Review Article

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Maternal Nutrition and Prenatal Growth*
Experimental Studies of Effects of Maternal Undernutrition on Fetal and Placental Growth

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We have known for some time that normal growth is not simply a uniform process of getting larger proportionately in all tissues (Scammon, 1930). The reproductive system, for example, achieves almost all of its growth during the few years of adolescence. The brain achieves a significant amount of its growth prenatally and reaches 60% of its total mass by 2 years of age in the human. Such mass measurements, however, tell us nothing regarding the growth of tissues at a cellular level.

Concepts of Cellular Growth

Through the use of measurement of total DNA and protein in the tissue in question, estimates of the average cell number and of the average mass or protein per cell can be obtained if one accepts the premise that DNA is located almost entirely within the nucleus and is constant in amount within the diploid nucleus of any species (Boivin, Vendrely, and Vendrely, 1948; Mirsky and Ris, 1949). Such calculations are valid in organs containing one diploid nucleus per cell, and fortunately most tissues meet this criterion. Moreover, regardless of whether the actual values for cell number and cell size are correct, the thesis that an increase in DNA content represents one aspect of cell growth, that mainly due to cell division, and that an increase in protein or weight out of proportion to an increase in DNA represents a second aspect of growth, namely that due to cell enlargement, seems valid.

Measurements of the DNA and protein content of normal rat (Enesco and Leblond, 1962; Winick and Noble, 1965) and human (Winick, 1968; Winick, Coscia, and Noble, 1967) tissues have shown that the timing or age at which each tissue reaches a particular stage of cellular growth varies considerably, but the general pattern is similar.

This pattern is characterized by four phases. In Stage I, parallel increases in DNA and protein with no change in the protein/DNA ratio results in new cells of the same average size: hyperplasia alone. In Stage II, growth in DNA decelerates, but protein accretion continues at rapid pace resulting in new cells at a slower rate than before and in larger cells as well: hyperplasia and hypertrophy. In Stage III, DNA growth ceases and cells only grow larger: hypertrophy alone. Finally by Stage IV, or maturity, all cell growth is complete and static. After outlining the timing of these stages of growth in a tissue in question, it is then possible to study the effect of nutrition on these parameters.

Postnatal Malnutrition

Postnatal malnutrition has been studied in the tissues of the rat (Winick and Noble, 1966a) and in human brain (Winick and Rosso, 1969; Winick, Rosso, and Waterlow, 1970). Malnutrition induced during the hyperplastic stage of growth will interfere with cell division leading to generally irreversible deficits in cell number. Malnutrition during the hypertrophic stage of growth will affect cell size which is easily reversible with refeeding. Thus the state of nutrition during the neonatal and infancy periods has an important effect in programming cellular growth, and effects on cell number made during the critical period of cell multiplication are, in general, permanent.

Can this type of programming take place during
prenatal life? Certainly all fetal organs are in the proliferative growth phase and therefore appear susceptible to permanent cellular effects. However the fetus is protected from the environment by the mother and the placenta. Thus one may ask, can the mother and/or the placenta protect the growing fetus from maternal malnutrition?

In animals it is possible to monitor the effects of various maternal stresses on the cellular growth of both placenta and fetal organs. In the human, the fetus is not available for these types of studies and therefore the placenta is the only organ which is accessible after birth to be examined when the fetus is born in a viable condition. Thus if changes in the pattern of cellular growth in placenta correlate with cellular growth in fetal organs in animals, perhaps similar changes in human placenta will give us a clue to the effects of maternal stresses on the human fetus.

Placental Growth

Normal placental growth, both in the rat and in the human, proceeds in the same previously described phases of cell growth. In the rat placenta, DNA synthesis and hence cell division stop at 17 days of a 21-day gestation (Winick and Noble, 1966b). In the human placenta, cell division continues until about a fetal weight of 2400 g or about the 34th to 36th week of gestation (Winick et al., 1967). In both cases net protein synthesis continues to term. Stimuli imposed before the 17th day or 36th week, respectively, should lead to a permanent reduction in placental cell number, whereas stimuli that are active only after cell division has stopped should affect the size of individual cells but not the total number of cells. Hence examination of the ultimate cellular make-up of the placenta should give us an idea of when, during the course of growth, a stimulus has been active.

Prenatal Malnutrition in Rats

Maternal protein restriction in rats will retard both placental and fetal growth (Winick, 1969; Zamenhof, Van Marthens, and Margolis, 1968; Zeman and Stanbrough, 1969; Zamenhof, Van Marthens, and Grauel, 1971). In placenta (Table I), cell number (DNA content) was reduced by 13 days after conception, cell size (protein/DNA) remained normal, and the RNA/DNA ratio was markedly raised (Winick, 1969). Retardation in fetal growth first became apparent at 15 days. After this there was a progressive decrease in cell number in all the organs studied. By term (Table II), there was only about 85% of the number of total brain cell number in term fetuses whose mothers were exposed to a slightly different type of nutritional deprivation. Thus the cellular changes produced by severe prenatal food restriction are reflected in placenta even earlier than in the fetus, but retardation of cell division in all fetal organs including brain can be clearly shown.

By employing radioautography after injecting the mother with tritiated thymidine, cell division can be assessed in various discrete brain regions. Differential regional sensitivity can be shown in this way by the 16th day of gestation in the brains of fetuses of protein-restricted mothers (Winick, 1970a) (Fig. I). The cerebral white and grey matter are mildly affected. The area adjacent to the third ventricle and the subiculum are moderately affected, whereas the cerebellum and the area directly adjacent to the lateral ventricle are markedly affected. These data again show that the magnitude of the effect produced on cell division is directly related to the actual rate of cell division at the time the stimulus is applied. Moreover they show that the maternal placental barrier in the rat is not effective in protecting the fetal brain from discrete cellular effects caused by maternal food restriction.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Rat Placenta in Maternal Malnutrition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control*</td>
</tr>
<tr>
<td>Weight</td>
<td>0.405</td>
</tr>
<tr>
<td>Protein</td>
<td>23.00</td>
</tr>
<tr>
<td>RNA</td>
<td>1.00</td>
</tr>
<tr>
<td>DNA</td>
<td>1.06</td>
</tr>
<tr>
<td>RNA/DNA</td>
<td>0.99</td>
</tr>
<tr>
<td>Protein/DNA</td>
<td>27.00</td>
</tr>
</tbody>
</table>

*Data expressed in mg per whole placenta.

<table>
<thead>
<tr>
<th>Table II</th>
<th>Fetal Organs (% of Normal Control) in Maternal Malnutrition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue</td>
<td>Weight</td>
</tr>
<tr>
<td>Whole animal</td>
<td>87</td>
</tr>
<tr>
<td>Brain</td>
<td>91</td>
</tr>
<tr>
<td>Heart</td>
<td>84</td>
</tr>
<tr>
<td>Lung</td>
<td>82</td>
</tr>
<tr>
<td>Liver</td>
<td>82</td>
</tr>
<tr>
<td>Kidney</td>
<td>84</td>
</tr>
</tbody>
</table>
Maternal Nutrition and Prenatal Growth

Subsequent Course After Prenatal Malnutrition

The subsequent course of these animals born of protein-restricted mothers can be examined. Lee and Chow (1965, 1968) have reported that even if these animals are raised normally on foster mothers, they show a permanent impairment in their ability to utilize nitrogen. Data from our own laboratory show that if these animals are nursed on normal foster mothers in normal-sized litters, they will remain with a deficit in total brain cell number at weaning (Winick, 1970a). Thus we can again see early programming of the ultimate number of brain cells. This programme, moreover, is written in utero in response to maternal nutrition.

These same newborn pups of protein-restricted mothers may be subjected to postnatal nutritional manipulation. If they are raised in litters of three on normal foster mothers until weaning, the deficit in total number of brain cells may be almost entirely reversed (Winick, 1970a). Though quantitatively the number of cells approaches normal, qualitatively the deficit at birth might very well be made up by an increase in cell number in different areas from those most affected in utero. Thus though it may appear by optimally nourishing pups after exposing them to prenatal undernutrition that the cellular effects will be reversed, this may not actually be so in specific brain areas.

Perhaps the most analogous situation to the situation in humans is exposing these pups, malnourished in utero, to subsequent postnatal deprivation. One can raise these animals on foster mothers in groups of 18. Animals so reared show a marked reduction in brain cell number by weaning. This effect is much more pronounced than the effect of either prenatal or postnatal undernutrition alone (Winick, 1970a). Animals subjected to prenatal malnutrition alone, as previously described, show a 15% reduction in total brain cell number at birth. Animals subjected only to postnatal malnutrition show a similar 15% to 20% reduction in cell number at weaning (Winick and Noble, 1966a). In contrast, these 'doubly deprived' animals show a 60% reduction in total brain cell number at weaning. These data show that malnutrition applied constantly throughout the entire period of brain cell proliferation will result in a profound reduction in brain cell number, greater than the sum of effects produced during various parts of the proliferative phase. It appears that the duration of malnutrition as well as the severity during this early critical period is extremely important in determining the ultimate cellular make-up of the brain.

Prenatal Malnutrition in Humans

The effects on cellular growth of the human fetus are more difficult to assess. Indirect evidence suggests that cell division in the human fetus might be retarded by maternal undernutrition. Prenatal growth is retarded and brain weight reduced (Smith, 1947). If one examines available data on infants who died after exposure to severe postnatal malnutrition, three separate patterns emerge (Winick, 1970b) (Fig. 2). Breast-fed infants mal-

![Graph showing radiothymidine uptake in normal and malnourished fetal brain regions.](http://adc.bmj.com)
nourished during the second year with kwashiorrork have a reduced protein/DNA ratio but a normal brain DNA content. Term infants of normal birthweight who subsequently died of marasmus during the first year of life had a 15% to 20% reduction in total brain cell number. Infants weighing 2000 g or less at birth who subsequently died of severe undernutrition during the first year of life showed a 60% reduction in total brain cell number. It is possible that these children were deprived in utero and represent a human clinical counterpart of the 'doubly deprived' animal. It is also possible that these were true premature infants and the premature is much more susceptible to postnatal malnutrition than the term infant.

Human placenta data are available which suggest that maternal malnutrition does affect placenta growth by decreasing the DNA content or cell number (Winick, 1970a; Dayton, Filer, and Canosa, 1969) (Fig. 3). Placentas from infants with intrauterine growth retardation (IUGR), but without gross congenital malformations, show a raised RNA/DNA ratio and decreased DNA content when compared to controls. 50% of placentas from an indigent population in Santiago, Chile, showed similar findings. Placentas from a documented malnourished population in Guatemala had even more reduction in DNA content. In a single case of anorexia nervosa in which a severely emaciated mother carried to term an infant weighing 2500 g at birth the placenta contained less than 50% of the expected DNA. These data strongly suggest that the stimuli have been active for some time before the 36th week of gestation.

The effects of prenatal malnutrition in the rat are summarized in Table III. The possible effects of prenatal malnutrition in the human are summarized in Table IV. It is apparent that there are a number of similarities between the rat and human data with regard to the effects of pre-natal malnutrition upon the developing fetus and placenta.

**TABLE III**

<table>
<thead>
<tr>
<th>Effects of Prenatal Malnutrition in the Rat</th>
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<tbody>
<tr>
<td>Reduces number of cells in placenta</td>
</tr>
<tr>
<td>Increases RNA/DNA ratio in placenta</td>
</tr>
<tr>
<td>Reduces birthweight of newborn</td>
</tr>
<tr>
<td>Reduces brain cell number at birth</td>
</tr>
<tr>
<td>Increases brain cell number at weaning</td>
</tr>
<tr>
<td>Increases brain response to postnatal malnutrition</td>
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**TABLE IV**

<table>
<thead>
<tr>
<th>Possible Effects of Prenatal Malnutrition in the Human</th>
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<tbody>
<tr>
<td>Reduces number of cells in placenta</td>
</tr>
<tr>
<td>Increases RNA/DNA ratio in placenta</td>
</tr>
<tr>
<td>Reduces birthweight of infant</td>
</tr>
<tr>
<td>Reduces brain cell number at birth</td>
</tr>
<tr>
<td>Increases brain response to postnatal malnutrition</td>
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</tbody>
</table>

**DNA Polymerase**

Since a number of stimuli including nutrition have been shown to affect the growth in DNA in tissues, some investigators have turned to the study of factors which control cell division. Hyperplastic growth, or growth by cell division, requires the biosynthesis of DNA. In vivo the details of this synthesis are not completely known. However, the system requires a DNA template or pattern to copy, the replication is semiconservative, i.e. each new cell contains a strand of DNA from its mother cell and a newly made strand of its own, and all four nucleoside triphosphates containing deoxyribonucleotides are necessary building blocks for synthesis (Keir, 1965). Kornberg (1961) first described the de novo synthesis of DNA by a highly purified DNA polymerase from *Esch. coli*. In a somewhat simple-minded manner the polymerase enzyme can be thought of as a 'zip' connecting the individual nucleotides together via phosphate linkage between the deoxyribonucleotides in a manner that faithfully complements the pattern supplied by the DNA template. Subsequent to Kornberg's work with *Esch. coli* other investigators have shown similar enzyme activity in extracts from mammalian tissues. In numerous instances of unphysiological stimulation of growth such as androgen-induced growth in prostate after castration (Coffey, Shimazaki, and

![Fig. 3.—Human placental DNA content. DNA content is expressed as percentage of normal control. IUGF = intrauterine growth failure without major congenital anomalies. Circumstances leading to fetal growth retardation or likely to produce fetal growth retardation are associated with a reduction in DNA content of placenta.](image-url)
Williams-Ashman, 1968), liver regeneration after partial hepatectomy (Bollum and Potter, 1959), and isoprenaline-induced growth in salivary gland (Barka, 1965), to name a few, increases in DNA polymerase activity have been associated with a net increase in DNA, leading the investigators to feel that enzyme activity may be a convenient ad hoc index of the proliferative phase of cellular growth.

Little was known however about changes in polymerase activity with normal growth and development, and no attempts had been made to correlate enzyme activity with the rate of net DNA synthesis in normal mammalian tissues. We therefore carried out serial studies of polymerase activity in whole brain of normal rats from -6 to 44 days of age (Brasel, Ehrenkranz, and Winick, 1970). Growth in mass of the rat brain is decelerating by 44 days but has not yet reached full adult values. Growth in DNA, on the other hand, reaches adult levels by 20 days of age. When the mean percentage increase in DNA in mg per day is plotted, the peak rates in DNA increase occur between minus 6 and 0 days and between 6 and 10 days of age. Between 6 and 10 days the total DNA content actually doubles, rising from 0·9 to 1·8 mg. After 20 days of age the rate curve drops to zero with the cessation of net DNA synthesis.

We have shown that DNA polymerase activity per mg DNA or per cell parallels the rate of DNA synthesis (Brasel et al., 1970; Brasel, Joh, and Ehrenkranz, 1972). These data for whole brain are shown in Fig. 4. Polymerase activity falls after birth from previously high prenatal levels, rises sharply to reach a peak at 10 days of age, and falls again sharply to a lower level at 20 days of age with the cessation of DNA synthesis. In Fig. 5 denatured polymerase activity in forebrain is plotted in association with its rate curve for DNA synthesis. Note that one peak of enzyme activity is seen postnatally at 12 to 14 days of age, which corresponds directly with the time of peak DNA synthesis. Levels of enzyme activity then decline in association with the diminishing rate of cell division. In Fig. 6 similar data are plotted for the cerebellum. In contrast to the forebrain, the cerebellum postnatally shows two separate and distinct, but directly corresponding, peaks of enzyme activity and rates of DNA synthesis occurring at 7 and at 13 days of age.

These relations between enzyme activity and rate of DNA synthesis in whole brain, forebrain, and cerebellum are valid whether the data are expressed per mg DNA, as shown, or per mg DNA.

**Fig. 4.**—DNA polymerase activity in normal rat whole brain during development. Polymerase activity is shown by open circles, with brackets representing range of data and not SDs. Rate of increase in DNA is shown by closed circles. Enzyme activity is seen to fall after birth, rise sharply to peak at 10 days, and fall sharply thereafter. DNA rate curve parallels curve for enzyme activity.

**Fig. 5.**—DNA polymerase activity in normal forebrain during development. See legend to Fig. 4 for explanation. DNA polymerase activity and DNA rate curve peak simultaneously at 10 to 14 days of age.
Brasel and Winick

Though none of these data proves that DNA polymerase is responsible for DNA replication in vivo, they do support the contention that enzyme activity is an index of cell proliferation, and we have therefore extended our studies to malnourished tissues, where, as already mentioned, there is evidence of disturbed cellular proliferation.

If normal rats are malnourished from birth to 32 days of age, total body weight is reduced by 50% (Brasel, 1972). There is a commensurate reduction in liver weight, liver protein, and liver RNA. Liver DNA is 65% of normal and the protein per cell and the RNA per cell are reduced to 80% of normal. There is reduction then in the number of cells as well as in the size of cells.

DNA polymerase activity in postnatal rat liver (Fig. 7) shows a preference for native DNA primer, and this primer preference is maintained in malnourished rat liver. However, these latter levels are significantly reduced from control values whether expressed per mg DNA, i.e. per cell, or per mg tissue protein. Thus this enzyme's activity is selectively reduced even more than the general tissue proteins. Changes in denatured primer activity, which is present at high levels in the liver only during fetal life, are less spectacular. Preliminary data (E. Velasco and J. A. Brasel, unpublished) in rats fed a low protein diet from day 5 of gestation reveal that polymerase levels at 12 days are reduced in placenta when compared to normally fed controls. This reduction in polymerase precedes by 24 to 48 hours any measurable reduction in placental DNA content. Further studies of enzyme levels in the fetus are currently underway. These data

supernatant protein, per mg tissue weight, or total organ. Additionally, cerebellum which shows the most rapid rates of DNA synthesis postnatally in the rat, actually nearly tenfold higher per g tissue than the forebrain, also shows the highest levels of enzyme activity per g tissue.

Further, we have worked out the characteristics and requirements of the enzyme in rat and human placenta. Our data confirm that the enzyme is indeed measurable in the rat and human placenta and that its characteristics are similar to those described in other mammalian tissues (E. Velasco and J. A. Brasel, unpublished). We have performed an additional study to examine the variability of enzyme activity in multiple small samples from various placental sites. We found, not unexpectedly in this heterogeneous tissue, widely variable results. Therefore, large placental samples of one-quarter to one-half of the organ will have to be assayed to gain an accurate estimate of total tissue activity. Preliminary data in rat placenta (E. Velasco and J. A. Brasel, unpublished) reveal a parallel relation between levels of polymerase and the rate of DNA increase from 12 to 19 days of gestation.

Fig. 6.—DNA polymerase activity in normal rat cerebellum during development. See legend to Fig. 4 for explanation. Two coinciding peaks of enzyme activity and rate of DNA increases are seen in the cerebellum postnatally.

Fig. 7.—Malnourished rat liver DNA polymerase activity. After malnutrition for first 32 days of postnatal life, DNA polymerase activity in rat liver is significantly reduced when native DNA primer is employed regardless of whether the reference unit is DNA or tissue protein. Denatured DNA polymerase activity, which is present in high amounts only in fetal liver, shows less severe reductions.
suggest that one manner by which malnutrition may interfere with the biosynthesis of DNA and subsequent cell multiplication is by affecting DNA polymerase activity.

**Summary**

Intrauterine development is characterized by proliferative cell growth in all tissues. As such, it is particularly vulnerable to adverse stimuli, such as malnutrition, which could disturb the normal development patterns leading to deficits in cell number in the placenta and organs of the fetus. Indeed evidence exists in both the rat and the human that this is the case. Additionally, prenatal malnutrition may predispose the animal to particularly severe consequences if exposed to postnatal malnutrition as well. Studies of DNA polymerase activity after malnutrition suggest that one way in which this stimulus interferes with proliferative cell growth may be by reducing the level of this enzyme’s activity.

**References**


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