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Light and electron microscopic study of fetal lung following maternal exposure to methylmercuric chloride

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Summary. Varying dose levels of methylmercuric chloride (MMC), 1000 ppm (5 mg through 15 mg/kg of body weight), were administered via an intragastric tube to pregnant ICR Swiss/Webster mice on day 9 of gestation. The animals were killed on gestational day 18 and the fetuses removed. Fetal lung sections were processed for light and electron microscopy. A group of animals treated with physiological saline in a similar mannner served as the controls. The fetal lungs from treated animals were hypoplastic and retarded in development. The severity of pulmonary changes increased with the dose-levels of MMC. Vacuolation and lysis of mitochondria were seen in fetal lungs. Mitochondrial damage increased in severity with dose-level of methylmercuric chloride.

Key words: Fetal lung - Methylmercuric chloride

Introduction

Microscopic studies have been carried out on the fetal nervous system following maternal exposure to methylmercuric chloride (Murakami, 1972). The most severe congenital anomalies induced by methylmercuric chloride (MMC) were exencephaly, encephocele and hydrocephalus (Harada, 1977). There is a paucity of information on the effects of MMC on the fetal lung. However, studies on the central nervous system showed a persistent hypoplasia related to MMC's antimitotic effect (Harada, 1977; Sager et al., 1984).

Ultrastructural studies have been done predominantly on nervous tissues following MMC intoxication (Chang and Hartmann, 1972; Jacobs et al., 1977; Miyakawa and Deshimaru, 1969; Miyakawa et al., 1970). It was noted that rough endoplasmic reticulum and abnormal lipid accumulation occured in neurons (Chang and Hartmann, 1972). Few experiments have been carried out examining the retinal and liver of rats (Braekevelt, 1982; Desnoyers and Chang, 1975). Visual defects were observed due to disruption of protein synthesis in the rod cells (Braekevelt, 1982). Hepatocytes of treated animals showed several early ultrastructural changes, accumulation of glycogen, and proliferation of smooth endoplasmic reticulum. Edema and degenerate sworls were observed in mitochondria of hepatocytes a month after treatment and these changes persisted throughout the recovery period (Desnoyers and Chang, 1975). Once again, there is a paucity of information availabe on ultrastructural changes in the lungs following MMC intoxication. The purpose of this study was to investigate the effects of methyl mercuric chloride on the growth and development of fetal lungs in ICR Swiss/Webster mice.

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Materials and methods

Male and female ICR Swiss Webster mice were caged together overnight and examined on subsequent mornings for copulatory plugs. The presence of a copulatory plug confirmed mating and indicated day 0 of gestation. Methylmercuric chloride (1000 ppm) was administered to the pregnant mice, using an intragastric tube, on gestational day 9, at dose levels of 5, 10 and 15 mg/kg body weight. Control animals received a corresponding volume of isotonic saline. On day 18 of gestation, the animals were killed and the fetuses removed. Sections of lungs were immediately preserved by immersion for 48 hours in 10% neutral buffered formalin and subsequently processed in a Histomatic tissue processor and embedded in paraplast. Tissue sections were prepared on an A-O Spencer rotory microtome and stained with Periodic-Acid-Schiff (PAS) to demonstrate glycogen (Pearce, 1968).

Sections of the fetal lung stained with PAS were

examined with a Leitz-Dialux-20 microscope. Black and white photomicrographs were taken of treatment and control groups with a Wild Lietz MPS-45 35 mm camera mounted on a Leitz Dialux microscope.

Lung tissues were removed from fetuses on day 18 of gestation and immediately fixed in 3% glutaraldehyde buffered at pH 7.4, using Millioning's buffer containing 10% dimethylsulfoxide and 7.5% sucrose, for 60 minutes at 4°C. The tissues were then diced into small blocks with a razor blade and fixation continued for another 1-3 hours at 4°C. The tissues were then rinsed in Millionig's buffer and postfixed in 1% osmium tetroxide buffered in 0.1 M Sorensen's pH 7.4 for 2 hours. Tissues were then dehydrated with graded series of ethanol/methanol solutions and embedded in small gelatin capsules with araldite (Sabatini, 1963).

The tissues were cured at 60°C for 18-24 hours. They were then sectioned on an LKB ultratome III and gold to silver sections were taken up on copper 200 mesh grids, blotted and dried. The grids were stained in aqueous uranyl acetate and lead citrate. Thin stained sections were examined using a Philips 201 electron microscope.

Results

I. Light microscopy

Figures 1-4 show fetal lungs on gestational day 18 from animals of the control, 5, 10 and 15mg/kg treatment groups. Morphological appearance of fetal lung was used to estimate its relative maturity and development. The location and amount of glycogen was also used to determine the rate of growth and the relative developmental stage of the fetal lungs.

Control lung was in the terminal air sac stage (tas), as can be seen in Figure 1. The presence of numerous terminal air sacs (tas) confirmed this morphological finding. Glycogen was not present in the flattened differentiating epithelial cells and was largely confined to interstitial mesenchymal cells in small amounts. The morphological appearance of lungs from animals of the 5 mg/kg treatment group showed the presence of some terminal air sacs (tas) and numerous unbranched alveolar tubules (t). There was a large amount of glycogen present in the epithelial cells of the alveolar tubules, with lesser amounts in the epithelia of the terminal air sacs and the interstitial cells. Lungs from fetuses of the 5 mg/kg treatment group were in a transitional phase between the more mature terminal air sac stage and the less mature pseudoglandular stage (Fig. 2).

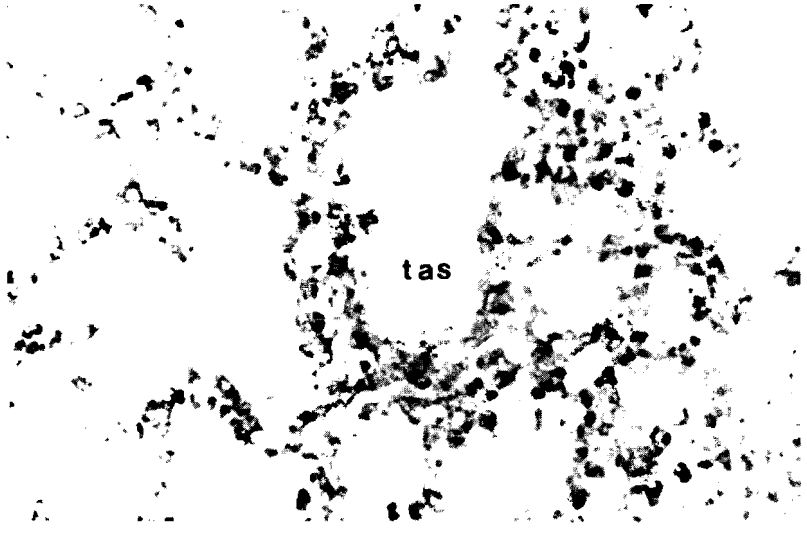
Fetal lungs obtained from the 10 mg/kg treatment group revealed a glandular appearance due to the presence of straight alveolar tubules (t) embedded in a thick mesenchyme. The epithelial lining of the alveolar tubules are columnar, filled with large amounts of glycogen. Morphologically these lungs appeared pseudoglandular (Fig. 3). Fetal lungs from mothers treated with 15 mg/kg of methylmercury appeared to have still fewer alveolar tubules. These alveolar tubules are lined by columnar epithelium distended with copious amounts of glycogen, while the very thick interstitial mesenchyme appeared to have no glycogen. These lungs appeared immature (Fig. 4) and were in an early pseudoglandular stage consisting of widely spaced straight alveolar tubules embedded in a thick mesenchyme.

II. Electron microscopy

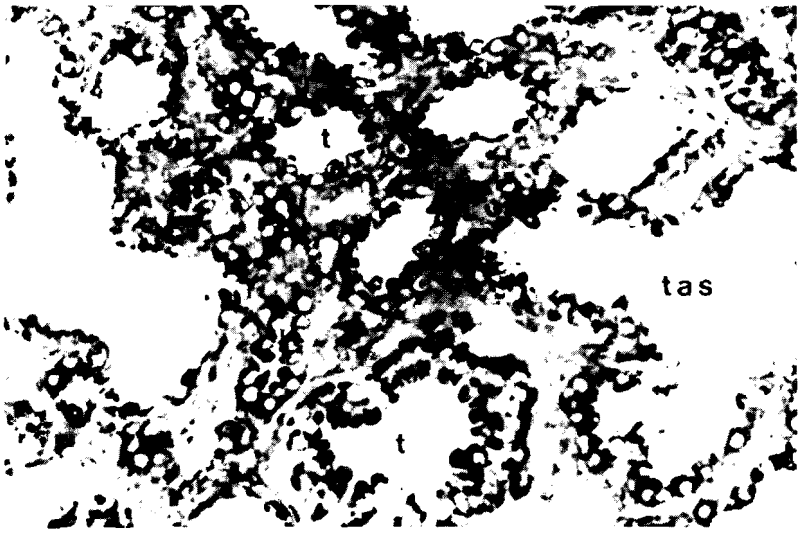
Electron microscopical examination of day 18 fetal lung following maternal treatment with MMC revealed several degenerative ultrastructural changes (Figs. 5-8).

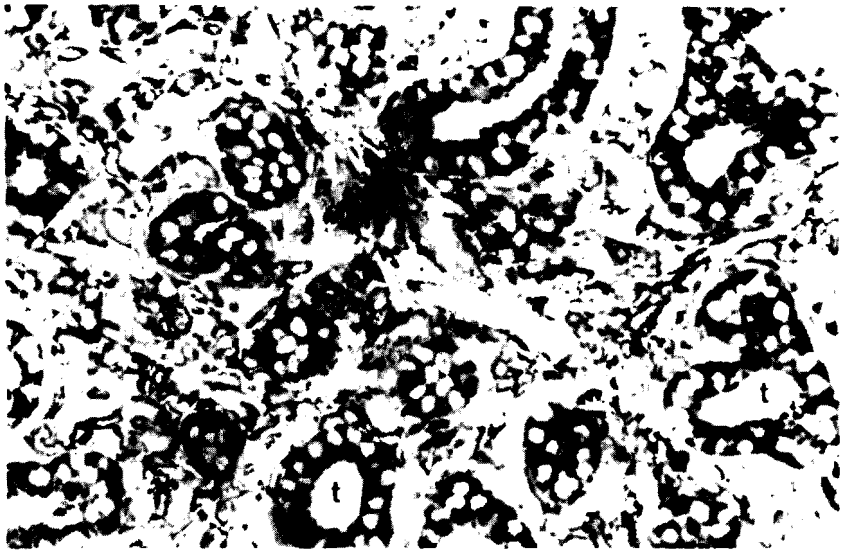
Control lungs appeared normal with no obvious ultrastructural changes in either the nucleus or cytoplasm (Fig. 5). Fetal lungs examined from animals of the 5 mg/kg treatment group showed the presence of numerous small vacuoles in the mitochondria, as well as slightly pleomorphic mitochondria. The remainder of the cellular organelles appear normal (Fig. 6).

Fetal lungs from animals of the 10 mg/kg treatment group (Fig. 7) showed prominent vacuolation in the mitochondria and also degenerating sworls within the



Figs. 1-4. Paraffin sections stained with P.A.S. to demonstrate glycogen. Control (Fig. 1) shows the presence of terminal air saccules (tas) and very little glycogen in epithelial cells (glycogen stains darkly). The 5 mg/kg tratment group (Fig. 2) shows a mixture of terminal air saccules (tas) and tubules (t). The epithelium of the airways are still filled with darkly stained glycogen. The 10 mg/kg group shows the presence of only tubules (t) lined by epithelium filled with glycogen (Fig. 3). The 15 mg/kg (Fig. 4) reveal fewer tubules (t) lined by glucogen filled epithelial cells. × 400


t
tas
t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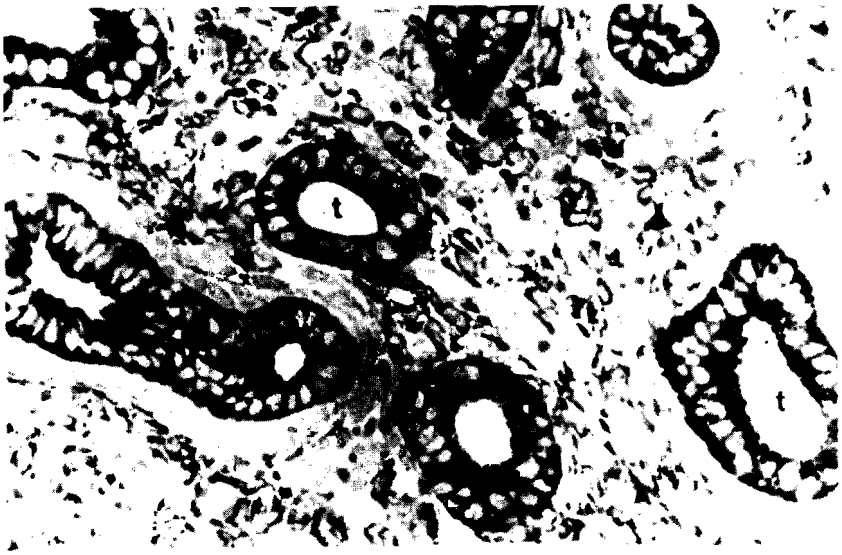
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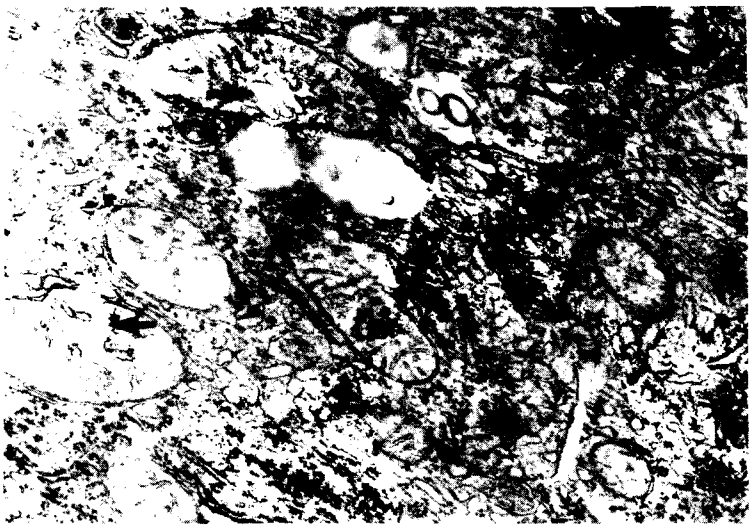
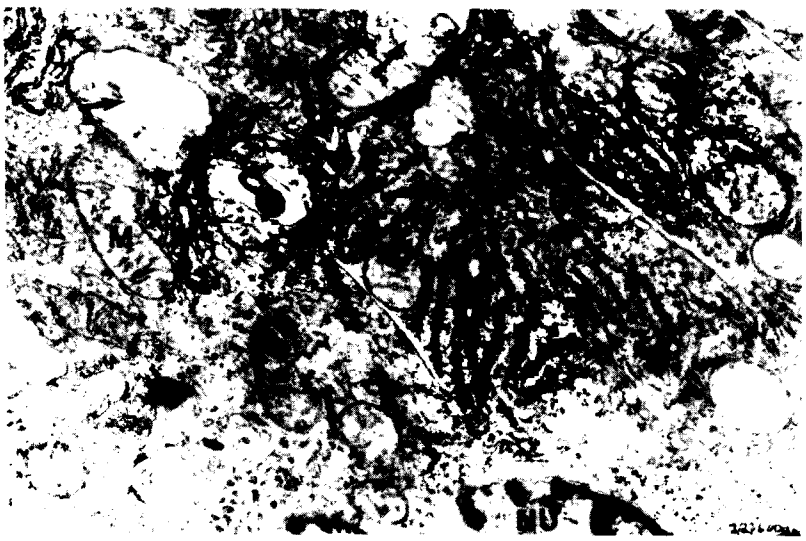
Figs. 5-8. Electron micrographs of fetal lung from control and three treatment groups. Control (Fig. 5) shows normal ultrastructural organelles, nucleus (NU) and mitochondria (M). The 5 mg/kg treatment group (Fig. 6) shows the presence of numerous small vacuoles (arrows) in the mitochondria. The 10 mg/kg (Fig. 7) shows large pheomorphoric mitochondria with large vacuoles (arrows) and sworls. The 15 mg/kg (Fig 8) shows degenerating mitochondria (M) which are undergoing lysis. × 22,610





mitochondrial vacuoles. The mitochondria are pleomorphoric and markedly degenerated, having assumed a rather bizarre shape. Other cellular organelles do not appear to have been adversely affected at this stage by MMC. At the highest dose level (15 mg/kg), fetal lungs appeared to be more adversely perturbed by MMC. The mitochondria underwent lysis having lost their smooth contour (Fig. 8). The mitochondria were degenerated and scalloped in appearance.

Discussion

The growth and development of the fetal lungs can be estimated by its morphological appearance and by the amount and location of intracellular glycogen. The lungs of fetuses recovered on day 18 of gestation were found to be at different stages of growth and development. Control lungs appeared most mature and developed as they were in the terminal air sac stage. The epithelial lining was flattened and contained little intracellular glycogen. The interstitial mesenchyme was relatively thin and contained some intracellular glycogen. There were no alveolar tubules present or cuboidal epithelial cells filled with glycogen. This type of respiratory morphology is associated with a relatively mature and well developed fetal lung just before parturition (Curle and Adamson, 1978).

Fetal lungs from the 5 mg/kg treatment group appeared less well developed. Both terminal air sacs and the more immature alveolar tubules were present. The epithelium was not as advanced in differentiation as determined by its cuboidal shape with abundant intracellular glycogen. The interstitial mesenchyme was thicker than in the control with some intracellular glycogen present. These findings suggested that these lungs are in a transitional phase betwen the more mature terminal air sac stage and the less mature pseudoglandular stage (Burri, 1974).

Fetal lungs from the 10 mg/kg treatment group of animals appeared to be delayed in growth and development compared to those from 5 mg/kg treatment group and the controls. They exhibited a distinct pseudo-glandular appearance with numerous alveolar tubules but no terminal air sacs. The alveolar tubules were lined with undifferentiated cuboidal epithelium filled with intracellular glycogen. The very thick intracellular mesenchyme had little or no glycogen. These findings suggest a relatively immature pseudoglandular lung, delayed in growth and development, for a fetus of this gestational age.

Lungs examined from the 15 mg/kg treatment group had a hypocellular tubular pseudo-glandular appearance. There were fewer alveolar tubules than in the 10 mg/kg group which gave a hypocellular appearance to the lung. The mesenchyme was thick with no glycogen present, while the epithelia cells were undifferentiated and distended with glycogen. The morphological appearance of the lung was that of an immature, underdeveloped lung not consistent with a fetal mouse lung just prior to parturition as was seen in the control.

These histochemical and morphological findings indicate that methylmercury has a perturbing effect on fetal lung growth and subsequent development. The immature appearance of the lungs from the treatment groups compared to the control support the contention that lung growth and development are retarded by methylmercury. The degree of retardation of fetal lung growth and development paralleled the increasing dosage of MMC. The highest treatment dose (15 mg/kg) caused the most retardation of pulmonary growth and development. The intermediate dose (10 mg/kg) resulted in less retardation of growth and development while the lowest dose (5 mg/kg) had the least effect. However, the lungs of fetuses from all three treatment groups appeared less developed compared to the controls.

Electron microscopical studies of fetal lung revealed that mitochondrial degeneration was the salient morphological change seen in fetal tissues examined from dams treated with methylmercuric chloride (O'Hare and Sheridam, 1970). Initial damage was seen as mild vacuolation in mitochondria at the lowest dosage

group (5 mg/kg). Mitochondrial ultrastructure damage was progressive as apparent in the intermediate treatment group (10 mg/kg); large vacuoles with sworls were seen inside pleomorphic mitochondria. The highest dose level (15 mg/kg) caused actual mitochondrial destruction as indicated by edema and lysis. The progressive destruction and loss of mitochondria would lead to entrophy and eventually irreversible cell necrosis.

Light microscopy revealed that methylmercury inhibited the growth and development of the fetal lungs. At the ultrastructural level, the lungs of treated fetuses showed evidence of cellular damage. In particular, the mitochondria revealed varying levels of vacuolation (Noack and Schwartz, 1976).

Most alveoli develop after birth. The degree of development has been retarded by maternal exposure to MMC and the relative maturity of the prenatal lung varies widely depending upon the dosage; the higher the dosage the more immature the lung appeared. In this manner the growth and development of fetal lungs are being compromised by maternal exposure to MMC. A reduced rate of cellular division and differentiation in the critical prenatal period would result in a more immature air-blood barrier at birth which could lead to severe respiratory diseases in the offspring.

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