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Distribution of Nutrients between Fetal Brain and Body during Rat Development[1]

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Abstract. During prenatal development, the distribution of nutrients between brain and body is influenced by many factors. Fetal and neonatal ratios may serve as indices of the final outcome: brain weight/body weight (Rw), brain DNA/body DNA (R_{DNA}) and brain protein/body protein (Rprot; similar to Rw). These ratios decrease with fetal age but this dependence on age disappears towards term. Within one age, these ratios show considerable variability (up to 14%), from litter to litter, and within litters. The variability is not due to variation in water contents (< 1%). An extensive study of 2,089 normal newborns revealed that 7.5% had Rw values significantly higher than the mean for the population. Of these, 35% had normal body weights but significantly higher brain weights. Thus, they do not represent previously studied cases of general (brain and body) overdevelopment; rather, they represent cases of favorable brain versus body growth, i.e. distribution of nutrients between brain and body that is more favorable for the brain. Such a favorable distribution may be caused by maternal factors if it affects entire litters (3% of all affected) or by fetal factors, if it affects only individuals within a litter (49%).

Introduction

During pregnancy, the nutrients that reach the fetus are distributed (partitioned) among various fetal organs according to a genetically and environmentally conditioned schedule. In the past it was suggested that the differential nutrient supply to various developing organs depends on the metabolic rates of these organs at that particular time, the tissue with highest metabolic rate receiving highest priority [4, 5]. It appears now that the schedule of nutrient distribution by its nature is very complex as it involves small differences in implantation times, individual blood supplies, spaces alloted, enzyme concentrations,

[1] A short abstract of this work has appeared [12].

mitotic potentials, tropic factors [9, 18] and other regulatory mechanisms, fetal age, as well as the total amount of nutrients available [16] and the environmental disturbances [15]. Of particular interest is the distribution (partition) of nutrients between fetal brain and fetal body, since it is this overall distribution that determines the final extent of prenatal brain growth.

Because of the aforesaid complexity, the individual components that determine this distribution (spaces alloted, mitotic potential, etc.) do not lend themselves to convenient quantitative study. On the other hand, the overall final outcome, postnatal (adult) brain weight to body weight ratio, has often been quoted, especially in comparative neurology – for reviews see *Blinkov and Glezer* [3] and *Jerison* [6]. Needless to say, such a ratio, especially in adult animals, does not reflect prenatal (developmental) nutrient distribution. Such a ratio is also open to objections that it includes water and fat contents which obscure true cell numbers of brain and body.

The present work is concerned with the outcome of prenatal nutrient distribution at various fetal ages and at birth. In addition to determination of brain weight to body weight ratio, as well as brain and body water contents, we have also determined prenatal (and neonatal) brain DNA (dry weight) to body DNA (dry weight) ratios. These ratios represent an index of the brain cell number to body cell number ratios because DNA content per cell is constant for a given species – for a review, see *Zamenhof and van Marthens* [17]. Such ratios could serve as indices of distribution, between brain and body, of nutrients that had served to produce these cell numbers. Another useful ratio is the prenatal brain protein (dry weight) to body protein (dry weight) ratios, as an indication of nutrient partition between brain and body. These studies revealed that such partition indices differ in individual fetuses (and newborns) of the same litter, or the same age, some fetuses having indices more favorable for the brain than the other fetuses. A study of 2,089 newborns revealed the following. Some fetuses have significantly higher brain weight to body weight ratios only because their body weight was low; however, 35% of newborns with significantly higher brain weight to body weight ratios have normal (average) body weight and high brain weight, brain DNA and brain protein. Thus, in these animals prenatal nutrient partition was more favorable for the brain than for the rest of the body.

Materials and Methods

The animals and their nutrition regimes were as described in previous work [10]. The rats were Sprague-Dawley derived. Virgin females, 3 months old and weighing 200–260 g, were mated; the presence of a vaginal plug was considered day 0 of pregnancy. The animals were fed a pelleted stock diet containing 20.5% protein (Wayne Mausebreeder Block, Allied Mills, Chicago, Ill.). At 16, 18, or 20 days of pregnancy the fetuses were removed by cesarean section. The fetuses, as well as the newborns (at term; natural birth) were then weighed, and their brains (cerebral hemispheres without olfactory lobes) were dissected out and weighed. The brains and the remaining bodies were then stored at –15 °C for subsequent biochemical determinations.

To determine water contents, brain and (separately) body were weighed, homogenized in 7 ml distilled water, dessicated in a vacuum over P_2O_5 in tared dishes, and then weighed again. DNA contents in the brain and separately in the body were determined by a modification of the diphenylamine colorimetric method [13]. Protein contents were determined by a modification of the colorimetric method of *Lowry* et al. [7].

Results and Discussion

The ratio of brain weights to body weights (body including brain; Rw) in function of fetal age (mean for groups of 60–120 rats at each prenatal age and 2,089 newborns), is represented in figure 1. As expected, this ratio rapidly decreases towards the end of pregnancy, and becomes practically constant at term. From figure 1 one can also see that a ± 0.5 day error in the *true age* of the fetus will result in a $\pm 0.77 \times 10^{-2}$ error in Rw at nominal (chronological) fetal age 16, $\pm 0.6 \times 10^{-2}$ at 18, $\pm 0.12 \times 10^{-2}$ at 20 days, and 0 at term. Such errors before birth must be taken into consideration, in view of the observation that, even within the same litter, the implantation times for individual fetuses may vary up to ± 0.5 day (unpublished observations).

Table I shows the Rw at various fetal ages and at term. It can be seen that, at the same chronological age, Rw varies from litter to litter; in addition, Rw shows considerable variation even for individual fetuses within any one litter, as evidenced by high values of standard deviations. These standard deviations are generally higher than the possible error caused by ± 0.5 day error in true age of the fetus, especially near term (fig. 1). Thus, the deviations must be due to the actual differences among growth rates for individual fetuses, i.e. also differences in distribution of nutrients between brain and body. This is under the assumption that this portion of the nutrients which is catabolized to generate energy for the growth of the fetal mass (and therefore is not scored in the increase in fetal mass), is in each fetus proportional to this mass. The significance of variations in Rw (wet weights) could be open to objections that they merely represent variations in water contents. That this is not the case is shown in table II. It can be seen that the variations in water contents are negligible. Considering that these figures also include the dissection errors of small organs (fetal brain), the actual variations in water content are even smaller. Brain water content is similar to body water content so that wet brain weight/wet body weight is similar to dry brain weight/dry body weight.

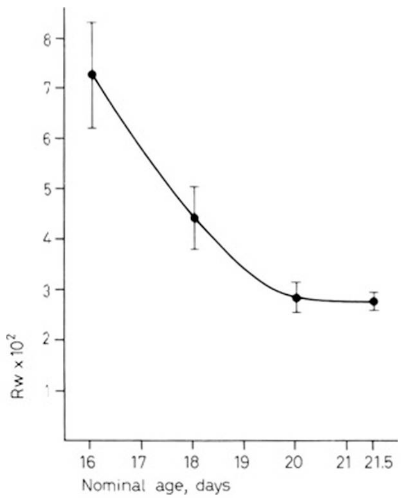

Fig. 1. The changes of ratios Rw (brain weight/body weight) towards the end of pregnancy in the rat. Body weight includes brain weight. Abscissa = nominal (chronological) fetal age; ordinate = $Rw \times 10^2$ (wet weight). Vertical lines indicate standard deviations.

The above variations are also confirmed by the study of brain DNA (dry weight) to body DNA (dry weight) ratios (R_{DNA}; table II), which are equal to brain cell number

Table I. Brain weight/body weight ratios (Rw) (wet weights) at various fetal ages and at term (mean ± SD)

Fetal age, days	Litter size	Total numbers examined		Rw × 10^2	SD, %[1]
		mothers	fetuses		
16	8.9 ± 2.7	8	70	7.29 ± 1.06	± 14.5
18	9.5 ± 2.6	13	123	4.43 ± 0.63	± 14.2
20	11.6 ± 3.4	5	58	2.86 ± 0.30	± 10.5
21½ (term)	9.6 ± 2.5	231	2,089	2.76 ± 0.19	± 6.9

[1] Standard deviation, in % of mean Rw for this age.

Table II. Water, DNA and protein contents of brain and body tissue of 18-day-old rat fetuses (mean ± SD of 13 animals)

Tissue	Water, %	DNA (dry) mg	Protein (dry), mg	Rw × 10^2		R_{DNA}[1] × 10^2	R_{prot}[2] × 10^2
				wet	dry	dry	dry
Brain	87.3 ± 0.5	0.418 ± 0.039	3.46 ± 0.51	4.43 ± 0.63	5.30 ± 0.76	7.36 ± 1.28	4.55 ± 1.10
Body[3]	89.4 ± 0.2	5.68 ± 1.08	76.1 ± 1.90				
SD, in % of mean				14.2	14.3	17.4	24.2

[1] Ratio brain DNA/body DNA.
[2] Ratio brain protein/body protein.
[3] Body including brain.

to body cell number ratios (see Introduction). It can be seen that standard deviations for R_{DNA} are even higher than those for Rw. As expected, brain protein (dry weight) to body protein (dry weights) ratio (Rprot.; table II) is itself similar to Rw; the percent standard deviations for Rprot are also higher than for Rw. These results for R_{DNA} and Rprot, both based on dry weights, indicate again that individual variations are not merely due to variations in water content.

Table III represents variability in Rw ratios in a larger study of 2,089 normal newborns from 231 mothers. It can be seen that 157 animals (N_1) or 7.5% (from 63 mothers, 27.3% of all mothers) had significantly higher Rw values (more than 2 SD above the mean for the entire population). These are the animals in which nutrient allotment to the brain was outstandingly high, as compared with the nutrient allotment to the rest of the body.

As can be further seen from table III, in some of these fetuses the high Rw value was associated with low body weight. These were *not* the animals whose actual (developmental) age was younger (implantation delay) because by the time the fetuses reach term, such animals could catch up with their litter-

Table III. Newborns with Rw values higher than 2 SD above the mean for the total population

n		% of the mean for the population				
		Rw	body weight	brain		
				weight	DNA	protein
2,089	n_1 = 157 (from 63 ♀)	121 ± 7	90 ± 11	109 ± 12	104 ± 9	104 ± 18
	n_2 = 55 (from 28 ♀)	117 ± 3	101 ± 4	118 ± 6	107 ± 9	112 ± 19

n = Total number examined; n_1 = number of animals with Rw higher than 2 SD above the mean for the total population (in parentheses – number of their mothers); n_2 = those of N_1 animals which had normal body weight.

mates [8]. At term, small differences in actual age do not affect the value of Rw (fig. 1). Thus, at term, higher Rw values must indeed reflect the distribution that during life was more favorable to the brain for them than in the cases of their littermates.

As can be seen from table III, 35.0% of newborns with higher Rw values had body weights which were average (101% of the mean) so that their high Rw ratio was solely due to their significantly higher brain weight (118% of the mean). This is also confirmed by the study of their brain DNA and brain protein (table III): they are also higher than the mean values for the population. Thus, again such fetuses cannot be 'younger', and their high Rw value must be due not to the actual younger age, but to nutrient distribution which was more favorable for the brain than in the case of their littermates.

In the past we have studied neonatal rats with outstanding values of brain and body parameters [14]. If these animals had higher values of brain weight, they also had higher values of body weight; thus, their Rw values were not necessarily high. They represented cases of general (brain and body) overdevelopment, rather than, as it is here, cases of favorable brain vs. body growth, i.e. distribution of nutrients between brain and body which is more favorable for the brain.

Out of 157 animals with significantly higher Rw, in 3% cases such a favorable distribution affected the entire litters; in such animals the causes probably involved maternal factors. In 49% of cases, only 1 individual per litter had such a favorable distribution; in such cases individual fetal factors were more likely to be the cause.

Further statistical study shows that 13 females had mean Rw values of their entire litters higher than the mean of the entire population plus 2 SD of this mean (maternal factors as probable causes). In addition, 28 newborns (from 28 different females) had their individual Rw values higher than the mean of their own litters + 2 SD of this mean (individual fetal factors as probable causes).

It must be pointed out that this more favorable nutrient supply for the brain occurs during prenatal neuron proliferation of the rat brain: depending on when it occurs during days 16 to term, it should favor different individual cortical layers, whose 'neuron births' happen to occur during that time [1, 2]. Thus, one can expect differences in neuron distribution in cortical layers among individual animals: such differences were indeed reported recently by us [11].

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