# THE EFFECTS OF TANNIC ACID AND CONCANAVALIN A ON GROWTH RATES, OXYGEN CONSUMPTION, AND ATP UTILIZATION OF *PARAMECIUM MULTIMICRONUCLEATUM*

Raymond D. Dillon and Brian D. Thrall
Department of Biology
University of South Dakota, Vermillion, S. D. 57069

## ABSTRACT

*Paramecium multimicronucleatum* cultures when treated with tannic acid or Concanavalin A responded in a dose-dependent growth pattern, higher concentrations inhibited growth and lower concentrations stimulated growth. Measured oxygen uptake and ATP levels were increased with increasing dosage. The rise in oxygen uptake and ATP levels were attributed to the paramecia attempting to reroute energy in order to survive.

The combined effects of 50 $\mu$g/ml Con-A and $10^{-4}$M tannic acid resulted in the lowest growth rates and the smallest increase in oxygen consumption and blistering of ciliate membranes.

Concentrations of 500 $\mu$g/ml Con-A were found to be inhibitory to culture growth at 24 hours.

## INTRODUCTION

In recent studies of phenolics on *Tetrahymena pyriformis*, Schultz and Dumont (1977) 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; but population densities were inversely related to the concentrations of phenolics used (5, 10, and 25 $\mu$g/1).

Protozoans, such as *Paramecium multimicronucleatum*, can serve as excellent organisms for studies in cellular biology because they are large single-celled organisms. Hence, they may provide valuable insight into the physiological mechanism of allelochemicals. This study focused on the effects of a phenolic (tannic acid) and a lectin (concanavalin A) on the respiration, ATP utilization, and growth rate of *Paramecium multimicronucleatum*. The work of Demos, et al. (1975) suggested phenolics reduced respiratory rates and released respiratory control through reduction of ADP/O and concluded the loss of respiratory control was not due to an uncoupling mechanism in mung bean hypocotyl studies.

Green and Corcoran (1975) studied the inhibitory effects of five tannins on gibberellin induced growth of rice seedlings that suggest an antagonistic action.

Lommen (1983) investigated the effects of tannic acid on *Paramecium multimicronucleatum* and found tannic acid to be inhibitory to growth at concentrations of $10^{-4}$M and respiration increasing to 130% of controls after only 3 hours of exposure, increasing consumption to 207% of controls after 48 hours of exposure.

When paramecia were exposed to $10^{-6}$M concentrations, there were no significant changes in oxygen consumption.

Balke (1985) reported certain flavonoid type compounds inhibit ATPase activity in oat roots. Since the mitochondria are the primary site and source of ATP and oxygen consumption any alteration of cellular or mitochondrial activity could affect ATP production.

Recent knowledge about the specific structure and activity of biological membranes is due to a large class of proteins called "lectins" (Sharon, 1977).

One such lectin, Concanavalin A, is produced by a jack bean (*Canavalia ensiformis*) and has been shown to precipitate polysaccharides at pH ranges of 6.5 - 8.0 (Agrawal, 1967).

Frisch, et al., (1976) showed "Con-A" inhibited conjugation of *Tetrahymena pyriformis* by binding to the conjugation area of the cellular membranes.

Lommen (1983) using Concanavalin A at dilutions of 250 and 500 $\mu$g/ml showed a marked inhibition to growth and at 50 $\mu$g/ml appeared to be stimulatory to growth.

## METHODS

Twelve, 250 ml flasks were prepared for testing tannic acid and Con-A on cultures of *P. multimicronucleatum* with 3 dilutions for each treatment and a control flask per lot similar to studies of Mulfinger (1973), Lommen (1983), and Dillon, et al., (1980).

Preliminary studies were conducted to confirm maximum concentrations that could be used without causing death of paramecia (i.e. 10 M tannic acid lethal within 3 hours in all test flasks). The measurements of ATP levels followed the luciferen-luciferase assay of Strehler and Trotter developed in 1952. This study included a standard curve so a coefficient of correlation could be calculated using std. ATP solutions from Calbiochem-Bering and Firefly luciferase using an Aminco Fluor-colorimeter with attachments for photometric recording.

Methods for extraction in this study were methods suggested by Tobin, et al. (1978), on a comparative review of various ATP extraction methods using 4 ml of 6% n-butanol per 1 ml of cultured protozoa sampled.



## RESULTS AND DISCUSSION

Figure 1 shows the effect of concanavalin A on the growth of *P. multimicronucleatum* with 50 $\mu$g/ml and 250 $\mu$g/ml being stimulatory on the growth of organisms and 500 $\mu$g/ml inhibitory.

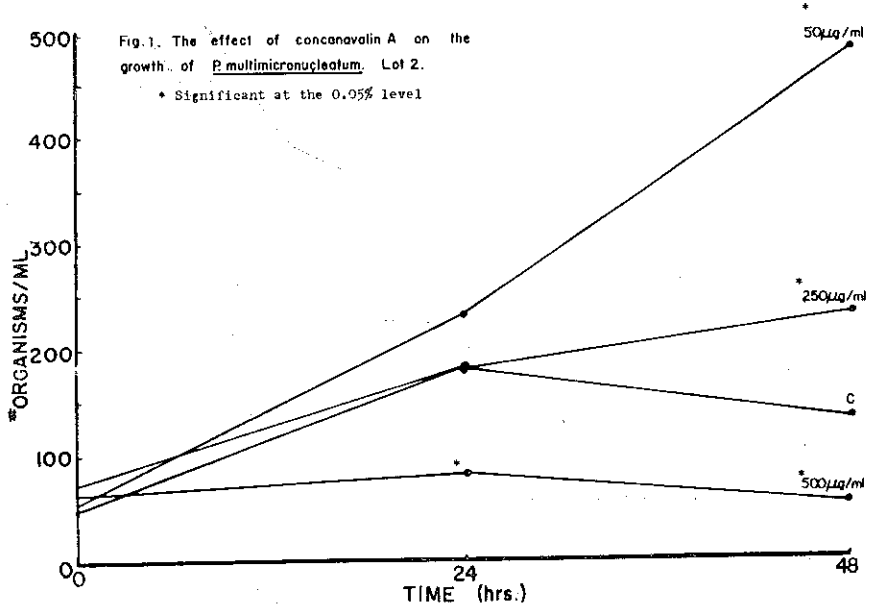


**Figure 1**

Figure 2 shows the combined effect of tannic acid and concanavalin-A concurrently in solutions with *P. multimicronucleatum* all were suppressing growth rates.

Table 1 expresses the effects of tannic acid and Con-A on oxygen consumption after 48 hours of exposure to these two chemicals as compared to the uptake by control organisms.

Figure 3 indicates log of relative light intensity plotted to ATP concentrations using the firefly luciferin-luciferase reaction.

Table 2 indicates the effect of tannic acid on the relative light intensity produced by the organisms and adjusted to light intensity per organism. Concentrations of $10^{-4}$ and $10^{-6}$ tannic acid solutions indicated by this method to stimulate ATP output per organism 3 to 4 fold.

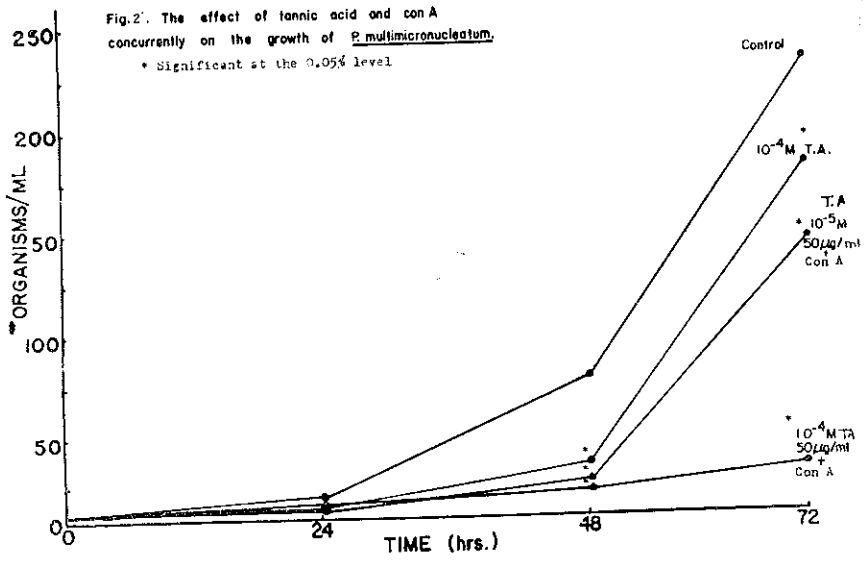


Figure 2

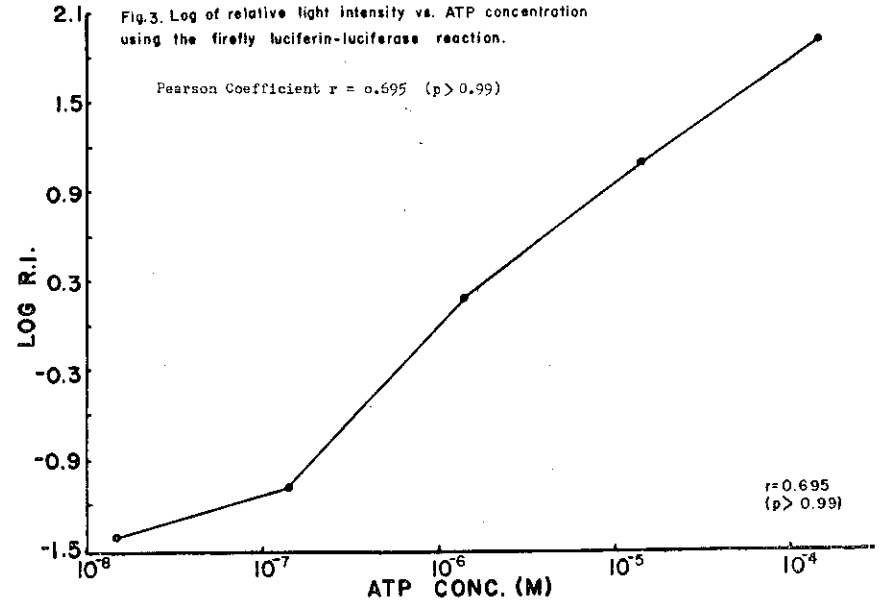


Figure 3



TABLE 1

The Effects of Tannic Acid and Concanavalin A on Oxygen Consumption of *P. multimicronucleatum* After 48 Hours of Exposure

| Treatment | Number of Organisms/ml | $\mu$l $O_2$/ml/org./hr | Percentage of Control |
|---|---|---|---|
| Control (none) | 78.7 ± 8.7 | 0.038 ± 0.005 | ..... |
| $10^{-4}$ M tannic acid | 35.3 ± 2.2 | 0.354 ± 0.022* | 831.6 |
| $10^{-5}$ M tannic acid | 85.3 ± 9.2 | 0.310 ± 0.051* | 715.8 |
| $10^{-4}$ M tannic acid and 50 $\mu$g/ml con A | 16.3 ± 2.6 | 0.229 ± 0.001* | 502.6 |
| $10^{-5}$ M tannic acid and 50 $\mu$g/ml con A | 25.3 ± 5.0 | 0.296 ± 0.057* | 678.9 |

*Significant at the 0.05% level (p>0.95)

TABLE II

The Effects of Tannic Acid on the Relative Light Intensity Produced by the Firefly Luciferin-Luciferase Reaction

| Treatment | Calculated Number of Organism/0.2 ml | Mean Relative Light Intensity | Adjusted Light Intensity Per Organism |
|---|---|---|---|
| Control | 54.4 | 0.037±0.003 | 0.0007 |
| $10^{-4}$ M tannic acid | 57.4 | 0.182±0.006 | 0.0032* |
| $10^{-5}$ M tannic acid | 65.6 | 0.142±0.006 | 0.0022* |
| $10^{-6}$ M tannic acid | 78.8 | 0.063±0.012 | 0.0008 |

*Significant at the 0.05% level ($p>0.95$)



## ATP STUDY

ATP levels of organisms grown in tannic acid were investigated using the firefly luciferin-luciferase method. Mean relative light intensity was measured, and this value was adjusted to represent relative light intensity produced per organism. Figure 3 demonstrates the light intensity is proportionally related to the ATP level of the organisms.

Organisms (Table II) treated with $10^{-4}$M tannic acid produced significantly higher relative light intensity values compared to controls. The $10^{-5}$M tannin treated paramecium also produced higher light intensities. Paramecium grown in $10^{-6}$M tannic acid did not differ significantly from the controls. The ATP levels per paramecium treated with mixtures of tannic acid and Con-A were not measurable with present instrumentation.

## LITERATURE CITED

Balke, Nelson E. 1985. Effects of allelochemicals on mineral uptake and associated physiological processes. In: Thompson, Alonzo (ed.). 1985. Chemistry of allelopathy. ACS Symposium 268. Am. Chem. Society.

Dillon, R. D., F. A. Einhellig and R. F. Mulfinger, 1980. Growth and respiratory rates of **Paramecium multimicronucleatum** cultured with p-coumaric and chlorogenic acids. Proc. S. Dak. Acad. Sci. 59:166-174.

Demos, E. K., M. Woolwine, R. H. Wilson, C. McMillan. 1975. The effects of ten phenolic compounds on hypocotyl growth and mitochondrial metabolism of mung bean. Amer. J. Bot. 62(1):97-102.

Frisch, A., W. Bessler, H. J. Lipps, D. Ammerman. 1976. Immobilization of ciliates by concanavalin A. J. Protozool. 23(3):427-430.

Lommen, Peter. 1983. The effects of selected phenolic compounds and lectin upon the growth and oxygen uptake of **Paramecium multimicronucleatum.** Unpublished M.A. Thesis, University of South Dakota.

Mulfinger, R. F. 1973. The effects of phenolic compounds upon the growth and respiration of **Paramecium multimicronucleatum.** Unpublished M.A. Thesis, University of South Dakota.

Sharon, N. 1977. Lectins. Sci. Am. 236:108-119.

Strehler, B. L. and R. Totter. 1952. Firefly luminescence in the study of energy transfer mechanisms. Firefly and other methods. Substrate and enzyme deterioration. Arch. Biochem. Biophysics 40:28-41.

Tobin, R. S., J. F. Ryan, B. K. Afghan. 1977. An improved method for the determination of adenosinetriphosphate in lake waters, activated sludges, and sediments. Environmental Analysis. pp. 29-45. Academic Press, New York, N. Y.

Webster, J. J., J. C. Change, J. L. Howard and F. R. Leach. 1979. Some characteristics of commercially available firefly luciferase preparations. J. Appl. Biochem. 1, 471-478.