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Effects of methylmercury on DNA synthesis of human fetal astrocytes: a radioautographic study

BEN H. CHOI, KYU H. CHO and LOWELL W. LAPHAM

Neuropathology Division, Department of Pathology, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642 (U.S.A.)

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A number of studies have demonstrated that prenatal intoxication by methylmercury (MeHg) significantly influences prenatal development of the central nervous system (CNS) both in human and experimental animals[3,4,9,13]. The mechanism or pathogenesis of this toxic influence remains obscure at the present time. One of the prominent features observed in the offspring of mothers who were intoxicated by MeHg during pregnancy has been a significant reduction in body weight as well as brain weight[3,4,9,13]. Weight reduction could be the result of diminished proliferation of cells, increased cell death, diminished biosynthetic activity of cells resulting in smaller cells or a combination of these mechanisms. No detailed information is currently available concerning the effects of MeHg on proliferating elements in developing CNS. This study was an attempt to investigate the pattern of DNA synthetic activity in human fetal brain cells, specifically astrocytes in these experiments, exposed to various concentrations of MeHg. Our previous studies of early human fetal brain[5,6] have indicated much earlier development of astroglial cells than traditionally believed, and have further suggested that these cells play important roles during neuronal migration as well as modulation of various CNS functions during development.

Human fetal astrocytes were established in cultures of human fetal brains obtained by hysterotomy*. The cells were grown routinely in medium containing 76 parts nutrient mixture F-12 (GIBCO), 20 parts fetal serum, one part L-glutamine, one part non-essential amino acids (GIBCO), one part 50% glucose in water, and one part antibiotics. Monolayer cultures of pure astrocytes were used in experiments. Identification of cell type was established by correlating features observed by phase contrast microscopy of living cultures with the findings by electron microscopy and immunohistochemistry using glial fibrillary acidic protein as a marker for astrocytes (Fig. 1).

* The fetal specimens used were received as surgical pathology specimens. This research project was approved by the Committee on Investigations Involving Human Subjects of the University of Rochester School of Medicine and Dentistry.

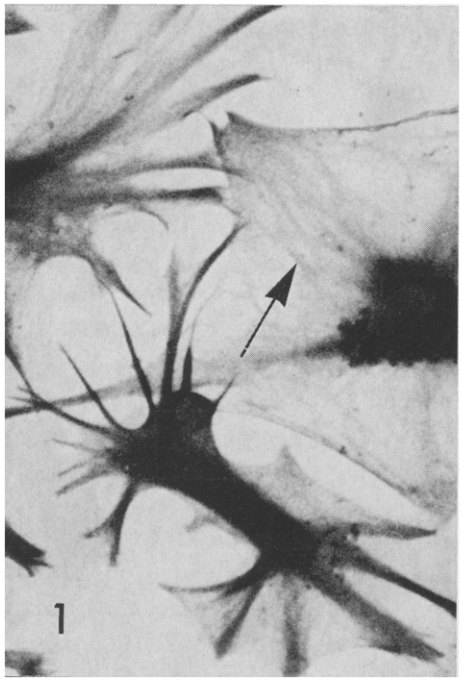

Fig. 1. Photomicrograph of astrocytes demonstrated by unlabeled antibody enzyme technique using anti-GFA protein serum. Note dark precipitates of immune reaction. Fibrillar network of GFA protein radiating toward the periphery of cytoplasm is well visualized. ×670.

Homogeneous suspensions of astrocytes (approximately 10,000 cell/ml) were aliquoted into either petri dishes or Leighton tubes with cover slips. Cultures were allowed to grow for two days and then exposed to either medium containing MeHg or control medium. Tritiated thymidine 1.0 μCi/ml was used for labeling. The cover slips were harvested at intervals, and were coated with Ilford L4 photographic emulsion. The cultures grown in petri dishes were used to study morphological details under electron microscopy after fixation in 4% glutaraldehyde and embedding in Epon.

After appropriate times in the dark at 4 °C, the cover slips were developed in Dektol and stained with either May–Grünwald–Giemsa or hematoxylin–eosin. The stained cover slips were then marked into many equal squares. The cells labeled with tritiated thymidine overlying nuclei and the total number of cells in each square were counted, and the labeling index of each sample was determined. The samples were coded and counted by 4 different individuals, all without knowledge of the code.

Methylmercuric chloride (MMC) dissolved in phosphate buffered saline (PBS) was mixed into the complete medium and incubated at 37.5 °C for 30–60 min before use. Control medium contained PBS in place of MMC. Mercury concentration in culture media was determined by the cold vapor atomic absorption technique.

When cultures were exposed to 0.1 mM and 0.2 mM MMC, the cells underwent rapid degenerative changes associated with almost total loss of DNA synthetic activity. The tritiated thymidine labeling indices in cultures exposed to 0.1 mM MMC were

0.3 and 0.4% after 30 min of pulse labeling. The control cultures in these experiments showed 5.8–6.2%. With cumulative labeling in 0.2 mM MMC, the index was 0.1% at 30 min and did not rise above 0.2% by the end of 4 h of exposure, whereas control cultures gave labeling indices of 7.8, 8.5 and 12.1% at 30 min 1h and 4 h respectively (total number of cells counted averaged 5000 cells in each).

When the concentration of MMC was reduced to much lower levels the cells maintained considerable DNA synthetic activity, though still with significant reduction in labeling indices as compared to controls. As shown in Fig. 2, the labeling index of astrocytes exposed to 5.97×10^{-3} mM MMC was significantly lower than the control at the end of 4 h.

In order to test the capacity for recovery, astrocytes exposed to MMC for 10 and 30 min were washed and replaced with normal complete medium containing tritiated thymidine. The labeling indices in these cultures returned promptly to normal or slightly above normal levels, suggesting stimulation of DNA synthesis following addition of fresh culture medium (Fig. 2).

To determine the threshold level of inhibitory effects of MMC on DNA synthesis, astrocytes were exposed for 1–6 h to concentrations ranging from 5.97×10^{-3} mM to 0.59×10^{-3} mM MMC with appropriate controls. Significant reduction in the labeling index was observed at the level of 1.19×10^{-3} mM MMC and above. No difference from the control was observed in cells exposed to 0.59×10^{-3} mM MMC (Fig. 3).

There have been conflicting reports in the literature on the effects of MeHg on macromolecular synthesis. Brubaker et al.[1,2] reported enhanced synthesis of DNA,

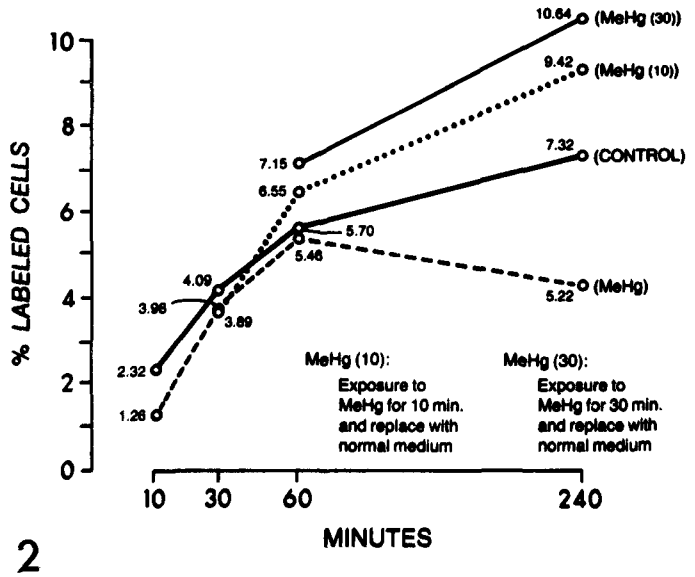

Fig. 2. Effects of methylmercury (5.97×10^{-3} mM) on [^{3}H]thymidine labeling index of human fetal astrocytes.

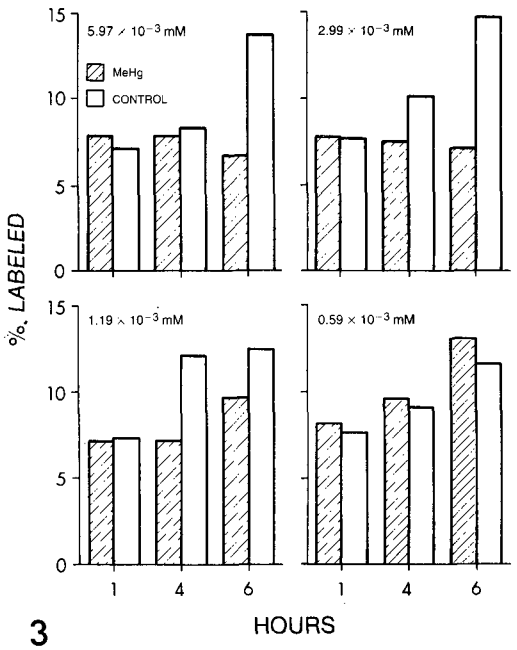

Fig. 3. Labeling indices of astrocytes in various concentrations of methylmercury.

RNA and protein in the liver and brain of rat with increasing tissue burden of mercury, while kidney was reported to show inhibitory effects. On the other hand, there are many other reports[7,8,10,11,14] claiming decreased macromolecular synthesis after MeHg exposure. Although a number of reports have dealt with inhibitory DNA synthesis of cultured cells under MeHg[7,8,10,12], this study is the first in which human fetal astrocytes were utilized. The levels of MeHg used in this study represent the total mercury concentration in the culture medium and not the levels actually taken up by the cells.

Our data are more or less similar to findings reported by Gruenwedel et al.[7] on inhibitory effects of DNA synthesis by MeHg in cells of human cancer origin. It is of interest to note that Prasad et al.[12] reported much more sensitive inhibitory effects of MMC in cell division in glioma cells than in neuroblastoma cells in culture.

It is clear from this study that within a small range of concentration, MeHg profoundly influences DNA synthesis of human fetal astrocytes. In view of the fact that these cells play a major role during development of CNS the effects of MeHg on DNA synthesis of astrocytes must be taken into account in the overall evaluation of prenatal effects of MeHg on growth and development of CNS.

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