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DETECTION OF HEAVY METAL TOXICITY BY *TETRAHYMENA PYRIFORMIS* CULTURE METHOD

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A rapid, inexpensive and simple method using *Tetrahymena pyriformis* has been established for the biological study on heavy metal toxicity. The growth of this organism was highly sensitive to various kinds of heavy metals and was affected at the concentration of 10^{-6} M of the metals. The strength of the toxicity was clearly expressed by the protein concentration of the cultured *Tetrahymena pyriformis* in medium containing the varying concentration of the metal.

Cadmium and mercury inhibited most remarkably the growth of *Tetrahymena pyriformis* and the complete inhibition was observed at the concentration of 4×10^{-6} M, while selenium, manganese, zinc and copper showed less toxic effects, and magnesium and calcium had no notable effect on the organism.

Zinc administered simultaneously with cadmium reversed the inhibitory effect of cadmium. On the other hand, mercury and cadmium additively inhibited the growth of the organism. It should be noted that the newly established method detects not only toxicity of heavy metals, but also the mutual effect of two kinds of metal compounds on organisms.

Examinations of heavy metal toxicity by using higher animals such as rats or mice have advantages that the results obtained from these animals could be applied fairly directly to human being, and also that the metabolic control system might be found as a whole animal. These animal examinations, however, need much cost, time or labour. Moreover, they have been shown to be inadequate to the studies concerning the direct toxicity of heavy metals to a cell, because of the complexity and buffer-actions contained in animals.

In this respect, a tissue culture method has been recently developed for the detection of heavy metal toxicity.[1)]

In this paper, we intended to develop a sensitive biological method using *Tetrahymena pyriformis* for the study on heavy metal toxicity, because this unicellular animal is highly sensitive to heavy metals and has less buffer-actions.

On the other hand, reciprocal effects of heavy metals has become a main

problem in the field of the metal poisoning research. In this connection, we studied the mutual effect of two kinds of heavy metals on living systems by newly established method.

Materials and Methods

Culture of Tetrahymena pyriformis

Tetrahymena pyriformis syngen 1, mating type III given by the courtesy of Dr. Takashi Mita of National Cancer Center Research Institute was used in the experiment. The medium used contains following materials in g per litre, proteose-peptone (Difco), 2; yeast extract (Difco), 0.5; glucose, 0.8. To 1 litre medium 0.8 ml of 1N HCl is added to adjust the pH of the medium to 6.5. $1\text{-}3\times10^5$ cells of *Tetrahymena pyriformis* were grown at 34°C for 17-24 hours in 5 ml of medium including certain tested metals, which were shown in Table 1.

Table 1. Metal-compounds used in the experiments.

Cd:	$Cd(CH_3COO)_2\cdot 2H_2O$
Hg:	$HgCl_2$
Se:	SeO_2
Mn:	$Mn(CH_3COO)_2\cdot 4H_2O$
Zn:	$Zn(CH_3COO)_2\cdot 2H_2O$
Cu:	$CuSO_4\cdot 5H_2O$
Ca:	$CaCl_2\cdot 2H_2O$
Mg:	$MgCl_2\cdot 6H_2O$

Determination of Tetrahymena pyriformis protein

After incubation, the cells were separated by centrifugation at 150 g for 4 minutes. The cells were washed twice with 10 ml of 50 mM potassium phosphate buffer (pH 6.5) containing 1 mM magnesium chloride. The protein of the final precipitate was measured by the method of Lowry *et al.*[2]

Results

Experiment 1. Toxic effect of various metals on Tetrahymena pyriformis

Fig. 1 shows the inhibitory effects of cadmium, mercury, selenium, manganese, zinc and copper ions on the growth of *Tetrahymena pyriformis*. As can be seen in Fig. 1, cadmium and mercury inhibited most remarkably the growth of the organism and the complete inhibition was observed at the concentration of 4×10^{-6} M of both metals (mercury: 0.8 ppm; cadmium: 0.44 ppm). On the other hand, selenium completely inhibited the growth of the organism at the concentration of 10^{-4} M, and manganese, zinc and copper completely inhibited the growth of organism at the concentration of 10^{-3} M. Magnesium and calcium had less toxic effects as compared with the metals mentioned above.

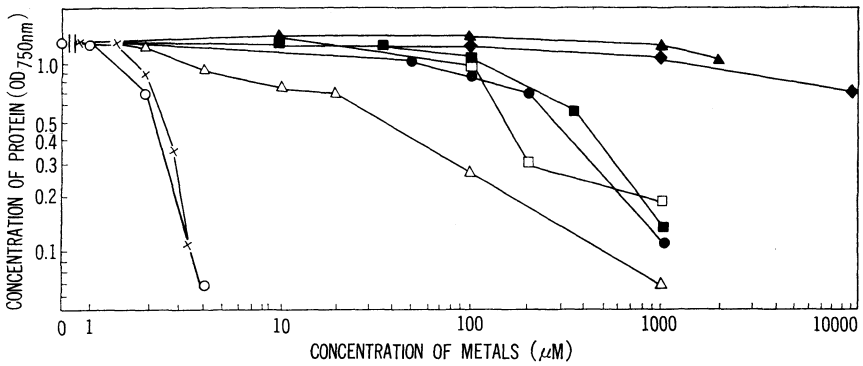

Fig. 1. Toxic effect of various metals on *Tetrahymena pyriformis*. Abscissa: concentration of metals administered. Ordinate: protein concentration of grown *Tetrahymena pyriformis*.
▲ magnesium; ◆ calcium; □ copper; ■ zinc;
● manganese; △ selenium; ○ mercury; × cadmium.

Experiment 2. Effect of zinc on cadmium toxicity to the organism

As can be seen in Fig. 2, administration of cadmium decreased the growth of the organism already at the concentration 3×10^{-6} M. On the other hand, zinc administered simultaneously at the concentration of 10^{-4} M reversed the inhibitory effect of cadmium and the concentration at which cadmium completely inhibited the growth of the organism shifted to 8×10^{-6} M.

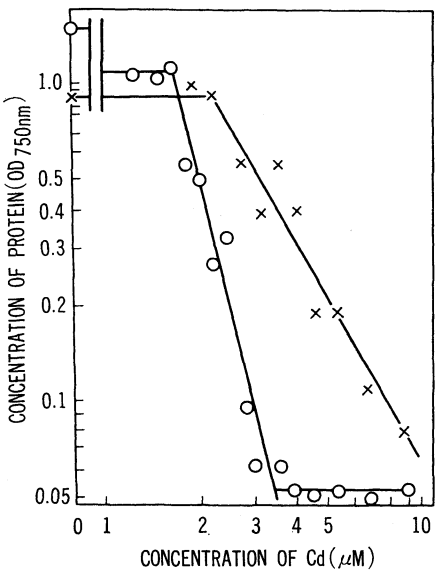

Fig. 2. Interaction of zinc and cadmium against growth of *Tetrahymena pyriformis*.
× cadmium + 10^{-4} M zinc;
○ cadmium only.

Experiment 3. Additive inhibitory effect of mercury on cadmium toxicity to the organism

As shown in Fig. 3, no notable change of cadmium toxicity was observed in presence of 10^{-7} M mercury as compared with that in absence of mercury. However, when 10^{-6} M mercury was administered simultaneously with cadmium, cadmium decreased the growth of the organism at the concentration of 2×10^{-6} M. And when 2×10^{-6} M of mercury was administered, 9×10^{-7} M of cadmium completely inhibited the growth of the organism.

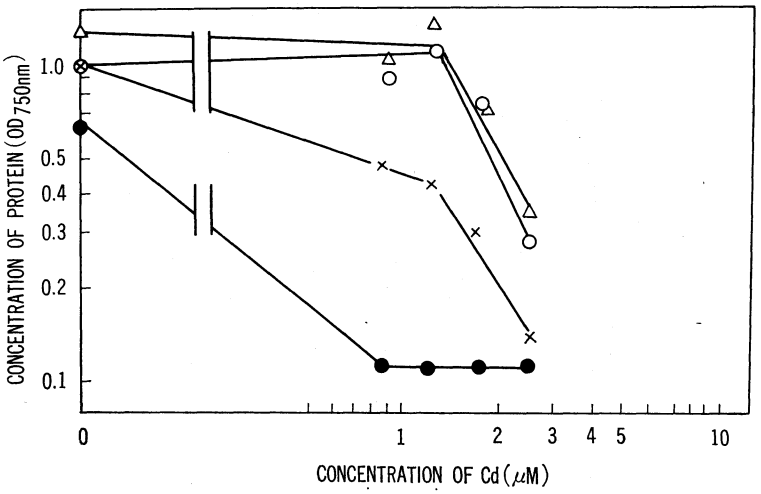

Fig. 3. Effect of mercury on cadmium toxicity to *Tetrahymena pyriformis*.
○ cadmium only; △ cadmium+10^{-7} M mercury;
× cadmium+10^{-6} M mercury;
● cadmium+2×10^{-6} M mercury.

Discussion

The present study showed that cadmium and mercury ions inhibited the growth of the organism at the concentration of 4×10^{-6} M. These results indicate that the growth of the organism is very sensitive to heavy metal ions. In this respect, Rutman *et al.*[3] observed the influence of metal ions on *de novo* synthesis of glucose, using cortex slices of rat kidney and they found that cadmium was inhibitory at 2×10^{-5} M. Ishizawa *et al.*[4] reported that the same concentration of cadmium is toxic on L-cells in Eagle's medium. Furthermore, Kajikawa *et al.*[1] described that an inhibitory effect of cadmium and mercury on the cultured cells was observed at the concentration of 0.1 ppm (cadmium: 9×10^{-7} M; mercury: 5×10^{-7} M). In comparison with these reported data, the method using *Tetrahymena pyriformis* was demonstrated to be more sensitive to heavy metals than the methods using rat kidney cortex or L-cells, though it has the same degree of sensitivity as

Kajikawa's cell culture method. Furthermore, the newly established method seems to afford us a rapid, inexpensive and simple procedure for study on heavy metal toxicity as compared with the cell culture method which is time-consuming and needs much training.

As shown in Fig. 2, zinc counteracts the toxic effect of cadmium administered simultaneously in the medium. Gunn *et al.*[5] reported that zinc, BAL or selenium reverses the lethality and testicular toxicity caused by cadmium in mice. Webb[6] also reported that zinc ion protects mice against the toxicity of cadmium ion. The result shown in Fig. 2 shows the same antagonistic effect of zinc against the toxicity of cadmium as these observed in the above reports. The evidence indicates that this organism is suitable for studying the effect of two kinds of metal compounds *in vivo*. Interaction of cadmium and mercury against growth of the organism is shown in Fig. 3. It is suggested that toxic effect of mercury and cadmium is additive for the organism. We are now under investigation to elucidate the mechanisms of the *in vivo* antagonism of cadmium and zinc and also of the additive toxicity of cadmium and mercury by using the method described in this paper.

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