Effect of Subchronic Administration of Methyl Parathion on in Vivo Protein Synthesis in Pregnant Rats and Their Conceptuses

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Some pregnant women are undoubtedly exposed to organophosphorous pesticides (OP) due to the widespread use and environmental persistence of these chemicals (Ferreira and Fernandes, 1980; Draper and Street, 1981). OP cause characteristic terata in chicks (see Misawa et al., 1981). The majority of studies in mammalian species suggest that OP have high maternal toxicity and are embryolethal but not teratogenic (Fish, 1966; Tanimura et al., 1967; Ackerman and Engst, 1970; Bus and Gibson, 1974; Harbison, 1975; Fuchs et al., 1976; Schwetz et al., 1979; Deacon et al., 1980). Some observations suggest, however, that OP can induce visible structural defects in laboratory animals (Kimbrough and Gaines, 1968; Martson and Voronina, 1976; Staples et al., 1976; Staples and Goulding, 1979). The limb defects observed after exposure of mouse limb buds in organ culture to methyl parathion (MPTH) support this contention (Welsch et al., 1978). Conceivably high embryotoxicity of many OP compounds precludes the expression of the teratogenic potential because embryolethality results.

The possibility that prenatal exposure to OP might cause persistent neurochemical abnormalities in offspring has not been examined, however. OP can alter protein synthesis both in vivo (Clouet and Waelsch, 1963; Domschke et al., 1970; Cevohic et al., 1972; Koelle et al., 1977) and in vitro (Welsch and...
Dettbarn, 1971). Moreover, Lajtha and Dunlop (1981) suggested that changes in levels of amino acids during development may cause permanent damage to the brain. Thus, the goal of the present experiments was to determine if administration of MPTH throughout the period of organogenesis alters in vivo protein synthesis in embryonic/fetal or maternal tissues.

METHODS

Animals:

Estrous cycle-controlled virgin inbred Wistar-Furth rats of 10–12 weeks of age were paired for 4–5 hr with inbred Fischer 344 males (one female with two males; both strains from Harlan Industries, Indianapolis, Ind.). The day spermatozoa were found in the vaginal smears was considered Day 0 of gestation. Animals were housed in a temperature, light, and humidity controlled room (22 ± 1°C; 50 ± 10% humidity; 12-hr light/12-hr dark cycle) and provided food (Wayne Lab-Blox) and water ad libitum.

Drug Administration

Inseminated female rats were given a daily dose of peanut butter (0.1 g/25 g body wt) beginning on Day 1 between 1300 and 1400 hr. From Day 6 through Day 15 or 19 of gestation, a dose of 1.0 mg MPTH/kg was provided in the peanut butter dietary supplement which was consumed in <2 min. MPTH (7.5 mg) was dissolved in 10 ml of peanut oil which was thoroughly mixed 1:10 (v/w) with peanut butter. This level of MPTH ingestion caused no visible signs of cholinergic toxicity. The higher dose of 1.5 mg MPTH/kg had to be given by gavage (0.1 ml peanut oil/50 g body wt) because preliminary experiments revealed that some rats refused to eat the drug-spiked peanut butter supplement after a few days of administration. Gavage with plain peanut oil began on Day 1 of gestation and as of Day 6 contained MPTH. The 1.5 mg/kg dose caused signs of maternal cholinergic toxicity in some of the animals (see below). Control animals received the dietary supplement of peanut butter for the duration of gestation.

Determination of Protein Synthesis

Protein synthesis was measured by an approach modified from Dunlop et al. (1975) and Henderson et al. (1980). The method is based on the incorporation of L-[1-14C]valine (obtained from Research Products International Corp., Mount Prospect, Ill., at a specific activity of 37.5 mCi/mmol and diluted with carrier L-valine from Sigma Chemical Co., St. Louis, Mo.) at stable precursor specific activity levels into maternal and embryonal/fetal proteins of various organs. One hour after the dose of MPTH on Day 15 or 19 of gestation, 1-[1-14C]valine (500 µCi, 10 µmol/g body wt) was injected sc into the inguinal fold at a dose of 5 µCi/mmol/100 g body wt. Because of the relatively large volume (typically 6 ml) to be injected, selection of this area was critical to prevent leakage from the injection site. Animals were decapitated 0.5, 1.0, and 2.0 hr after isotope administration. On Day 15, maternal brain regions, liver, heart, kidneys, placenta, and whole embryos were dissected over a cold plate, weighed, and immediately frozen on dry ice. On Day 19, brain, liver, heart, and kidneys were also obtained from all fetuses of a litter and were pooled for one determination. Tissues were stored at −80°C and processed for protein extraction within 72 hr of their collection.

The method of Dunlop et al. (1975) was used to study the incorporation of [14C]valine into total extracted proteins of brain and viscera. Net protein was extracted by homogenizing the tissue samples with a Polytron PT10 homogenizer (Brinkmann Instruments, Westbury, N.Y.) in 3% sulfosalicylic acid (SSA, 1:5, w/v). The homogenate was centrifuged at 10,000g for 10 min. The SSA supernatant fraction constituted the free amino acid pool and was processed for HPLC analysis. The pellet was further extracted with cold and hot 5% trichloroacetic acid and washed with methanol, chloroform:methanol (1:1, v/v), and ether (Dunlop et al., 1975). The remaining powder was dried and the amount of protein determined by weight.

Determination of Net Protein and Acid-Soluble Radioactivity

Weighed aliquots (4–30 mg) of dry protein powder were transferred to scintillation glass vials and wetted with 0.2 ml of distilled water for 30 min prior to adding 1.5 ml of Soluene 350 (Packard Instruments, Downers Grove, Ill.). The vials were incubated for 4 hr with gentle agitation in a shaking water bath at 45–50°C. To each sample, isopropanol (0.4 ml) and hydrogen peroxide (0.2 ml) were added gently, and the vials remained at room temperature for 15 min before they were placed back into the warm water bath for 15 min. Then 15 ml of ACS scintillation fluid (Amersham Corporation, Arlington Heights, Ill.) was added, and radioactivity was quantified by liquid scintillation spectrometry (Tri-Carb 460 CD, Packard Instruments). The values for net protein incorporation of [14C]valine were expressed in dpm per milligram. Radioactivity in the SSA-soluble free pool was measured in aliquots which were diluted with water and mixed with ACS scintillation fluid. The measurements obtained were...
expressed as dpm per milliliter of SSA and/or as dpm per milligram fresh tissue weight.

**Analysis of Valine by HPLC and Specific Radioactivity Determinations**

(a) Acid-soluble fraction. A 500-μl aliquot of the 10,000g SSA supernatant fraction was mixed with 100 μl of acetonitrile and 100 μl of 0.5 M potassium borate buffer (pH 10.5). This sample was filtered through a 0.2-μm Teflon filter (Schleicher and Schuell, Keene, N.H.). To 200 μl of filtrate, 50 μl of methanol was added just before derivatization of the free amino acids.

(b) Protein (bound pool). Powdered protein was dissolved in 88% formic acid (1 mg/100 μl). A 20-μl aliquot was hydrolyzed in 200 μl of 6 M HCl per protein sample by heating in a tightly sealed 1-ml conical Kontes microfuge container (Kontes Glass, Vineland, N.J.) for 24 hr in a sand bath at 110°C. The hydrolysate was dried under nitrogen and immediately prepared for derivatization of the free amino acids by addition of 110 μl of 0.5 M potassium borate buffer (pH 10.5), 40 μl of acetonitrile, and 50 μl of methanol with gentle shaking after each addition.

Pre-column derivatization of amino acids was achieved by the method of Hill et al. (1979) with minor modifications. To each sample of SSA extract or hydrolyzed protein, 100 μl of 1% ethanethiol (Mallinckrodt, St. Louis, Mo.) was added, followed by 100 μl of 1% o-phthalaldehyde (Eastman Kodak, Rochester, N.Y.) (both freshly dissolved in methanol). The reaction was complete in 5 min at room temperature. A 200-μl aliquot of each derivatized sample was injected into the HPLC inlet port.

**HPLC analysis of valine.** The chromatographic system consisted of two 6000-A pumps, a Model 660 automatic solvent programmer, a Model 440 UV absorbance detector, and a Bondapak C18 reversed phase column, 3.9-mm i.d. and 30-cm length (Waters Associates, Milford, Mass.). Two buffers were used to obtain separation of the amino acids. Buffer A was 15 mM sodium phosphate (pH 7.2) and buffer B consisted of 55% acetonitrile in 15 mM sodium phosphate. The gradient system had an initial concentration of buffer B of 35% and a final one of 65%. At a flow rate of 2 ml/min, the total analysis time required was about 25 min.

The specific radioactivity of valine in the SSA extract was determined by measuring the 14C in the column effluent corresponding to the valine peak, and the values were expressed in dpm per nanomole of valine. This method, however, could not be applied to the acid-soluble pool of maternal brain regions because the levels of valine and thus of radioactivity were too low for HPLC analysis. Therefore, radioactivity in maternal brain was expressed 8 dpm per milligram wet tissue. A similar technical problem arose with the protein hydrolysates. To ascertain that radioactivity in protein (bound pool) was solely associated with valine, a few representative samples of relatively high radioactivity (150-400 dpm/mg dry protein) were selected, and 0.5-1 mg of this material was analyzed with a Beckman Model 121 amino acid analyzer. The amino acids in the protein hydrolysates were identified by comparison to the elution characteristics of authentic standards by the post-column ninhydrin derivatization method. This procedure confirmed that the amount of 14C associated with valine was equal to that present in the corresponding amount of protein powder prepared to obtain the net protein synthesis data. All other protein hydrolysates were analyzed by HPLC to quantify valine. Specific radioactivity was then calculated as dpm per micromole of valine based on radioactivity determinations in accurately weighed amounts of protein powder and by extrapolation from those values.

**Statistical Treatment of Experimental Observations**

All data were analyzed by analysis of variance and Dunnett's t test. The slopes of the lines were calculated by linear regression analysis and compared by Student's t test (Steel and Torrie, 1960).

**RESULTS**

Pregnant rats which ingested MPTH at a dose of 1 mg/kg each day beginning on Day 6 of gestation showed no signs of toxicity. At the higher dose of 1.5 mg/kg, however, signs typical of diffuse cholinergic stimulation were observed in some but not all dams. Muscle fasciculations and tremors occurred following the third or fourth dose of MPTH. More severe signs of toxicity including occasional mild clonic convulsions and prostration were observed on subsequent gestation days. Signs of toxicity appeared 15-30 min after ingestion of MPTH and lasted for 2-4 hr. The percentage weight gain between Days 0 and 15 in pregnant dams treated with 1.5 mg/kg MPTH was 11% which was significantly less than the 16% weight gain of control dams. The weight gain was 23% for rats given 1.5 mg/kg MPTH on Gestation Day 19 compared to 29% in controls. Among 14 dams treated with 1.5 mg/kg MPTH and having 123 visible implantation sites, 10 dams had a total of 36 resorptions (29%; 8 early, 28 late), compared
to 14 control dams having 111 implantations but no resorptions on Day 15. On Day 19 there were 120 implantation sites in 14 control rats and no resorptions, while 14 dams given 1.5 mg/kg MPTH had 107 implantation sites with 27 resorptions (25%). Inspection of all embryos and fetuses revealed no gross structural abnormalities in any control or treated animal. In a preliminary experiment, the effects of 1.0 and 1.5 mg/kg MPTH on rectal temperatures in pregnant rats were determined with a thermistor probe. Neither dose of MPTH altered rectal temperature (data not shown).

MPTH caused a maximal inhibition of blood cholinesterase activity (Potter, 1967) of 25–30% on the first day of administration (Day 6) and of 60–75% on Day 15.

Effects of MPTH on Protein Synthesis

A. Specific Radioactivity of [14C]Valine in Free Amino Acid Pool

The concentration of [14C]valine in SSA extracts rose rapidly and attained a stable level in many tissues within 30 min. This effect lasted for up to 2 hr (Tables 1, 2). Note that in maternal cerebral cortex, particularly on Day 19, the concentration of [14C]valine in the free pool continued to increase for 1–2 hr.

TABLE 1

EFFECT OF METHYL PARATHION (MPTH) ON SPECIFIC RADIOACTIVITY OF [14C]VALINE (FREE AMINO ACID POOL) IN TISSUES OF PREGNANT RATS, PLACENTA, AND WHOLE EMBRYOS ON DAY 15 OF GESTATION

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MPHTH (mg/kg)</th>
<th>Time (hr) after injection of [14C]valine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Liver</td>
<td>0</td>
<td>7.58 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>7.49 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>6.60 ± 0.23*</td>
</tr>
<tr>
<td>Heart</td>
<td>0</td>
<td>7.41 ± 0.73</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5.61 ± 0.14*</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>4.47 ± 0.57*</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0</td>
<td>6.60 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6.77 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>6.17 ± 0.16</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>0</td>
<td>7.92 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>8.19 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>6.66 ± 0.23*</td>
</tr>
<tr>
<td>Placenta</td>
<td>0</td>
<td>6.69 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6.77 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>4.66 ± 0.07*</td>
</tr>
<tr>
<td>Embryo</td>
<td>0</td>
<td>5.66 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5.48 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.97 ± 0.37*</td>
</tr>
</tbody>
</table>

Note. Values are expressed as dpm/nmol valine ± SE and represent the averages of four or five pregnant animals and of the placentas and embryos from entire individual litters.

*Significantly different from control at this incorporation time (p < 0.05).
TABLE 2

EFFECT OF METHYL PARATHION (MPTH) ON SPECIFIC RADIOACTIVITY OF [14C]VALINE (FREE AMINO ACID POOL) IN VISCERA OF PREGNANT RATS AND IN FETAL TISSUES ON DAY 19 OF GESTATION

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MPTH (mg/kg)</th>
<th>0</th>
<th>1.0</th>
<th>1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8.35 ± 0.40</td>
<td>5.59 ± 0.38</td>
<td>6.46 ± 0.19</td>
</tr>
<tr>
<td>Maternal</td>
<td>1.0</td>
<td>6.29 ± 0.43</td>
<td>6.72 ± 0.44</td>
<td>6.44 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>6.99 ± 0.23</td>
<td>6.82 ± 0.07</td>
<td>5.45 ± 0.03</td>
</tr>
<tr>
<td>Heart</td>
<td>0</td>
<td>8.07 ± 0.64</td>
<td>8.12 ± 0.29</td>
<td>6.34 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5.44 ± 0.28</td>
<td>6.14 ± 0.64</td>
<td>6.23 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>5.12 ± 0.15</td>
<td>6.35 ± 0.17</td>
<td>4.99 ± 0.11</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0</td>
<td>7.92 ± 0.61</td>
<td>8.18 ± 0.47</td>
<td>5.91 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6.06 ± 0.48</td>
<td>5.42 ± 0.71</td>
<td>5.50 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>5.83 ± 0.26</td>
<td>5.83 ± 0.16</td>
<td>4.98 ± 0.18</td>
</tr>
<tr>
<td>Cortex</td>
<td>0</td>
<td>6.75 ± 0.21</td>
<td>11.10 ± 0.64</td>
<td>11.77 ± 0.53</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6.36 ± 0.38</td>
<td>9.71 ± 0.47</td>
<td>11.22 ± 0.84</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>6.37 ± 0.26</td>
<td>10.31 ± 0.16</td>
<td>10.78 ± 0.24</td>
</tr>
<tr>
<td>Fetal</td>
<td>Liver</td>
<td>6.06 ± 0.51</td>
<td>6.77 ± 0.28</td>
<td>6.63 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>4.54 ± 0.27</td>
<td>5.24 ± 0.47</td>
<td>5.72 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>4.03 ± 0.68</td>
<td>3.60 ± 0.19</td>
<td>3.65 ± 0.39</td>
</tr>
<tr>
<td>Brain</td>
<td>0</td>
<td>6.84 ± 0.33</td>
<td>7.81 ± 0.20</td>
<td>7.32 ± 0.31</td>
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<td>5.37 ± 0.34</td>
<td>5.98 ± 0.63</td>
<td>6.88 ± 0.50</td>
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<tr>
<td></td>
<td>1.5</td>
<td>4.94 ± 0.13</td>
<td>5.93 ± 0.12</td>
<td>5.40 ± 0.06</td>
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<tr>
<td>Heart</td>
<td>0</td>
<td>6.98 ± 0.48</td>
<td>8.19 ± 0.07</td>
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</tr>
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<td></td>
<td>1.0</td>
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<td>5.80 ± 0.69</td>
<td>7.22 ± 0.22</td>
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<tr>
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<td>5.17 ± 0.16</td>
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<tr>
<td>Kidneys</td>
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<td>7.91 ± 0.30</td>
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<tr>
<td></td>
<td>1.0</td>
<td>6.12 ± 0.49</td>
<td>6.60 ± 0.68</td>
<td>7.10 ± 0.64</td>
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<tr>
<td></td>
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<td>4.87 ± 0.16</td>
<td>4.76 ± 0.37</td>
<td>3.22 ± 0.56</td>
</tr>
<tr>
<td>Placenta</td>
<td>0</td>
<td>7.91 ± 0.51</td>
<td>8.49 ± 0.46</td>
<td>7.65 ± 0.24</td>
</tr>
<tr>
<td></td>
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<td>5.70 ± 0.46</td>
<td>6.87 ± 0.61</td>
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<tr>
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<td>5.19 ± 0.22</td>
<td>6.70 ± 0.09</td>
<td>5.46 ± 0.36</td>
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</table>

Note: Values are expressed as dpm/nmole valine ± SE and represent the averages of four or five pregnant animals and of the organs pooled from entire individual litters.

*Significantly different from control at this incorporation time (p < 0.05).

The 1-mg/kg dose of MPHT did not significantly alter the initial concentration of [14C]valine in the free pool of maternal and embryonal tissue on Day 15, except for maternal heart. By Day 19, however, this dose significantly reduced the initial concentration (at 30 min) of [14C]valine in maternal viscera but not brain structures and in all fetal tissues.
except kidneys (Tables 1, 2). These differences tended to disappear at 1 hr and especially at 2 hr.

The higher dose of 1.5 mg MP/TH/kg caused greater and longer lasting decreases in free \([^{14}C]valine\) pool size in both maternal and fetal tissues than did 1 mg MP/TH/kg. On Day 15, striking reductions were observed in placenta (35%) and embryos (65%) (Table 1). On Day 19, there were significant reductions in SSA pool sizes in all the organs; reduced pool sizes persisted throughout the 2-hr incorporation period (Table 2). Greatest reductions of specific radioactivity were observed in fetal brain (45%) and fetal kidneys (59%).

**B. Specific Radioactivity of \([^{14}C]valine\) in Proteins**

Protein hydrolysis followed by HPLC quantitation of valine and measurement of its specific radioactivity proved to be a much more sensitive indicator of the interference by MP/TH with protein synthesis than measurement of changes in the free pool presented above. While incorporation of \([^{14}C]valine\) proceeded in a fairly linear fashion between 0.5 and 2 hr in most of the organs of control dams on both Days 15 and 19 of gestation, the specific activity of \([^{14}C]valine\) increased slowly and erratically in MP/TH-treated preg-

![Figure 1](image-url)

**FIG. 1.** Effect of methyl parathion on specific radioactivity of \([^{14}C]valine\) in proteins extracted from brain regions of rats on Day 15 of gestation. Pregnant animals received daily administrations of 0, 1.0, or 1.5 mg/kg of the drug beginning on Day 6 of gestation. One hour after methyl parathion dosing on Day 15, rats received a sc injection of \([^{14}C]valine\), and 0.5, 1, and 2 hr later, proteins were extracted from various brain regions and organs. Specific radioactivity of valine in proteins was determined after acid hydrolysis and separation of valine by HPLC. Values shown are means ± SE of four or five rats, and asterisks denote significant differences from control animals (p < 0.05).
Prenatal Toxicity of Methyl Parathion

Fig. 2. Effect of methyl parathion on specific radioactivity of [14C]valine in proteins extracted from viscera and placenta of rats on Day 15 of gestation. Pregnant animals received daily administrations of 0, 1.0, or 1.5 mg/kg of the drug beginning on Day 6 of gestation. For further details see Fig. 1.

Fig. 3. Effect of methyl parathion on specific radioactivity of [14C]valine in proteins extracted from whole rat embryos on Day 15 of gestation. Pregnant animals received daily administrations of 0, 1.0, or 1.5 mg/kg of the drug beginning on Day 6 of gestation. Embryos were obtained from rats treated as described in Fig. 1.
gested, the inhibitory effects of the 1-mg/kg dose of MPTH were more pronounced on Day 19 than on Day 15 in both maternal and fetal tissues and were significant in all organs (Figs. 4–6). Comparison of the effect of MPTH on the specific radioactivity of valine in maternal and fetal proteins revealed that the inhibition of valine incorporation in fetal brain (54%) was similar to that in maternal subcortical tissue (51%), which was the most severely affected brain region (Figs. 4 and 6). Fetal organs in general had greater reductions in valine specific radioactivity than did the corresponding maternal tissues (Figs. 4–6).

Linear regression analysis of the same data enabled comparison of the rates of [14C]valine incorporation into protein and revealed a qualitatively similar pattern. The 1.5-mg/kg dose of MPTH significantly reduced the rate of [14C]valine incorporation into proteins in all tissues examined on Days 15 and 19. The lower dose of 1.0 mg MPTH/kg reduced the rate of protein synthesis in only maternal liver, embryo, and placenta on Day 15 and in all tissues except cerebellum and fetal heart on Day 19.

DISCUSSION

In this study, administration of MPTH daily from Day 6 to Day 15 or 19 of gestation inhibited incorporation of [14C]valine into protein in maternal, placental, and embryonal/fetal tissues. The mechanism involved is not known, although there are several possibilities.

An inadequate fetal supply of amino acids can alter protein synthesis and impair in utero growth (Dancis et al., 1968; Young, 1979). If
the initial valine specific activity in the free 
amino acid pool is taken as an index of trans­
port into tissues, then transport of valine into 
maternal, placental, and fetal tissues was quite 
rapid and was significantly reduced by MPTH 
exposure (Tables 1 and 2). Whether this is a 
valid criterion of amino acid transport remains 
to be determined. Sershen et al. (1982) ob­
served that prenatal administration of nicotine 
throughout the period of brain development 
hindered brain protein synthesis in fetal and 
newborn animals by inhibiting both its syn­
thesis and breakdown. They attributed this 
effect to some metabolic changes and not to 
inhibition of amino acid transport.

In in vitro studies of lobster walking leg 
nerves, Welsch and Dettbarn (1971) found 
that $10^{-3}$ M paraoxon caused an initial increase 
in $^{14}$C-amino acid incorporation into total 
protein, but when present for $>$4 hr the drug 
caused a marked inhibition. The uptake of 
$^{14}$C-amino acids into the soluble pool was not 
affected by paraoxon. The relationship of those 
in vitro data to the present observations ob­
tained in a subchronic, in vivo experimental 
design is not clear.

A variety of drugs including ethanol, chlor­
promazine, barbiturates, and other depressants 
have been reported to alter protein synthesis 
indirectly by changing brain or body temper­
ature (Shuster and Hannam, 1964; Henderson 
et al., 1980). However, the doses of MPTH 
used in this study did not affect body tem­
perature. Other drugs such as L-DOPA (Roel et al., 1978), L-5-hydroxytryptophan (Weiss et al. 1973), d-amphetamine (Moskowitz et al., 1975), and LSD (Brown et al., 1982) have 
been reported to decrease in vivo brain protein 
synthesis by disaggregation of polysomes and 
reduction in initiation of protein synthesis.
Whether this hypothesis holds true for MPTH-induced inhibition of protein synthesis is not yet known.

The incorporation of $[^{14}\text{C}]$valine into proteins was significantly higher in whole embryos or fetal brain and fetal visceral tissues compared to the respective maternal tissues. Recently, such high protein synthesis rates in young compared to adult brain were reported by Lajtha and Dunlop (1981). Hayashi and Macfarlane (1980) explained this phenomenon for the amino acid leucine as priority of placental and fetal growth requirement over the maternal needs. Furthermore, rates of net protein synthesis have been reported to remain relatively constant in adult tissue (Dunlop et al., 1975; Henderson et al., 1980), which suggests equal rates of protein synthesis and degradation. In rapidly growing embryonal/fetal tissues, however, protein accrual exceeds protein degradation. The adult protein labeled following injection of $[^{14}\text{C}]$valine is largely replacement; whereas in the embryo/fetus, it primarily corresponds with growth.

The method employed to determine protein synthesis is a modification of that of Dunlop et al. (1975) and Henderson et al. (1980). It is based on the use of a large dose (500 mM, 10 $\mu$mol/g rat) of valine sufficient to flood the endogenous valine pool and avoid isotope dilution. The specific radioactivity in brain is immediately equal to that of the injected amino acid and remains constant for hours, assuming that all the radioactivity in the protein is associated with valine. Similar observations for $[^{14}\text{C}]$valine (Henderson et al., 1980) and other amino acids (isoleucine, methionine, tryptophan, etc.) have been reported for mouse brain (Battistin et al., 1971). The possibility of inhibition of protein synthesis by large doses of valine itself (up to 15 $\mu$mol/g body wt) has been ruled out by Dunlop et al.
(1975). This technique has adequate sensitivity to detect small changes in protein synthesis in various maternal and fetal tissues resulting from ethanol administration (Henderson et al., 1980). The use of HPLC required a smaller amount of protein and a shorter duration of time to separate and quantify valine compared to use of an amino acid analyzer and also permitted determination of valine specific radioactivity in both free and protein bound pools.

In summary, prenatal exposure to MPTH resulted in reduced incorporation of [14C]valine into maternal, placental, and fetal proteins. The effect was not specific to developing tissues and was most pronounced at a dose causing overt maternal toxicity. We have yet to determine if reduced protein synthesis persists postnatally in MPTH-exposed offspring.

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