Maternal Hyperphenylalaninemia Induced Experimentally: Decreased Incorporation of \(^{14}C\)-Leucine into Protein in the Brain of the Fetus

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\(^{14}C\)-leucine was intraperitoneally injected to pregnant rats with experimental hyperphenylalaninemia. One hour following the \(^{14}C\)-leucine injection the radioactivities of amino acid fraction and protein fraction of the brain and liver were measured both in maternal and in fetal rats. Little inhibitory effect of an excess of phenylalanine was observed on the uptake of \(^{14}C\)-leucine by the liver or on its incorporation into the liver protein in the maternal rats. The striking inhibition of the uptake and incorporation of \(^{14}C\)-leucine was found in the brain of fetuses from the phenylalanine-loaded rats, in contrast with those of controls (p<0.001). In the fetal liver and maternal brain a similar inhibitory effect was also noted (p<0.05). These findings suggest that an excess of phenylalanine in maternal blood transfers across the placenta into the fetus, concentrates in fetal blood, and interferes with protein synthesis in fetal organs, particularly in the brain. These mechanisms may play an important role on the development of mental retardation and microcephaly of fetus in maternal PKU.

There are increasing number of reports concerning the deleterious effects of maternal PKU on embryogenesis and fetal development. On the other hand, it has been known that maternal PKU can be prevented by a low phenylalanine diet during pregnancy (Arthur and Hulme 1970; Howell and Stevenson 1971; MacCready and Levy 1972; Goldstein et al. 1973). Experimental study on monkeys showed that elevation of maternal serum phenylalanine could interfere with fetal growth and subsequent learning ability (Kerr et al. 1968). From these findings, it seems to be clear that the maternal biochemical abnormalities cause irreversible damages to the offspring.

Weber (1969) reported an inhibitory effect of phenylpyruvate on hexokinase of the human brain. More recently, Patel et al. (1973) documented that pyruvate metabolism by homogenates of the human brain could be disturbed in the presence of 5 mM phenylpyruvate. There is, however, no evidence that abnormal metabolites of phenylalanine such as phenylpyruvate can penetrate the placenta and accumulate in the fetal body fluids. Brown and Waisman (1971) reported that

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mental retardation occurred in four offspring of a hyperphenylalaninemic mother, without any excretion of phenylalanine metabolites in her urine.

It is well known that amino acids are actively transported across the placenta, with accumulation in fetal blood at a level two to three times higher than those in maternal blood (Glendening et al. 1961; Ghadimi and Pecora 1964). When an excess of phenylalanine is present in the maternal circulation, it can be transferred in the same manner and rate and thus accumulates in the fetal blood, amniotic fluid and fetal cerebrospinal fluid (Kerr and Waisman 1966; Thomas et al. 1971; Cockburn et al. 1972; Emery et al. 1972; Kuroda and Miyao 1973). The findings that the activity of phenylalanine hydroxylase is very low in the liver of human fetus (Räihä 1973) may contribute to accumulation of phenylalanine in the fetus of maternal PKU. These findings suggest that a high level of phenylalanine itself is detrimental to the fetal growth.

Our previous studies of experimental hyperaminoacidemia showed that intraperitoneal injection of a single amino acid in excess (such as phenylalanine, methionine, valine, histidine or glycine) commonly produced an imbalance of amino acid pools in the brain and resulted in a reduction of protein synthesis of the brain. It was presumed that such an inhibition of brain protein synthesis secondary to a shortage of substrates might be a common mechanism of development of the brain dysfunction in inherited hyperaminoacidemias (Takada and Tada 1970; Tada et al. 1971).

The present study was designed to examine the rate of protein synthesis in the fetal brain in experimentally induced maternal hyperphenylalaninemia.

**Materials and Methods**

Pregnant albino rats (S-D strain) were fed 7% L-phenylalanine from the 19th to the 21st day of timed gestation. On the 22nd day of gestation, the rats received a single intraperitoneal injection of 2.5% L-phenylalanine solution (5 ml/350 g of body weight). The injection was done in order to obtain an invariable elevation of plasma phenylalanine at the time of experiments. Thirty minutes following the injection, the rats received another intraperitoneal injection of 14C-leucine (25 μCi/350 g of body weight). One hour after the injection of radioisotope, the blood was obtained directly from the heart under a light anesthesia with ether. The fetuses consisting of 8 to 14 litter-mates were removed by the cesarean section. The liver and brain were immediately excised from maternal and fetal rats, washed in cold saline, weighed, and homogenized with 0.25 M sucrose adding 0.5% triton X-100. The maternal and fetal brains were divided into cerebrum and the other parts of the brain ("the others"), and were treated separately. The homogenates were centrifuged at 15,000×g for 30 minutes. The supernatant thus obtained was added with an equal volume of 10% trichloroacetic acid (TCA) for deproteinization. The supernatant (free amino acid fraction) and the precipitant (protein fraction) after washing with 5% TCA were assayed for radioactivity by gas flow counter (Aloka DTC-6). The protein content was measured according to the method of Lowry et al. (1951). The concentration of phenylalanine was determined by the method of McCaman and Robins (1962). Comparable pregnant females fed on the unsupplemented diet and injected of the equal volume of physiological saline on the 22nd day of gestation were served as controls.

Preliminary experiment revealed that contamination of radioactivity by lipid contained in TCA-precipitated fraction was negligible.
TABLE 1. Radioactivity found in various tissues of fetal and maternal rats with or without phenylalanine loading

<table>
<thead>
<tr>
<th></th>
<th>Radioactivity in the free amino acid fraction (cpm/g of wet weight)</th>
<th>Radioactivity in the protein fraction (cpm/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Loaded</td>
<td>Control</td>
</tr>
<tr>
<td>Maternal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>2475±494</td>
<td>2224±341</td>
</tr>
<tr>
<td>Liver</td>
<td>2625±143</td>
<td>2403±249</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>1011±198</td>
<td>1445±156</td>
</tr>
<tr>
<td>&quot;The others*&quot;</td>
<td>918±310</td>
<td>1368±199</td>
</tr>
<tr>
<td>Fetal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>2014±287</td>
<td>2745±359</td>
</tr>
<tr>
<td>Liver</td>
<td>1382±154</td>
<td>1933±136</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>695±536</td>
<td>1775±407</td>
</tr>
<tr>
<td>&quot;The others&quot;</td>
<td>669±230</td>
<td>1637±417</td>
</tr>
</tbody>
</table>

Values represent the average±s.d. from 5 rats.
* "The others" means the other parts of brain except for cerebrum.

Fig. 1. Phenylalanine levels in various tissues following an intraperitoneal injection of phenylalanine (125 mg/350 g of body weight) to pregnant rats. Tissue phenylalanine was determined by the fluorometric method of McCaman and Robins (1962).

Radioactivity in free amino acid fraction of maternal serum one hour following an intraperitoneal injection of 14C-leucine was estimated in both phenylalanine-loaded group and control group. There was no significant difference between both groups as shown in Table 1. More than 85% of radioactivity in free amino acid fraction was found to be recovered in leucine fraction. This indicates that phenylalanine loaded does not interfere with the peritoneal absorption of 14C-leucine in the present experiment.

RESULTS

1) Pregnant female rats fed on excess phenylalanine showed a slight degree of anorexia but little loss of body weight compared with controls. The fetuses from
maternal rats with phenylalanine loading exhibited neither gross anomalies nor fetal resorption. Their body weights were well comparable to controls.

2) Fig. 1 showed phenylalanine levels in serum, liver and brain from maternal and fetal rats following an intraperitoneal injection of phenylalanine. It was found that phenylalanine injected to pregnant rats was rapidly taken up by the placenta and reached its peak at 60 to 90 min following the injection in the fetal serum, liver and brain. Phenylalanine levels were found to be far higher in the fetal brain than in the maternal brain whereas there was no great difference in phenylalanine levels of the serum and liver between maternal and fetal rats; indicating an accumulation of phenylalanine to the fetal brain.

3) Fate of 14C-leucine in the maternal side: As seen from Table 1 and Figs. 2 and 3, no significant difference in the uptake of 14C-leucine by the liver nor in its incorporation into liver protein was detected between the loaded and the control group. On the other hand, both the uptake and incorporation of 14C-leucine were reduced in the maternal cerebrum and "the others" of the experimental group (p<0.05).

4) Fate of 14C-leucine in the fetal side: Reduced rates of both the uptake of 14C-leucine in the liver and its incorporation into the liver protein were found in the fetal rats as compared with controls (p<0.05 and <0.01, respectively). The mostgrave inhibitory effects on the uptake and incorporation were noted in the fetal brain (p<0.001).

Although it was not significant (p>0.05), the transport of 14C-leucine across
The placenta tended to decrease in phenylalanine loading group (Table 1 and Fig. 2).

**COMMENTS**

Analysis of the documented cases so far has uncovered that the maternal PKU may influence its progeny more profoundly than mother herself; average score of IQ may be 20 points below that of mothers, and offspring may frequently be suffered from abortion, still birth, congenital anomalies, microcephaly and intrauterine growth retardation (Stevenson and Huntley 1967; Fish et al. 1969; Hansen 1970; Yu and O'Halloran 1970). The present studies showed that the fetus could concentrate amino acids and synthesize protein to a greater extent than did maternal rats (Figs. 2 and 3). It is not surprising, therefore, that a normal placental process and such fetal ability may thus magnify maternal biochemical abnormalities resulting in a more serious disturbance in the fetus in respect to protein synthesis.

The active transport of amino acids in the immature brain is thought to be present from an early stage of fetal life (Baerlocher 1973). Oldendorf (1973) demonstrated that high level of phenylalanine (25 mg/100 ml) inhibited uptake of isotope-labeled ten neutral amino acids into the brain of rats, but had little influence on the basic amino acids such as lysine, arginine and ornithine. In the fetal brain of rat, Carver et al. (1965) noted an amino acid imbalance as a result of the daily injection of phenylalanine to the maternal rat throughout pregnancy. Barra et al. (1973) noted that reciprocal inhibition by phenylalanine and tyrosine...
of their respective incorporations into rat brain protein in cell-free system. Jones (1972) reported that addition of valine (2.5–10 mM) or tryptophan (1 mM) to the incubation medium was found to depress the brain leucine content, and also to decrease the incorporation of leucine and lysine into brain protein of guinea pig. These in vitro studies suggest that the protein synthesis in the brain is vulnerable to imbalance in amino acids.

The present study indicates that an excess of phenylalanine in maternal rats causes in vivo a profound effect on brain protein synthesis of the fetus.

References

Experimentally Induced Maternal PKU


