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The Effect of Temperature on Copper Tolerance of *Paramecium*

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The biological impact of heavy metals is a more complex problem than measurement of total concentrations of metals, for tolerance may be a complex function of internal physiological states and environmental conditions. If the magnitudes of the interactions of the variables determining tolerance are not great, then one may be justified in concentrating study on a single variable. The toxicity of copper and zinc to a variety of ciliate protozoans has been previously studied by NYBERG (1974). Within Paramecium tetraurelia two classes of tolerance to copper were found; the resistant lines had a 24 hr TL_{50} of 30 µM and the sensitive lines had a TLm of 4.0 µM cupric ion at 27 C. The resistant phenotype was due to a single recessive gene (NYBERG 1975).

Temperature is a particularly important environmental variable for aquatic organisms in temperate climates. CAIRNS et al. (1975) have reviewed the influence of temperature on heavy metal toxicity and conclude that while there may be effects on some species in some circumstances, in general variations in temperature within normal survival ranges do not consistently modify copper and zinc toxicity. In particular REHWOLDT et al. (1972) found no effect of a 13 C temperature difference on copper toxicity of six species of fish. CAIRNS et al. (1976) do report, however, a significant effect of prior and concomitant exposure to copper on survival duration under acute thermal stress. Because temperature is an important environmental variable and because the response to temperature gives clues about the physiological mechanisms of toxicity we have investigated the change in the copper TL_{50} (24 hr) as a function of temperature. Increases in temperature dramatically decrease the tolerance of Paramecium tetraurelia to copper.

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MATERIALS AND METHODS

A copper sensitive line, stock 51, of Paramecium tetraurelia (SONNEBORN 1975) was used in all experiments. The cells to be tested were grown at 27 C in 0.25% Cerophyl previously inoculated with Klebsiella aerogenes. All cells used for metal tolerance tests on a given day were members of a clone and were rapidly growing in excess food at the time they were placed in the test solution. Individual cells were manipulated using a micropipet.

All copper tolerance tests were done in 0.10% Cerophyl to maintain consistency with earlier studies (NYBERG 1975). The test Cerophyl was inoculated with Klebsiella 24 hr prior to preparing the test solutions. Before adding the $CuSO_4$ solution, the pH of the Cerophyl was adjusted to between 5.8 and 6.0. The test solutions were made by adding an amount of 0.01 M $CuSO_4$ solution appropriate to produce the highest test concentration, 20 µM. Lower concentrations were prepared by serial diultion with 0.10% Cerophyl of the highest concentration in steps of 20.9%. Thus a series of concentrations equally spaced on the log scale were prepared, specifically: approximately 20, 16, 12.6, 10.0, 8.0, 6.3, 5.0, 4.0, 3.16, 2.5, and 2.0 µM cupric ion. These solutions were then dispensed into three depression spot plates which had incubated overnight at the test temperature. The slides, now containing the test solutions, were returned to the appropriate incubator. Two hours later cells were transferred to the test solutions. We always used a single cell per well and normally had six wells for each concentration at each temperature. The cells were incubated inside a moist chamber at the temperature desired. After 24 ± 1 hr each well was scored for the presence or absence of a viable cell. From the survival probabilities at each concentration the TL_{50} was estimated using probit analysis (FINNEY 1971).

RESULTS

The copper concentrations in micromoles per liter estimated to produce 50% mortality for each temperature on different repetitions are shown in Table 1. As in previous tests (NYBERG 1975) repetitions of the same stock on different days show a variance of approximately 0.01 when the log TL_{50} values are used, i.e. standard deviation of 0.10. The mean and standard deviation of the estimated TL_{50} is shown for each temperature in Table 1, and in addition the means and standard

deviations of the log TL_{50} values are shown. The log values are given because the variance among repetitions seems to be distributed normally on the log scale. As expected if this were true, the log of the arithmatic mean is greater than the mean of the TL_{50} estimates.

TABLE 1

Copper TL_{50} of Paramecium at Four Temperatures

	Temperature			
Date	12°C	20°C	27°C	34°C
2/16		18.0 µM		6.4 µM
2/25		12.6 µM	7.8 µM	4.9 µM
3/29	12.5 µM		5.5 µM	4.9 µM
5/19		6.7 µM	6.3 µM	4.8 µM
5/25		10.4 µM	9.0 µM	5.2 µM
6/9	11.9 µM		6.6 µM	4.0 µM
6/16	9.8 µM	7.0 µM		
6/21	12.5 µM	7.8 µM	6.3 µM	2.8 µM
Mean±S.D.	11.7±1.4	10.4±3.4	6.9±1.0	4.7±1.0
Mean±S.D. of log TL_{50}	1.07±0.05	0.99±0.15	0.83±0.07	0.66±0.10

Graphical representation of this data indicated a linear effect of temperature on copper tolerance, and we proceeded to calculate the correlation and regression of mean copper TL_{50} on temperature. The results of these calculations are shown in Table 2.

TABLE 2

Correlation and Regression of Copper TL_{50} on Temperature

	Correlation	Regression Equation
Copper TL_{50} in µM	- 0.98	$16.6 - 0.332T^*$
log TL_{50} in µM	- 0.98	$1.327 - 0.0189T^*$

*Temperature in °C

The correlations are very high, supporting the hypothesis of a linear relationship. We had hoped to discriminate between the procedures of expressing the TL_{50} in micromoles Cu^{++}/liter and log TL_{50} in micro-

moles, but both methods of expressing the copper tolerance gave equivalently high correlations. The results indicate that there is a factor of about 3.5 difference between the copper tolerance at 4 C and 35 C, the maximum temperature tolerated in this stock.

DISCUSSION

These experiments have shown that copper tolerance in Paramecium tetraurelia linearly decreases with increases in temperature. This contrasts with the lack of an effect of temperature on copper tolerance in fish reported by REHWOLDT et al. (1972). The increased susceptibility at higher temperatures is consisent with the results of MACEK et al. (1969) using a variety of pesticides and the results of MACLEOD and PESSAH (1973) on the effect of temperature on mercury tolerance. Generally, an increased susceptibility in short term tests at higher temperatures may be attributed to a greater entry of the toxic substance into the organism. Though there is no concrete evidence, the temperature effect supports the hypothesis that uptake of copper by Paramecium is an active process, feeding or active transport, as opposed to a process like diffusion.

Finally, these results indicate that copper toxicity to at least some organisms can be considerably modified by other environmental parameters. The same concentration of copper that produced no mortality in winter could be toxic in the summer. Water standards should take cognizance of these facts.

SUMMARY

The copper tolerance of Paramecium tetraurelia decreases with increased temperatures over the range of 12 C to 34 C. The relationship is linear and the correlation = - 0.98. The regression equation has an intercept of 16 µM Cu^{++} at 0 C, and tolerance is reduced by 0.33 µM for each degree increase in temperature.

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