Some Pathophysiologic Changes in Experimental Intrauterine Malnutrition

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The two major causes (48) of intrauterine human growth retardation (IUGR) (63), which has become a major concern to neonatalogists (3,4), are a reduction in the blood flow to the fetus and maternal malnutrition. The physiological and biological changes observed in both human and animal species include a delay or arrest in cell multiplication, which may spare some organs while affecting others. Experimentation on intrauterine retarded growth has been conducted on rats (48,67), guinea pigs (46), lambs, monkeys, and pigs; IUGR has been induced either by maternal proteinocaloric deprivation or by vascular ligature or microemboli caused by plastic microspheres during gestation (Fig. 1). The following consequences have been observed in the animal and occasionally also in the human being (48):

FIG. 1. Schematic diagram of the experimental model. The fetal weights are taken from one experiment. (Modified from Wigglesworth, ref. 63.)
1. Relative preservation of the brain volume and weight versus excessive reduction of the liver (19,39,43,48)
2. Hypoglycemia (17,20,49)
3. Reduction in cell number (DNA) in the liver and preservation of cell size (18)
4. No catch-up growth when the total body weight reduction exceeds 30% (in rats) (51,61)
5. Specific pathological involvement of the cerebellum
6. Alteration of brown fat
7. Hypoproteinemia and alterations of the plasma aminogram
8. Premature appearance of the surfactant
9. Early opening of the eyes

Experimentally produced IUGR is now commonly induced by: (a) reducing the blood supply to the uterus (ligature of the uterine artery), or (b) total or selective deprivation of nutrients in the gestant animal. Sheep have been used for the experimental production of IUGR either by ligaturing one umbilical artery or by producing microemboli by injecting small plastic spheres into the uterine artery. This procedure also provides a means of measuring uterine blood flow and its distribution. Rhesus monkeys have been used to produce IUGR at 100 days gestation by ligaturing the fetal umbilical vessels leading to the secondary placental disk. The multiple pregnancy of the pig sometimes results in "runts" that weigh about 50% of the normal weight. Widdowson and Adams have used such material for their studies.

In general, most of the factors producing IUGR are environmental, either vascular or nutritional (63). The role of hormones is rather obscure; genetic factors are certainly to be taken into consideration, and it is well known that weight and body length of the offspring are dependent on maternal weight and size.

THE GENERAL PROBLEM OF ORGAN REDUCTION

If we accept a certain reduction in body weight (below the 10th percentile or 2 SD in the human, below 30% in rats, etc.) as indicative of IUGR, we can describe a general "large brain, small liver" syndrome, which is encountered in various species. This pattern is observed in all animals (48) whether IUGR is spontaneous or provoked, or of vascular or nutritional origin. In the human being, the brain is usually relatively large although it may sometimes be reduced; in contrast, the liver is more reduced than the rest of the body. In addition to the liver, the thymus and the lungs are also very reduced in size. In the rat, spleen and liver are reduced. In the monkey the spleen is markedly affected, whereas the piglet is the only animal in which the heart is also reduced (Fig. 2).
FIG. 2. Mean body and organ weight of IUGR rats, monkeys, lambs, and pigs, plotted as percentages of the control values.
The protein/DNA ratio and total RNA are unaffected in the liver of animals with IUGR which is related to the preservation of cell function (11). Liver glycogen is also markedly decreased in the rat. Glycogen content per unit wet weight of liver is not modified, but the total content is diminished because the liver is reduced in size.

**The Reduction of Blood Flow to the Organs**

The reduction of blood flow to the organs is one of the main mechanisms involved in the production of IUGR. Vascular lesions in the human being were mentioned by Gruenwald. Ligature of the uterine artery (rat, monkey, etc.) and nonradioactive-microsphere emboli obviously raise the problem of hemodynamic factors.

**Some Peculiarities in Brain Involvement (8–10)**

Winick (64) has shown that there are differences in the decrease of cell multiplication in various parts of the brain in intrauterine malnutrition. Whereas the cerebral cortex is hardly decreased in IUGR, the cerebellum (6,7,21,55) is markedly affected (it is the organ where cell multiplication is most rapid during fetal life). These differences have been studied by different methods and it has been shown (48,61) that oxygen uptake is the same in the cerebrum of IUGR rats and controls. On the other hand in our group, Privat (unpublished data) has shown, by using tissue cultures of IUGR rat brains, that there is a sharp decrease in the growth pattern of the cerebellum.

Another important point made by Winick (65,68) is that whereas prenatal or postnatal malnutrition in the pregnant mother produces very little decrease (15%) in the multiplication of the cells of the cerebrum in the offspring, the combination of both produces an enormous decrease in DNA.

**Glucose Metabolism (17,45,48,49)**

IUGR individuals can be very hypoglycemic. In the human, Sabata and Stembera have described a decrease in the glucose supply from the mother to the fetus and the absence of an arterial–venous difference in glucose, indicative of an absence of glucose utilization by the fetus; both features are abolished by a continuous infusion of glucose to the mother for short periods during labor. Lactate and pyruvate are high in the umbilical arterial blood of IUGR, a sign of chronic fetal distress. Melichar has studied hypoglycemia in IUGR newborns and has shown that intravenous administration of glucose is followed by a higher blood glucose level than in controls.

We have studied (19,20,59) enzymatic activities and their relation to blood glucose levels and hepatic glycogen content in the rat. The IUGR rat fetus has
a very low plasma glucose level. After birth, the level does not rise as in the control, but remains low until the 10th day. In contrast, the lactate level is quite normal, except at day 3, when IUGR animals have significantly higher levels than do control. In spite of a normal glycogen concentration, as expressed per gram of wet tissue, the total glycogen stores of the liver are decreased by the reduction of the liver size. The IUGR newborn rat has only 60 mg of glycogen per gram body weight, which is only half that of the control. Glycogen is normally mobilized within 24 hr and becomes similar soon afterwards in both groups. It thus appears that the hypotrophic liver is enzymically well equipped for the synthesis of glycogen.

Glucose-6-phosphatase activity in fetal liver is extremely low. It increases just after birth in both IUGR rats and controls. The activity of fructose-1,6-diphosphatase is also very low in fetal liver and rises soon after birth, the increase being slower in the IUGR newborn; 48 hr after birth it is significantly lower than in controls \((p < 0.01)\). Normal levels are reached 3 days later. At 10 days of age a significant difference in lactate dehydrogenase activity is found, the IUGR having a higher level of activity than the control animals. No modifications in the pattern of glucose-6-phosphate dehydrogenase have been found in hypotrophic liver. Asparte aminotransferase, which is involved in the conversion of amino acid to carbohydrates, has the same activity in both IUGR and controls.

Lipids, Free Fatty Acids, Glycerol, and Brown Fat \((12,13,23-25,37,44)\)

The three main functions of lipids are to act as insulation, energy stores, and structural components of certain membranes. At birth the first two functions predominate. The newborn rat is deprived of lipid stores, mainly white adipose tissue. One can follow the utilization of stores by studying the levels of free fatty acids and glycerol in the plasma. During development, the level in IUGR rats and controls is the same. In the IUGR rabbit (caused by hyponutrition of the pregnant mother during last period of gestation) the fatty acid level in the fetus is unmodified.

In the human, Sabata and Stembera have shown that in umbilical artery samples, the levels of esterified and free fatty acids are higher in the IUGR newborn than in the control. The same observation has been made for free fatty acids in the serum of the hypoglycemic IUGR newborn. This could be interpreted as a mobilization of fat reserves in the absence of sufficient carbohydrate stores (the decreased rate of glucose supply from the mother to the fetus; the small liver with a reduced total amount of available glycogen). Within 12 hr after birth, blood ketone bodies rise due to an increased breakdown of fatty acids in the IUGR newborn.

In IUGR rats, total body lipids decrease until the age of 10 days. This is associated with an increase in water content. The livers of IUGR rats contain
more total lipids 48 hr after birth than do the livers of controls. This tendency to develop fatty liver has also been found in newborn rabbits born from malnourished mothers. A decrease in hepatic hydroxybutyrate dehydrogenase has been found in spontaneous IUGR rabbits. This could be a consequence of an impaired catabolism of fatty acids. Lipogenesis from glucose is unaffected in the liver of IUGR rats (the activities of glucose-6-phosphate dehydrogenase and ATP citrate lyase are identical with those in controls). The percentage of fatty acids is identical in both IUGR and controls. The composition of brain lipids is unchanged in IUGR.

Interscapular brown fat in the IUGR rat is reduced from 12 hr before birth until 5 days after (60). Until that date the percentage of water is higher and triglyceride levels lower in IUGR. The activities of glucose-6-phosphate dehydrogenase and glycerokinase in brown fat tissue are lower. The incorporation of labeled glucose into brown fat lipids is very much reduced in IUGR. This indicates a marked reduction in the metabolism of brown fat.

**Long-Lasting Effects of Intrauterine Malnutrition on Neurotransmitter Metabolism in the Brain of Developing Rats (16)**

A number of studies have shown that malnutrition during the perinatal period may result in long-term alterations of the central nervous system (CNS) (31–34,52). Studies on neurotransmitter metabolism (1,2,53,54,57,58) in the CNS of undernourished developing rats have been conducted using food-deprived gestating or lactating mothers. However, the biochemical alterations observed may result not only from food deprivation of the fetus or newborn animal, but also from secondary changes in the food-deprived maternal organism. This led us to reinvestigate the effects of undernutrition on the metabolism of monoaminergic neurotransmitters in the CNS of developing rats using the uterine vessel ligature model (Fig. 3).

Although the blood supply was restricted for the last 5 days of gestation only, marked alterations in the levels of serotonin (5-HT) (35,36) dopamine (DA) and norepinephrine (NE) were noted in the offspring for at least the first 2 postnatal weeks. The high concentrations of tryptophan and 5-hydroxyindoleacetic acid (5-HIAA) (26) detected in the brain of IUGR rats strongly suggest that the turnover of 5-HT is accelerated in IUGR as compared with control rats.

**MATERIALS AND METHODS (see also ref. 16)**

Female rats of the Sherman strain were used. IUGR of the fetus was obtained according to Wigglesworth’s method (63). On the 17th day of gestation, the artery and vein of one uterine horn were ligatured after laparotomy under
light ether anesthesia; the opposite horn was left as a control. The fetus next to the ligature died and was partially resorbed.

In many cases, there was a gradient in fetal size between the upper and lower end of the experimental horn. No postoperative problems occurred, and about 58% of the operated mothers gave birth to IUGR rats. Eighty-seven percent of the IUGR offspring were viable and exhibited no malformation. At
birth, an animal was considered as being IUGR when the body weight was
reduced by at least 40% compared with controls. During development the
IUGR rats never reached the weight of control rats, whatever the rearing
conditions. All organs presented an important reduction, particularly the liver
and the brown adipose tissue. The brain weight was reduced by about 10%
(up to 20%). At weaning, the average weight reduction of the organs was
approximately 30%.

Throughout the study, all mother rats were fed a normal diet ad libitum
and were housed in separate cages with 6 to 8 pups. Lactation was normal
and weaning was established at 22 days. Both male and female offspring were
used at random and killed at 1, 8, 15, or 22 days (between 10 a.m. and 12
a.m.). Blood was collected from trunk vessels, and serum was obtained by
centrifugation at 3,000 × g for 30 min at 6°C.

All values were expressed as milligrams of amine, amino acid, or acid per
gram of fresh tissue or per milliliter of serum. They were corrected for recov-
erries calculated with internal standards. In all cases these recoveries were at
least equal to 75%.

Statistical calculations were performed according to Snedecor and Cochran
(56). When the p value was higher than 0.05 (Student’s t-test), the difference
was considered to be nonsignificant.

RESULTS

Whole Body, Brainstem, and Forebrain Weight Gain
in Developing Controls and IUGR Rats

Although body weight was reduced by about 40% at birth in IUGR com-
pared with control rats, differences in brain weights were rather small (Table
1). The reduction affected primarily the forebrain mass (−20%); the brainstem
weight of IUGR rats did not differ from controls. The differences noted at
birth were still present at the end of the third postnatal week. The precursors
of 5-HT have been estimated in blood and forebrain of IUGR and controls
(see Figs. 4 and 5).

Ontogenic Changes in 5-HT, DA, and NE Levels in the Brainstem
and the Forebrain of Control and IUGR Rats

5-HT levels increased markedly for the first 2 postnatal weeks and then
leveled off in the brainstem; they slightly decreased in the forebrain during the
following week (Fig. 6). Although this developmental pattern was similar in
both groups of rats, absolute 5-HT levels were generally higher in the IUGR
animals. This was particularly striking in the brainstem since the concentration
TABLE 1. Body and brain growth of control and IUGR rats during the first 3 postnatal weeks

<table>
<thead>
<tr>
<th>Age</th>
<th>Body wt (g)</th>
<th>Forebrain wt (mg)</th>
<th>Brain stem wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.21 ± 0.09 (40)</td>
<td>174.19 ± 1.58 (47)</td>
<td>47.11 ± 1.20 (17)</td>
</tr>
<tr>
<td>IUGR</td>
<td>4.46 ± 0.12 (40)</td>
<td>139.51 ± 1.95 (41)</td>
<td>46.88 ± 0.77 (18)</td>
</tr>
<tr>
<td>8 Days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>19.20 ± 0.10 (30)</td>
<td>616.55 ± 6.69 (20)</td>
<td>94.00 ± 4.19 (14)</td>
</tr>
<tr>
<td>IUGR</td>
<td>10.90 ± 0.24 (29)</td>
<td>519.89 ± 6.81 (19)</td>
<td>87.06 ± 2.53 (16)</td>
</tr>
<tr>
<td>15 Days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>34.27 ± 0.08 (30)</td>
<td>857.97 ± 10.87 (37)</td>
<td>124.15 ± 3.0 (13)</td>
</tr>
<tr>
<td>IUGR</td>
<td>23.61 ± 0.36 (30)</td>
<td>746.65 ± 9.22 (40)</td>
<td>123.86 ± 2.52 (15)</td>
</tr>
<tr>
<td>22 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>54.39 ± 0.87 (30)</td>
<td>1022.42 ± 8.78 (40)</td>
<td>155.92 ± 2.69 (25)</td>
</tr>
<tr>
<td>IUGR</td>
<td>36.85 ± 0.51 (30)</td>
<td>905.74 ± 9.65 (43)</td>
<td>151.88 ± 2.20 (36)</td>
</tr>
</tbody>
</table>

IUGR rats were selected at birth as those exhibiting a reduction in body weight at least equal to 40% as compared with control animals in the same litter. For the whole lactating period until weaning (28th postnatal day), IUGR and control developing rats were fed by mothers maintained under the same environmental conditions. Each value is the mean ± SEM of N individual determinations at each age (N expressed in parentheses).

* p ≤ 0.001, when compared with values from pair-aged control rats.

of the indoleamine in this region was significantly higher in IUGR than in control rats at all ages examined.

Little difference between control and IUGR rats was noted in the levels of the two other monoamine neurotransmitters, NE and DA (Table 2). NE levels were generally higher in IUGR than in control rats; the difference, however, was not statistically significant except in the forebrain at day 1. As already noted for 5-HT, the ontogenetic evolution of the concentration of DA followed the same pattern in the forebrain of controls and IUGR rats. DA levels were significantly higher in IUGR animals, except on day 8.

![FIG. 4. Percentage of serum free tryptophan in IUGR rats (white bars) and control rats (black bars) at various ages after birth.](image)
Ontogenetic Changes in Tryptophan and 5-HIAA Levels in the Brainstem and the Forebrain of Controls and IUGR Rats (14,15,27,28,30,38,40,41,47)

Since an increase in 5-HT levels can result from either a reduction or an acceleration (42) of the turnover of this neurotransmitter, it was of interest to measure the concentration of 5-HIAA in tissues to explain the differences between controls and IUGR rats. The ontogenetic evolution of 5-HIAA levels were markedly different in the brainstem and the forebrain in both groups of rats. Thus, at birth, the concentration of the 5-HT metabolite in the brainstem was already as high as in weaning rats and only discrete changes were noted during the first 3 postnatal weeks. In contrast, the concentration of 5-HIAA gradually increased in the forebrain during the same period and reached, on the 22nd postnatal day, a level twice as high as that found at birth. Although
these evolution patterns were very similar in controls and IUGR rats, absolute levels were markedly higher in hypotrophic animals.

A comparison of data indicates that the ratio of 5-HIAA to 5-HT levels is more elevated during the early period of life. Except at 8 days of age, this ratio is higher (10% to 36%) in IUGR than in control rats.

Under many circumstances, parallel fluctuations in the respective levels of 5-HIAA and tryptophan have been observed in the CNS leading to the proposal that an acceleration of 5-HT turnover may be due to an increased availability of the precursor amino acid in the tissues. In the present case, IUGR-induced increases in 5-HIAA levels were associated with significant elevations in the concentration of tryptophan in the brainstem and in the forebrain. Before tryptophan level fell to the adult value, that is, on the 22nd postnatal day, it
TABLE 2. NE and DA levels in the brain of IUGR and control rats at various postnatal ages

<table>
<thead>
<tr>
<th>Age</th>
<th>NE Forebrain</th>
<th>NE Brainstem</th>
<th>DA Forebrain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.127 (N = 18)</td>
<td>0.300 (N = 13)</td>
<td>0.232 (N = 18)</td>
</tr>
<tr>
<td>IUGR</td>
<td>0.009 (N = 19)</td>
<td>0.010 (N = 18)</td>
<td>0.010 (N = 18)</td>
</tr>
<tr>
<td></td>
<td>0.158* (N = 19)</td>
<td>0.326 (N = 18)</td>
<td>0.282* (N = 18)</td>
</tr>
<tr>
<td></td>
<td>0.016* (N = 19)</td>
<td>0.029 (N = 18)</td>
<td>0.013* (N = 18)</td>
</tr>
<tr>
<td>8 Days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.131 (N = 19)</td>
<td>0.315 (N = 18)</td>
<td>0.339 (N = 18)</td>
</tr>
<tr>
<td>IUGR</td>
<td>0.005 (N = 18)</td>
<td>0.021 (N = 18)</td>
<td>0.012 (N = 18)</td>
</tr>
<tr>
<td></td>
<td>0.146 (N = 18)</td>
<td>0.362 (N = 18)</td>
<td>0.348 (N = 18)</td>
</tr>
<tr>
<td></td>
<td>0.008 (N = 18)</td>
<td>0.015 (N = 18)</td>
<td>0.007 (N = 18)</td>
</tr>
<tr>
<td>14 Days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.151 (N = 12)</td>
<td>0.420 (N = 16)</td>
<td>0.421 (N = 14)</td>
</tr>
<tr>
<td>IUGR</td>
<td>0.008 (N = 18)</td>
<td>0.018 (N = 18)</td>
<td>0.014 (N = 14)</td>
</tr>
<tr>
<td></td>
<td>0.154 (N = 15)</td>
<td>0.472 (N = 14)</td>
<td>0.470* (N = 14)</td>
</tr>
<tr>
<td></td>
<td>0.008 (N = 15)</td>
<td>0.025 (N = 14)</td>
<td>0.015* (N = 14)</td>
</tr>
<tr>
<td>22 Days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.191 (N = 14)</td>
<td>0.555 (N = 14)</td>
<td>0.491 (N = 13)</td>
</tr>
<tr>
<td>IUGR</td>
<td>0.008 (N = 14)</td>
<td>0.021 (N = 14)</td>
<td>0.012 (N = 14)</td>
</tr>
<tr>
<td></td>
<td>0.187 (N = 14)</td>
<td>0.559 (N = 14)</td>
<td>0.564* (N = 14)</td>
</tr>
<tr>
<td></td>
<td>0.012 (N = 14)</td>
<td>0.029 (N = 14)</td>
<td>0.022* (N = 14)</td>
</tr>
</tbody>
</table>

Each value, expressed as micrograms of NE or DA per gram of fresh tissue, is the mean of N separate determinations. The SEM is indicated underneath.

* p ≤ 0.05 when compared with respective control values.

b p ≤ 0.01 when compared with respective control values.

remained significantly higher in tissues of hypotrophic compared with control growing animals (Fig. 7).

Ontogenetic Changes in the Concentration of Tryptophan and Tyrosine in the Serum of Control and IUGR Rats

In both control and IUGR rats, the concentration of free tryptophan progressively decreased as a function of age to reach adult values at weaning. These changes were mainly due to the progressive increase in the capacity of serum proteins to bind tryptophan since the concentration of total tryptophan and of tyrosine did not follow the same evolution pattern. A reduction in their concentration was noted only at weaning.

Except at 22 days of age, marked differences were detected between control and IUGR rats. Both the absolute concentration and the relative proportion of free tryptophan in serum were significantly higher in hypotrophic than in control animals. These changes in peripheral free tryptophan were closely related to those previously observed in brain. Such a relationship between tissue and serum free levels of tryptophan is particularly obvious when comparing values in 8- and 15-day-old animals in both groups (Table 3).
The reduction in tryptophan levels occurring in tissues of 15-day-old compared with 8-day-old control rats only paralleled that found in the concentration of free amino acids in serum, since that of total tryptophan remained constant for the period considered. In IUGR rats, tryptophan levels in tissue and concentrations of free tryptophan in serum were not significantly different in 8- and 15-day-old rats.

DISCUSSION

A restriction of the maternal blood flow during the last 5 days of pregnancy markedly affects the growth of the offspring. In spite of a normal lactation for the first 3 postnatal weeks, body weight is still more than 30% lower in IUGR compared with control rats. The brain growth is apparently comparatively less affected since a slight reduction (10% to 20%) is noted only for the forebrain mass in IUGR animals.
TABLE 3. Tryptophan and tyrosine concentrations in the serum of control and IUGR rats

<table>
<thead>
<tr>
<th>Age</th>
<th>8 Days</th>
<th>15 Days</th>
<th>22 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Control</td>
<td>IUGR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total tryptophan</td>
<td>Free tryptophan</td>
</tr>
<tr>
<td>8 Days</td>
<td>(15)</td>
<td>29.59 ± 3.41</td>
<td>9.60 ± 1.62</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>25.42 ± 2.62</td>
<td>13.14 ± 0.72*</td>
</tr>
<tr>
<td>15 Days</td>
<td>(12)</td>
<td>27.85 ± 1.48</td>
<td>3.65 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>31.57 ± 2.73</td>
<td>9.82 ± 1.19*</td>
</tr>
<tr>
<td>22 Days</td>
<td>(12)</td>
<td>22.46 ± 2.67</td>
<td>1.94 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>(19)</td>
<td>21.07 ± 1.50</td>
<td>1.93 ± 0.30</td>
</tr>
</tbody>
</table>

Control and IUGR rats were killed on the 8th, 15th, or 22nd postnatal day and blood was collected from trunk vessels. Whole and free tryptophan and tyrosine levels were measured in the serum as described in Materials and Methods. Each value (in μg/ml) is the mean ± SEM of N individual determinations.

*p < 0.05 when compared with respective control values.

During that time, changes in monoamines—particularly 5-HT, its precursor, and its metabolite—are detected in the CNS of IUGR animals. The increased levels of 5-HT, 5-HIAA, and tryptophan in IUGR rats are real since the most striking differences between IUGR and control animals are observed in the brainstem, that is, a region with the same weight in both groups of rats. Therefore, the simultaneous increases in tryptophan, 5-HT, and 5-HIAA levels very likely reflect an acceleration of 5-HT turnover in the CNS of IUGR rats. However, this acceleration may not necessarily represent an overall increase in 5-HT-dependent synaptic activity.

Two factors could be responsible for the changes in 5-HT turnover in the developing rat. Indeed, the restriction of the maternal blood flow not only results in undernutrition but also in partial hypoxia of the fetus. Although the latter can produce a marked increase in the turnover of 5-HT in the immature rat brain, it is not likely to be responsible for the long-term alterations presently observed, since no significant changes in brain tryptophan, 5-HT, and 5-HIAA levels are detected 24 days after resuscitation. Several authors have already mentioned that undernutrition can increase tryptophan, 5-HT, and 5-HIAA levels in the CNS of developing rats. Furthermore, in contrast to hypoxia, undernutrition exerts long-lasting effects since significant increases in 5-HT turnover are observed in several brain areas at 20 days after birth. The present results indicate that undernutrition for a short period (i.e., the last 5 days) during gestation induces a long-lasting acceleration of 5-HT turnover in the CNS of growing rats.

As already observed in adult as well as in normal developing rats, a close relationship exists between the concentration of circulating free tryptophan and the level of this amino acid in the brain tissue of IUGR rats. We have
confirmed that the evolutionary pattern of tissue tryptophan parallels that of the free form of the amino acid in the serum of developing animals. Although other factors, notably the level of neutral amino acids in serum, may be involved in the transport of tryptophan to brain, the present findings strongly suggest that the increased accumulation of tryptophan in brain tissues of IUGR rats is due to a higher concentration of peripheral free tryptophan. Previous studies have shown that several factors are responsible for the high level of serum free tryptophan in newborn animals. The concentration of unesterified fatty acids, which competes with tryptophan for its specific binding site onto serum albumin, is elevated during the lactation period. In addition, the concentration of the binding protein, serum albumin, is rather low in young as compared with adult rats. Accordingly, the higher level of serum free tryptophan in IUGR rats might result from changes in the concentration of unesterified fatty acids and/or serum albumin. However, neither of these two parameters is different in developing control and IUGR rats. Bourgoin et al. (15) have reported that a particular form of serum albumin with less capacity to bind tryptophan is present in the serum of normal developing rats during the early postnatal period. Since a pronounced retardation in liver maturation occurs in IUGR rats, a delay in the synthesis of the adult form of serum albumin with maximal capacity to bind tryptophan might well be responsible for the relative lack of tryptophan binding in hypotrophic animals. However, further experiments are required to establish this point.

On the basis of experiments using drugs, particularly 5-HT, which reduce the brain concentration of monoamines during development, several authors have proposed that these molecules may exert a trophic effect on brain maturation. One may thus speculate that the increased 5-HT turnover in IUGR rats could correspond to a faster maturation of the CNS in those animals, and thus represent a compensatory mechanism for retarded brain growth. Although such speculation has to be looked at with great caution, one may recall that IUGR animals open their eyes 1.5 days before controls do. A similar precocity in eye opening is obtained by daily administration of 5-HT during the early postnatal period. In this respect, IUGR rats differ markedly from offspring of mothers subjected to a low-protein diet during lactation where a delay of 2 days in eye opening is observed. This further illustrates the point that protein restriction in the mother may produce effects different from those observed in undernutrition of the offspring.

Although informative with regard to the level of neurological maturation of animals, data on the time of eye opening are, however, not precise enough to reveal alterations in the functional status of the brain of IUGR rats. In this respect the increased levels of DA and NE might be due to a more rapid differentiation of the storage capacities of the catecholaminergic neuron in developing IUGR rats. However, further studies with more appropriate neuronal markers are necessary to characterize the temporal changes in brain maturation in hypotrophic animals.
REFERENCES


**DISCUSSION**

*Dr. Semenza:* Do you have any data on the degree of respiratory control of the brown fat during development, and particularly when you say that the animals are very sensitive to cold, because obviously the physiological function of brown fat is supposed to be that.

*Dr. Minkowski:* I think you are right. It has not been looked at for the moment, but it should.
Dr. Rey: Dr. Minkowski, you used a special model to produce small-for-date rats or animals. Did you study the carcass composition of these animals at birth, because it seems to me that the main difference between small-for-dates infants and normal babies is a low content of fat in the organism. It seems also that the number of adipocytes is related to gestational age and that the size of the cells depends on the rate of growth. I wonder if with this special model you don't produce alterations of the body composition that are different in your experimental animals from what they are in humans.

Dr. Minkowski: I will try to reply to your question concerning fats. As you know, the rat does not deposit fat in intrauterine life. There are species that do this, for example, the human, guinea pig, and bat. Lafeber has studied it in guinea pigs because this animal deposits large amounts of fats, and as you would rightly suppose, the deposit of fat in the carcass is very much diminished in IUGR animals. The epidermal growth factor has been described by Dr. Stanley Cohen in the United States and, at the moment, its study requires a sophisticated radioimmunoassay. The interesting thing is that epidermal growth factor is activated by a certain number of compounds like hydroxytryptophane and thyroxine.

Dr. Greene: We have just finished a study which will be published in Endocrinology in which we gave pharmacological doses of epidermal growth factor to rats that were 12 days postdelivery, and we were able to demonstrate that at least calcium transport in an in vivo perfusion is converted from an almost passive process to an active one. In other words, we moved the development of the gastrointestinal tract as far as calcium transport was concerned backwards by a factor of 5 days, and this would tend to coincide with another observation we made, i.e., that those animals that were small, those that were in the horn of the uterus, tended to have a more rapid onset of calcium transport from the passive to the active phase. So it may be that your observations with epidermal growth factor, as far as the eyes opening is concerned, may, in fact, have some relevance as far as rapid onset of the maturation of the gastrointestinal tract of rats anyway is concerned.

Dr. Jacquot: Just a comment concerning the glucogen and the apparent lack of activity of the enzymes of the gluconeogenic pathway. I believe it has been clearly demonstrated in the rat that glucagon does not show the gluconeogenetic effects you might expect if the newborn is deprived of fatty acids. Since these small newborns do not get access to milk, they are in competition with the others, they don't suckle normally, they have a rather low content of free fatty acids in their blood, and glucagon cannot induce a gluconeogenetic pathway. As far as rats and guinea pigs are concerned, they are just as different as a newborn infant is from an adolescent. A newborn rat has nothing to do with a newborn guinea pig.

Dr. Minkowski: This is quite obvious. Nevertheless, comparative physiology does not deal necessarily with animals of some degree of maturity.