Short Communication

TOXIC EFFECTS OF METHYLMERCURY
ON THE LIVER IN EXPLANT
TISSUE CULTURE

肝培養組織に対するメチル水銀の毒性

Despite the rapid accumulation of methylmercury in the liver,^{1,2)} few detailed investigations have been performed to study the toxic effects of methylmercury on the hepatic tissues. Most alkyl mercury compounds are eliminated *via* the bile. As the bile enters the small intestine, a diffusible complex of methylmercury is rapidly reabsorbed into the bloodstream giving rise to a large enterohepatic circulation of methylmercury.³⁾

Takeuchi *et al.*⁴⁻¹⁶⁾ reported that the striking feature of Minamata Disease in humans is the preferential degeneration in both calcarine regions and other cortical areas of the brain. They reported further that the liver showed only minor changes. However, it is not clear whether the variety of the degree of injury is due to the difference in the distribution of mercury compounds or due to the difference in the vulnerability of these organs to the compounds.

The present investigation was designed to study the toxic effects of methylmercuric chloride (MMC) on the newborn rat liver in tissue culture.

Materials and methods

Livers from newborn rats (5 and 7 days old) were cut into small fragments of 1–2 mm³, and cultured on plastic dishes (Falcon dishes for cell culture, 35 × 10 mm) at 35°C for 1–4 days in a CO₂-incubator. The culture medium was a mixture of calf serum and Eagle's MEM (1:1).

As hepatocytes have many microbodies containing catalase, the peroxidatic activity of the enzyme was measured using 3,3'-diaminobenzidine (3,3'-DAB) as a hydrogen donor.⁷⁾ Fibroblasts took a characteristic trabecular pattern, and formed collagen fibers.

The degree of outgrowth was expressed by the following conditions of both hepatocytes and fibroblasts. I: No growth of hepatocytes and no growth of fibroblasts (without the formation of collagen fibers). II: Formation of only collagen fibers with degenerated fibroblasts and no out-

growth of hepatocytes. III: Depressed outgrowth of both hepatocytes and fibroblasts. IV: Normal outgrowth of fibroblasts and depressed outgrowth of hepatocytes (in rare cases, depressed outgrowth of fibroblasts and normal outgrowth of hepatocytes). V: Normal outgrowth of hepatocytes and fibroblasts.

Results

A few hours after plating, the cells were fixed to the dish; small spherical cells from the peripheral portions of the explants spread out on the dish surfaces. Fibroblasts formed collagen fibers, but identification of the cells was difficult. After 24–48 hr the morphological differences between the outgrowths began to appear. After 3–4 days culture, fibroblasts arranged in a typical trabecular pattern and the presence of epithelium-like cells was partially observed.

Fibroblasts arranged in a trabecular pattern were stained with 3,3'-DAB diffusely and faintly, while epithelium-like cells showed obviously positive granules. These epithelium-like cells were considered as hepatocytes.

At low concentrations of MMC ($0.2\text{--}0.5 \times 10^{-5}$ M) no detectable changes were found after 24–48 hr culture. After 3–4 days culture, degenerative changes of some of hepatocytes and fibroblasts were evident, in contrast to the cells in the control culture. Neither the trabecular pattern of fibroblasts nor the epithelium-like morphology of the cells became obscure.

At high concentrations of MMC ($1.0\text{--}4.0 \times 10^{-5}$ M) no outgrowth of cells was seen during 24–48 hr culture. After 3–4 days culture, degenerative changes of fibroblasts and a decrease in the formation of collagen fibers were observed in a few explants.

These results are summarized in Tables 1 and

Table 1. Normal outgrowth of liver and effects of MMC in explant culture (after 3 days incubation).

Degree of outgrowth	Control	MMC ($\times 10^{-5}$ M)			
		0.5	1.0	2.0	4.0
V	4	2			
IV	1	1			
III		1	1		
II			1	1	1
I			2	3	4
U-value		7.0	0.5 [#]	0 [#]	0 [#]

The Mann-Whitney U test. Critical value of U for a two-tailed test at $\alpha=0.05$, is 2.

[#]Significantly different from the control.

Numerals in the table show the number of explants of liver. As to the degree of the outgrowth, see the text. The liver from a 7-day old newborn rat was used.

Table 2. Normal outgrowth of liver tissues as explant culture and effects of MMC (after 4 days incubation).

Degree of outgrowth	Control	MMC ($\times 10^{-5}$ M)					
		0.2	0.5	0.7	1.0	2.0	4.0
V	5	1					
IV	4	7	9				
III			1		1		
II				3	5	1	
I	1	2	7	4	9	10	
U-value		29.5	28.0	7.5 [#]	10.0 [#]	6.5 [#]	5.0 [#]

The Mann-Whitney U test. Critical value of U for a two-tailed test at $\alpha=0.05$, is 23.

[#]Significantly different from the control.

Numerals in the table show the number of explants of liver. As to the degree of the outgrowth, see the test. The liver from a 5-day old newborn rat was used.

2. It is suggested that the minimal dose of MMC for injurious effect on the liver is between 0.5×10^{-5} M and 0.7×10^{-5} M.

Discussion

Clinical aspects of mercurial intoxication were studied mainly in the nervous system. As shown in the research of Minamata Disease, both in acute and subacute cases, the most conspicuous changes were seen in the cerebellum and carcaline regions. However, in the liver, only fatty degeneration of parenchymatous cells in general has been reported.

Changes in the liver due to acute and chronic exposure to methylmercury *in vivo* have been investigated.⁸⁾ These studies suggested that the hepatotoxicity of methylmercury is also important. However, in these experiments *in vivo*, it has not as yet been clarified whether the selective toxicity of mercury compounds is due to the particular distribution of the compounds in the body or to the specific vulnerability of tissues to the compounds.

Kasuya reported previously, that the lowest dose for total inhibition of MMC on cerebellum from newborn rats was about 1.5×10^{-5} M.⁹⁾ The lowest dose for total inhibition of MMC on the liver from newborn rats was about $2.0-4.0 \times 10^{-5}$ M (Tables 1 and 2). Although the dose on the liver was higher than that on the cerebellum, more detailed investigations are necessary to conclude that the differences in resistivity to organic mercurials of the liver and the nervous tissues are due not to the different distribution of the chemicals but to the different vulnerability of these organs.

Summary

At the high concentrations of MMC, after 3-4

days culture of newborn rat liver, degenerative changes of fibroblasts and a decrease in the formation of collagen fibers were observed. It is suggested that the minimal dose of MMC for injurious effect on the liver in culture is between concentrations at 0.5 and 0.7×10^{-5} M, and the lowest dose for total inhibition was about $2.0 \times 4.0 \times 10^{-5}$ M.

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