# SELECTED PHENOLIC COMPOUNDS AND LECTINS ON THE GROWTH AND OXYGEN UPTAKE OF *PARAMECIUM MULTIMICRONUCLEATUM*

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## ABSTRACT

The growth response of *Paramecium multimicronucleatum* varied from stimulatory with dilute solutions of ferulic acid to inhibitory for tannic acid ($10^{-3}$).

Lectin (Concanavalin A) at high concentrations (250 and 500 mg/ml) curtailed population growth without causing the death of the paramecia, and at 50 mg/ml stimulated growth.

When used alone, tannic acid ($10^{-4}$ M) Glutathione ($10^{-6}$ M), and 2,4,dinitrophenol (10 to 50 mg/ml) all demonstrated elevated respiratory rates. The effect of test compounds on oxygen uptake of paramecia was generally greater at the beginning of log phase than at three hours of testing. Ferulic acid at no time caused the respiration rate to increase over that of the controls.

## INTRODUCTION

This study was divided into several projects to ascertain the role of selected compounds on the oxygen uptake and growth rate on a large ciliate, *Paramecium multimicronucleatum*. The compounds used were selected for a variety of reasons: (1) the probable contact between compounds and ciliates in their natural environment, (2) current availability of the purified substances, and (3) current research suggests these chemicals act on receptor sites on cellular membranes.

Phenolics have been implicated in playing a major role in plant environmental interactions by hindering the growth and distribution of competing species. The phenomenon of inhibition of one plant species upon another has been termed allelopathy (Muller, 1966). A variety of phenolic acids have been found in high concentration in plant tissue and root exudates.

Parks and Rice (1970) tested eight phenolic acids on blue-green algae *Anabaena* and *Lyngbya* (a nitrogent fixing algae) with inhibition of growth at $.66 \times 10^{-7}$ M concentrations of gallotannic acid.

Phenolic compounds were implicated in the altering of respiratory rates by Gauer and Beevers (1959) while studying substituted phenols on the oxygen uptake of carrot discs with the greatest increase in uptake (which was 218% of control) being 2,4-dinitrophenol. It was thought that all of these compounds were acting as DNP, by uncoupling oxidative phosphorylation.



Demos, Woolwine, and Wilson (1975) looked at the effects of ten phenolic compounds on hypocotyl growth and mitochondrial metabolism of the mung bean. Tannic acid was found to inhibit the respiratory rates of isolated mitochondria at 115 micromoles dilution to 38% of control.

Glass, 1973, indicated phenolic compounds in plants have an inhibiting effect on uptake of ions such as potassium and phosphate and suggested several mechanisms for phenolic inhibition.

(1) a denaturation of specific membrane carriers

(2) an uncoupling of mitochondrial electron transfer

(3) the utilization of ATP equivalence; and

(4) the alteration of membrane properties by the solution of the phenolic compound in the lipid component of the membrane

While literature of the effects of phenolics on plants abounds, little has been done to test the effects of phenolic compounds on protozoa. Shaw and Geppert (1937) were the first to report exposure to phenol of *Paramecium caudatum* resulted in swelling and precipitation of proteins by phenol.

In recent studies of phenolics on ciliates, Schultz and Dumont (1977), while studying phenol as a by-product in scrubwater in fossil fuel conversion systems used *Tetrahymena* as their test organism. They found concentrations of 10 mg/liter reduced respiration the least and at concentrations of 100 mg/liter all were killed after one hour of exposure.

## METHODS

Population studies by Schultz and Dumont (1977) exposed *Tetrahymena pyriformis* to various concentrations of phenols and found population densities were inversely related to the concentrations of phenolics used (5, 10, and 25 $\mu$g/1).

In our studies we chose the larger ciliate *Paramecium multimicronucleatum*. The test organism were grown to log phase and transferred to sterile hay infusion broth and replicate (3) units tested similar to our previous studies (Mulfinger, 1973; Dillon, et al., 1980). Growth studies were carried to the stationary plateau or until a gradual decline had been reached. This usually occurred between 120 and 150 hours following the inoculation of paramecia

into 150 ml flasks and placed into a 23° C growth chamber. The counting process began three hours after inoculation (average of 3 counts from 3 flasks for each concentration), and additional counts were made every 24 hours until stationary phase had been reached up to 168 hours. To measure oxygen utilization a YSI model 53 was equipped with two teflon membrane Clark electrodes (with recorder B and L VOMS) to record the slopes of oxygen uptake and results calculated to microliters ($0_2$/ml consumer per minute).

## RESULTS AND DISCUSSION

Concentrations of $10^{-3}$ M ferulic acid (Figure 1) were lethal to paramecia, lesser concentrations ($10^{-4}$ M and $10^{-6}$ M) were found to stimulate population growth. Ferulic acid has only one hydroxyl group (tannic acid has three) and has been implicated in cross-linking of proteins and could interact with membrane elements.

The concentrations of tannic acid of $10^{-2}$ M (Figure 2) caused inhibition of growth and selective lethality for some of the paramecia, along with a retardation of the logarithmic log stage. This sublethal dose of tannic acid appears to cause an added stress to the organisms.

Dilutions of $10^{-3}$ M glutathione were found to be lethal to paramecia within 3 hours of inoculation (Figure 3). A $10^{-3}$ M test expressed inhibition in numbers and an increase in oxygen utilization of over 300% of controls (Table 1).

Results using DNP 2,4-dinitrophenol (Figure 4) were predictable in that by increasing concentrations of DNP suppressed the growth of paramecia and labored movement was observed in 20 and 50 micrograms/ml of DNP cultures.

The lectin Concanavalin A (Con A) at concentrations of 50 micrograms/ml stimulated growth of paramecia (Figure 5) but solutions with 250 and 500 mg/ml severely reduced population growth. This would suggest that Con-A may be binding with specific carbohydrate residues on the plasma membrane of paramecia and low dosages may facilitate transport of metabolic precursers into the cells.

If competition for such binding sites using 50 mg/ml of Con-A (stimulatory alone) were tested with varying concentrations of tannic acid the lectin might alleviate the toxic effects of tannic acid. Figure 6 suggests the specific binding sites for Con-A and tannic acid may prevent tannic acid from crossbonding protein or absorbing the lipid component of the paramecia membrane.



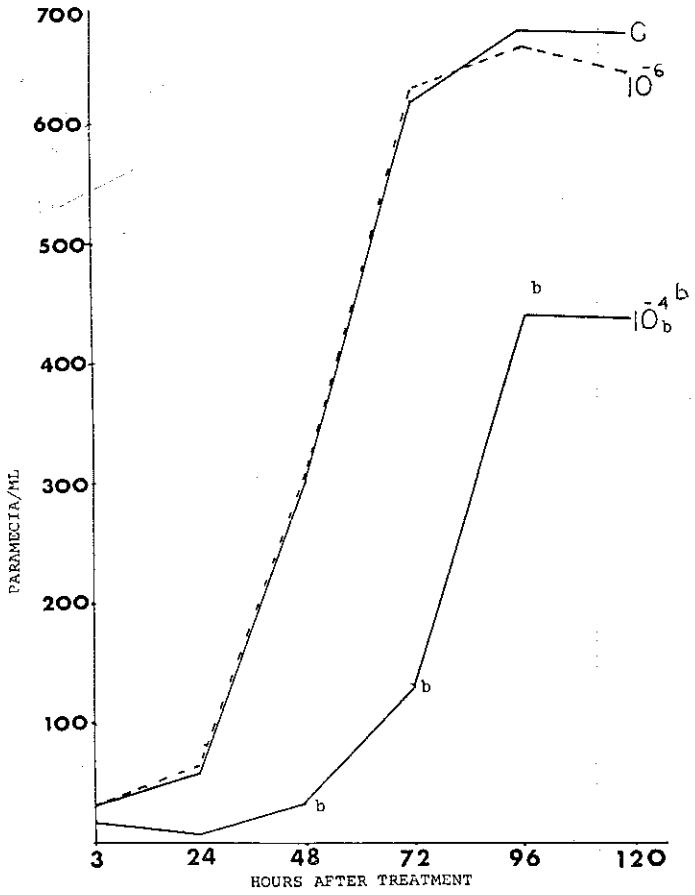


FIGURE 2. The effect of tannic acid upon growth of Paramecia.
b. Significant at the .05 % level

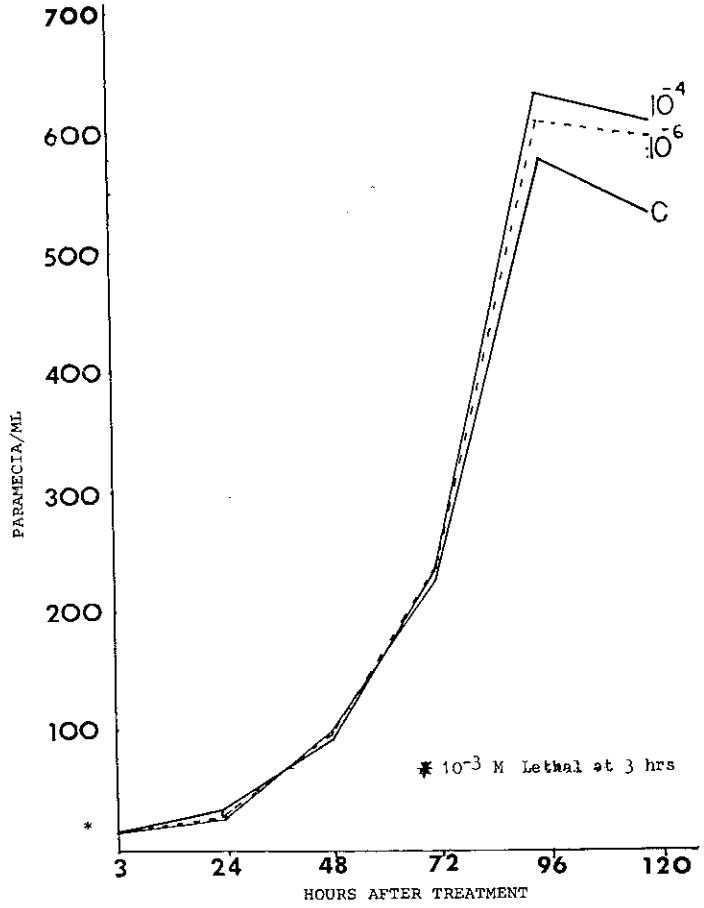


FIGURE 1. The effect of ferulic acid upon growth of paramecia.



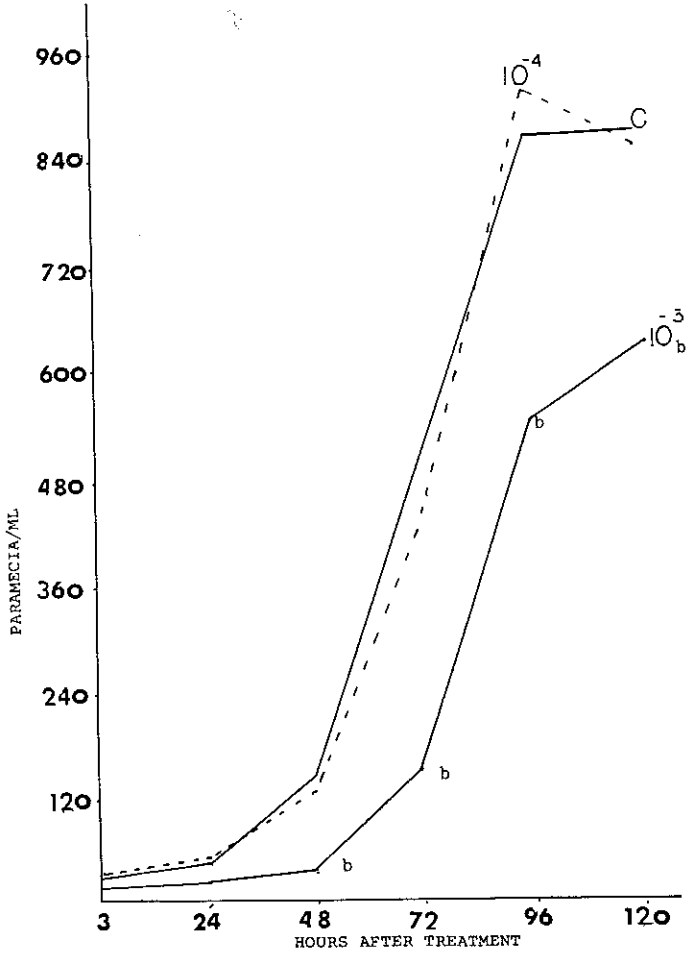


FIGURE 3. The effect of glutathione upon growth of paramecia.
b. Significant at the .05% level

TABLE 1

The Effects of Test Compounds Upon the Oxygen Consumption of *P. multimicronucleatum*[a]

| Test and Dilution | 3 hours | Log Phase | |
|---|---|---|---|
| Ferulic acid | | | |
| $10^{-4}$M | 99% | 82% | (72 hours) |
| $10^{-6}$M | 90% | 87% | |
| Tannic acid | | | |
| $10^{-6}$M | 93% | 56%[b] | |
| $10^{-4}$M | 130%[b] | 208% | (48 hours) |
| $10^{-6}$M | 101% | 102% | |
| Glutathione | | | |
| $10^{-3}$M | 147%[b] | 392%[b] | (48 hours) |
| $10^{-4}$M | 110% | 157%[b] | |
| $10^{-6}$M | 103% | 101% | |
| DNP | | | |
| 50 $\mu$/ml | 124% | 225%[b] | (72 hours) |
| 20 $\mu$/ml | 141% | 172%[b] | |
| 10 $\mu$/ml | 111% | 77.45[b] | |
| Tannic acid and Lectin | | | |
| $10^{-4}$M | 215%[b] | 51%[b] | (96 hours) |
| 50 $\mu$/ml | 38%[b] | 18%[b] | |
| $10^{-4}$M + | 132% | 21%[b] | |
| $10^{-6}$M + | 94% | 37%[b] | |
| Con A | no data | no data | |

[a]Results are expressed as percentage of control

[b]Significant at the .05 level



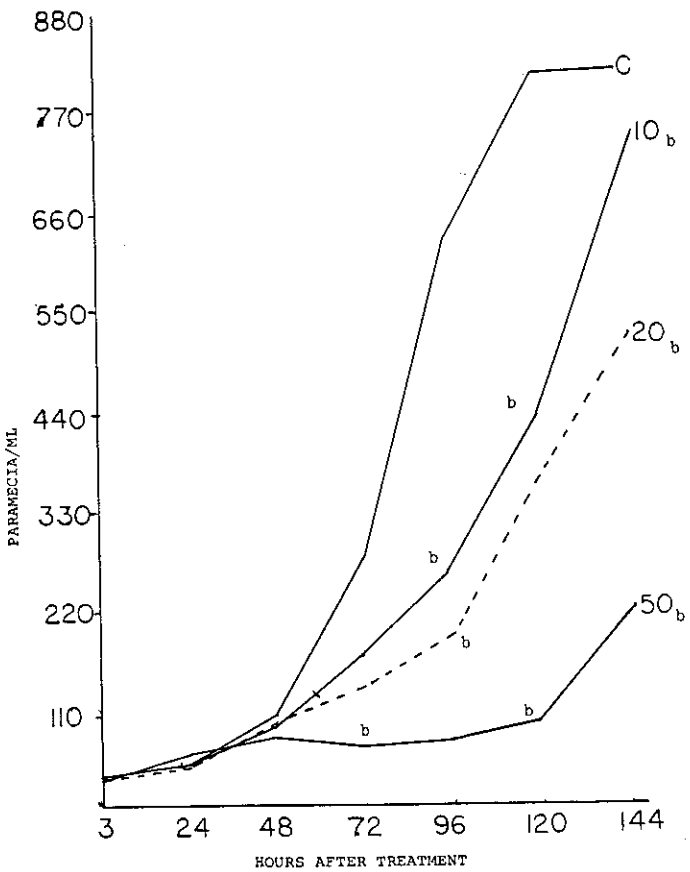


FIGURE 4. The effect of DNP upon growth of paramecia.
b. Significant at the .05% level

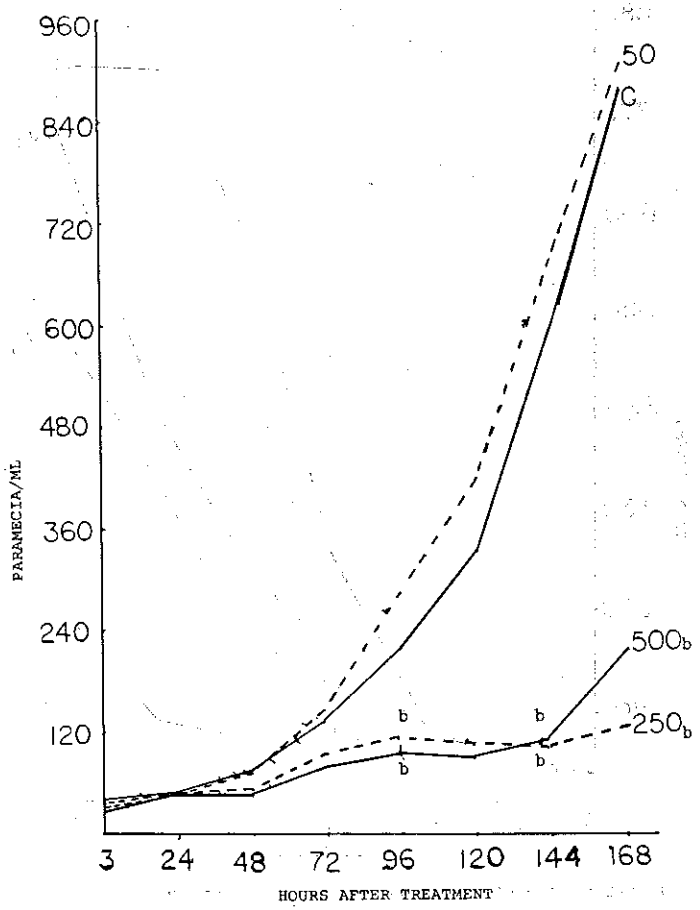


FIGURE 5. The effect of Con A upon the growth of paramecia.
b. Significant at the .05% level



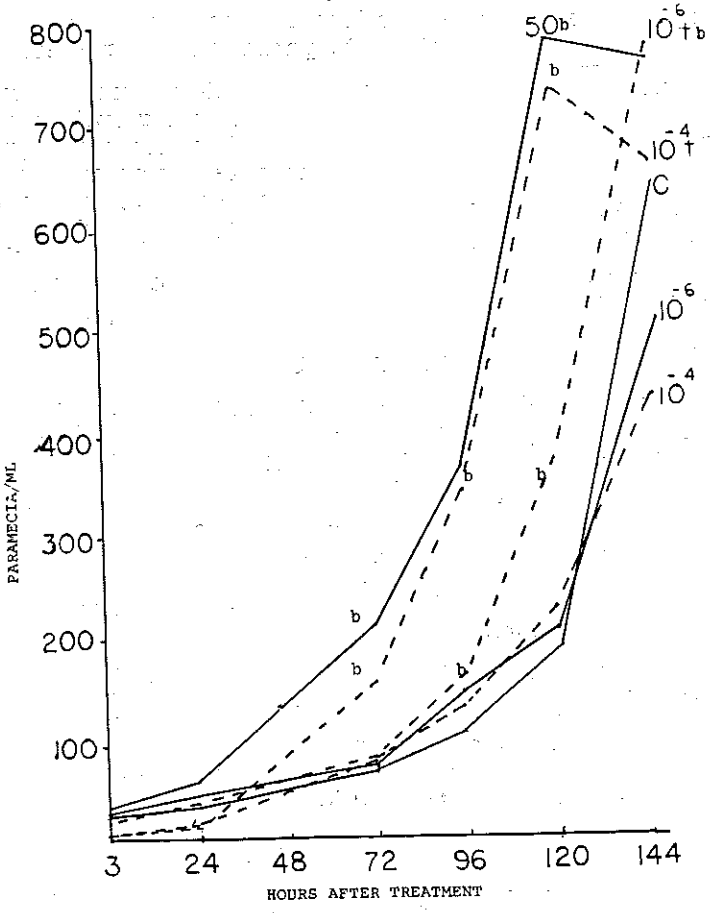


FIGURE 6. The effect of Con A and tannic acid upon growth of paramecia concurrently.
b. Significant at the .05% level

Table 1 summarizes the effects of test compounds on the oxygen consumption of *P. multimicronucleatum* at three hours and at log phase of growth. The variation of compounds and results suggest the probable relationships leading to increases in oxygen utilization is attributable to paramecia being stressed.

It would appear that organisms are being forced to reroute their energy resources from multiplication and growth processes to those involved in maintenance and repair of structural protein. If certain metabolic pathways are inactivated, more energy would be required to bypass that pathway as alternate routes would be less efficient. This would require more oxygen be made available to function as an oxidizing agent and suggest the difficulty in identifying a singular relationship to oxygen consumption by the compounds tested.

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