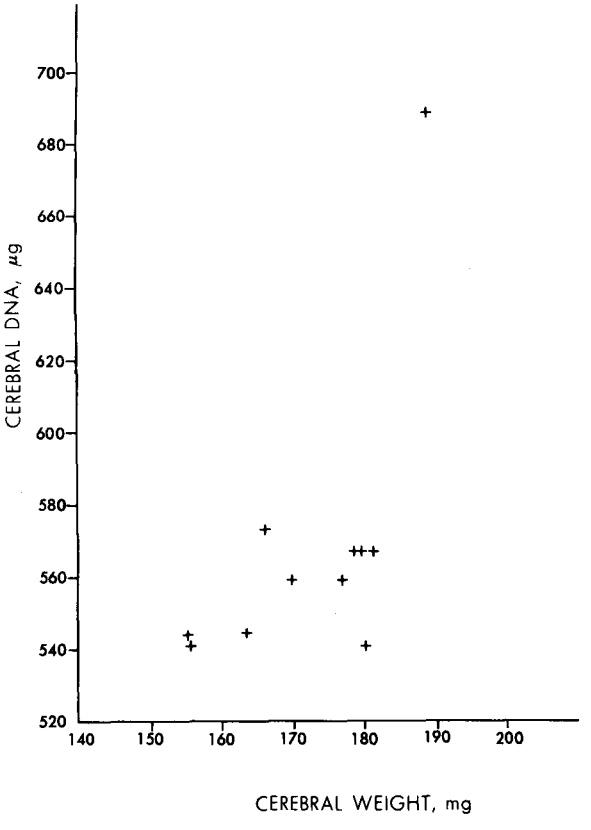

Fig. 4. Individual neonatal rat cerebral weights and neonatal cerebral DNA (cell number): One of the litters in which one fetus has cerebral DNA more than 2 std. dev. above the mean. See text for explanation. From[1].

Table 6

Effect of Operative Restriction of the Number in the Litter on Cerebral DNA Content in the Newborn[1]

Exp.[2]	Group[3]	No. rats		Offspring weights (± S.D.)		Offspring cerebral content (± S.D.)	
		Mothers	Offspring	Body (gm)	Cerebrum (gm)	DNA (μg)	Protein (mg)
1	Control	2	28	5.0±0.2	0.1277±0.0049	497±19	—
	Exptl.	3	16	5.4±0.3	0.1465±0.0065	536±21	—
	Increase			8%	15%	8%	—
				p<0.001	p<0.001	p<0.001	—
2	Control.	4	43	6.1±0.6	0.1705±0.008	539±17	9.6±0.3
	Exptl.	4	23	6.5±0.4	0.1890±0.0076	604±17	10.6±0.6
	Increase			6.5%	10.8%	12%	11%
				p = 0.01	p<0.001	p<0.001	p<0.001

[1] From van Marthens and Zamenhof[25].
[2] Exp. 1, cesarean section; Exp. 2, spontaneous delivery.
[3] Experimental, unilateral ligation of the uterine horn; Control, sham-operated.

centa[53, 54]. The primary action of the hormone might have been on the mother, by mobilization of maternal nutrient reserves, especially fat deposits.

The levels of growth hormone are known to increase during pregnancy and during fasting[55]. Perhaps this is for the purpose of mobilizing maternal nutrient reserves, or, at least, of preventing deposition of fat. Thus, mothers of similar genome but different pituitary development might produce offspring of different brain development.

Enhancement of prenatal brain development

As can be seen from Figure 4, in a genetically uniform population sporadically, animals can be found which have brain DNA well above the range of others (more than 2 std. dev.) from the same litter[1]. Such spontaneous occurrences are rare: in 0.4% to 2% of the cases in the rat. The causes of such high DNA are completely unknown, but their occurrence indicates that the precise mechanisms of regulation of DNA synthesis are not completely inviolable.

Cerebral parameters can be also increased by special laboratory treatments.

One method for increasing cerebral weight and cerebral protein content of neonatal animals is to treat normal pregnant animals with pituitary growth hormone[10] (Table 4, line C + GH and ΔC): Such treatment produced a significant increase in cerebral weight. This increase (ΔC) was not due to water but to increased content of cerebral protein. As explained above, the primary action of this hormone might have been on the mother, by mobilization of maternal nutrient reserves, especially fat deposits. Thus, conceivably, each fetus received more nutrients which stimulated its prenatal brain development.

Another method to enhance this development is to reduce operatively the number of fetuses during pregnancy: presumably such procedure also provides more nutrients per surviving fetus. The result is the significant increase in neonatal body weight, placental weight, cerebral weight, cerebral DNA (cell number) and cerebral protein[25–27]. One method to achieve this reduction consists in tying one of the two uterine horns (rat) prior to mating[25] (Table 6). Another method, used in rabbits[26] and rats[27], consists in destroying some implantation sites soon after implantation. In this case (rabbit) the increases in placental weight were up to 105%, in neonatal body weight – up to 50%, in cerebral DNA (cell number) – up to 21%, and in cerebral protein – up to 46%[26]. In the rat[27], the increases in placental weights closely followed the degree of reduction of number of fetuses.

Discussion and conclusions

This article attempts to assess some of the factors which may affect brain development, in either direction, deterioration or improvement.

The brain starts to develop very early, and its foundations and potential for further development are partially laid already at birth; thus, our considerations have also to refer to prenatal period. The evidence, admittedly very limited, suggests that environmental, rather than genetic factors, are of primary importance in that period. Genetic factors affecting prenatal brain development are essentially unknown and do not lend themselves to manipulation; this also applies to the factors that determine gene derepression and re-repression and thus start and stop neuronal DNA synthesis and neuron proliferation. On the other hand there is some information concerning the effects of environmental factors and their manipulation. Of these, embryonal and fetal nutrition has been discussed first.

The maternal nutrient supply to the fetus (embryo) offers an easy target for interference with prenatal brain development. To quote a recent study by NAEYE et al[56]: "The larger brain size in newborns of mothers who were best nourished raises the possibility that fetal brain growth may reach its full genetic potential *only* under such circumstances of full nutrition".

The actual nutrient requirements of the fetus (embryo) are not well known, and a biochemical study of "unknown growth factors" may still yield some surprises. This also applies to the multitude of regulatory factors which control the complex flow of nutrients to the site of synthesis of components in the proliferating neuron (neuroblast). This control is not always for the benefit of the fetus. For example, one of the complex controlling mechanisms described here (resorption of fetuses) has been developed during evolution to protect the mother against the organisms of different genomes (fetuses). Evidently, in times of emergency (protein deprivation) it is better to preserve a normal mother than to produce subnormal offspring. Obviously, the evolutionary driving force here was the selection for the features fittest to the species as a whole, rather than the selection of the fittest individual genomes (fetus versus fetus or fetus versus mother).

Other studies presented here indicate the transfer of the effects of prenatal malnutrition in one generation, on brain development in the next generation; the mechanism involved is a non-mendelian, maternal inheritance. These studies reveal the existence of the long-range regulatory mechanisms which, generation after generation, cumulatively adjust the size of individuals and their organs (within genetic limits) to the nutritional opportunities confronting a given strain. Thus, what we consider a deficiency may actually mean an adjustment that has a selective value for survival of an animal species. A recent study of birth weights (OUNSTED[57]) indicates that all such phenomena may be also operating in humans : the degree of constraint imposed on the fetus is correlated with the degree of constraint experienced by the mother when she herself was a fetus.

The deterioration of prenatal brain development in general may result in typical mental retardation. More numerous, and therefore more important for society are the borderline (subclinical) cases: the non-fulfillment of the genetic potential of the individual. TOWBIN[58], on the basis of 600 neonatal cases, has concluded that such subclinical minimal cerebral damages are of common occurrence, and in such cases "the potential of performance may be reduced from that of a genius to that of a plain child, or less'.

The *deterioration* of prenatal brain development by a *single* factor, e.g. deficiency of one nutrient, is biochemically easier to understand: this factor (nutrient) soon becomes the rate limiting step in the synthesis of components of the neuron (neuroblast). On the other hand, the *enhancement* of brain development may *a priori* appear impossible: the system as complex as this may consist of a multitude of closely overlapping checks so that if one factor is enhanced, the next one becomes the rate limiting step, and so on. Nevertheless, cases of enhanced brain development (admittedly rare), in genetically uniform strains, do occur naturally; possibly, many factors have changed in concert. Moreover, such enhancement can be produced at will in the laboratory, as presented here. Thus, the limits imposed on prenatal brain development are not completely precise and inviolable.

In this review, many of the particular facets are often presented in biological, rather than strictly biochemical terms. This is by necessity since even the biochemical basis of control of cellular proliferation and differentiation is still unknown. One might thus surmise that it is too early for any such studies: After all, it is only twentieth century. On the other hand, since the brain is the organ of primary im-

portance to our civilization, any progress towards its improvement appears worthy of our endeavors.

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