Graph II: The rate of respiration of *Blepharisma intermedium* in cystine and mercaptoethanol supplemented media expressed in ul O$_2$/hour/5000 ciliates.
GRAPH II

[Graph showing bar chart with categories CONTROL, CYS, C₂H₆Os, CYS + C₂H₆Os.]
Chapter VII

Volume changes in Blepharisma intermedium
Volume changes in Belpharisma intermedium

INTRODUCTION:

Growth and division are two distinct phenomena of life. These two processes are affected by many factors. The rate of growth in protozoans alters with the changes in the culture medium. One of the parameters employed to measure growth rate is by determining the volume of the cell. In this investigation the cell volume of the ciliate Belpharisma intermedium grown at different temperatures, different buffers of varying molarity and pH has been studied. Since traces of many heavy metals are known to promote growth, a study of the effects of copper and zinc on volume changes has also been included.

MATERIAL AND METHODS:

Stock cultures of Belpharisma intermedium were grown in hay infusion fortified with horlicks as described earlier. These cultures were transferred to a thermostat in which incubations could be made simultaneously at two different temperatures, 32°C and 35°C. After incubation at these temperatures for seven days, the ciliates were fixed and stained and cell volume studies were made.
Blepharisma intermedium culture was synchronised by temperature treatment as described in Chapter I. Volume was measured on the ciliates exposed only to the cold temperature (12°C for 95 hours), cold and warm temperatures (12°C for 95 hours and 38°C for 24 hours).

From the stock culture, the ciliates were inoculated to fresh hay infusion, organic medium, 1% Cramer Myers medium (1) inorganic medium, and filtered stream water, natural medium. These three media were supplemented with zinc at a concentration of 1 mg/100 ml and with copper separately at a concentration of 1 mg/1000 ml in hay infusion, 1 mg/10,000 ml in Cramer Myers and stream water as described in Chapter VII. The ciliates grown in these supplemented media for seven days were isolated and fixed to study the volume changes.

To test the effect of pH on growth, Blepharisma intermedium was grown in hay infusion in three different buffers at different pH and molarity and fortified with horlicks. The buffer components were separately dissolved in this medium and mixed in requisite proportions to get the required pH and was diluted with the original medium when necessary, to the required molarity. The following buffer systems were used: Citrate phosphate buffer system at 1 mM molarity, the pH tried was 5 and 6. At 1 mM concentration in acetate buffer 5 and 5.6 pH were used. In tris maleate sodium hydroxide buffer, the pH and molarity used were 5.2 and 5 mM, 6 and 1 mM, 6 and 10 mM, 9 and 5 mM. After 48 hours, the ciliates were fixed and stained for volume studies. Ciliates grown in hay infusion with a pH of 6.8 were kept as controls for all the experiments in the present study.
Controls were maintained at room temperature, they fixed and stained to study volume changes.

The ciliates exposed to the different temperatures, metals and buffers with varying pH and molarity were fixed in Caroys fluid, stained in Schiffs reagent and counterstained in light green. The length and the width of the stained ciliates were measured on Camera Lucida drawings in millimeters, converted to microns using a stage micrometer (8 X eye piece and 10 X objective) and the cell volumes in \( u^3 \) were computed from the formula for a prolate spheroid

\[
V = \frac{4}{3} \pi \left( \frac{A}{2} \right) \left( \frac{B}{2} \right)^2
\]

in which \( A \) is the major axis and \( B \) is the minor axis. This formula has been employed by Scherbaum (15) and Thorar (18) for similar studies on *Tetrahymena pyriformis*.

**Observations:**

*Blepharisma intermedium* grown at higher temperature shows reduction in volume as shown in Fig.1, where the cell volume is plotted versus the incubation temperature, Fig.2 represents the volume of the ciliates at 12°C, 12°C and 38°C. Fig.3 shows the volume changes with copper and zinc in the three different media. Fig.4 indicates the volume at different buffers, pH and molarity.
DISCUSSION:

The findings of several workers studying the effect of temperature (5, 6, 13) on the size of various protozoa indicate that there is an increase in size at low temperatures. Nemeth (12) has found decrease in cell size in *Tetrahymena pyriformis* exposed to cold shock 25°C and heat shock - 39°C. However Thormars (18) observations are different. He has observed an increase in cell size in the ciliate *Tetrahymena pyriformis* at 34°C when compared to 10°C. In an earlier investigation (7), it was seen that the number of doublings at 32°C and 35°C were the same - three doublings per day whereas at room temperature, there was only one doubling per day. The present investigation shows that there is a decrease in cell size in *Blepharisma intermedium* incubated at 32°C and 35°C when compared to the control. This decrease in size is more marked at 35°C than at 32°C. Growth in *Blepharisma intermedium* is temperature sensitive and it decreases with increasing temperatures (7). This investigation also showed that cell size decreased in *Blepharisma intermedium* exposed to cold shock - 12°C. However after cold and heat shock - 12°C and 38°C, the cell size increased when compared to the control.

It has been found that copper and zinc in traces are very essential for the growth of most of the protozoans (4). Copper is necessary for the enzymes polyphenol oxidase, ascorbic acid oxidase and tyrosinase (14). Zinc is found widely in higher concentrations than copper in animal tissues and is an essential component of the enzymes carbonic anhydrase, alcohol dehydrogenase. Recently copper is known to play a
role in stimulating RNA and protein synthesis (19). However at the concentrations tried in this investigation, both these metals reduced the growth rate. There was a considerable decrease in the volume of the treated ciliates (Fig.3) at room temperature.

The effect of pH of the medium upon the growth of several protozoan species has been studied (3, 8, 9, 10, 20). It is established that there is relationship between the pH of the medium and growth (2). The results of this investigation confirms that pH and different buffer systems at varying molarities affect growth. There is considerable decrease in volume in all the three buffers when compared to the control - $3.4 \times 10^6$. However the decrease in cell size is less in tris maleate - sodium hydroxide buffer among the buffers tried. In the acetate and citrate buffers, the higher concentrations in the molarity proved lethal to the ciliates and hence cell size is determined at only 1 mM concentration.

Cytochemical studies on ciliates grown in hay infusion with buffers show that the lipid, carbohydrate and protein contents and enzymes - succinic dehydrogenase, alkaline and acid phosphatases of Blepharisma intermedium are dependent on the nature of the buffer (16). The fission rate is also dependent on the molarity, pH and nature of the buffers (17). Nemeth (11) has observed changes in size, fission rate as well as activity of some enzymes in Tetrahymena pyriformis exposed to cold and heat shocks.
The results of this investigation confirm that growth is sensitive to many factors in the medium. A slight change in any one of the factors like temperature, pH, buffer systems and increase in the concentration of trace metals bring about a considerable change in the growth rate which is reflected in the volume of the cell.
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Fig. 1: The volume change of *Blepharisma intermedium* exposed to higher temperatures.
VOLUME CHANGES IN BLEPHARISMA INTERMEDIUM AT DIFFERENT TEMPERATURES
Fig. 2: The volume change of *Blepharisma intermedium* acclimated to 12°C, 12°C and 38°C.
Fig. 3: The volume change of *Blepharisma intermedium* grown in three different media supplemented with copper and zinc.
VOLUME CHANGES IN BLEPHARISMA INTERMEDIUM GROWN IN DIFFERENT MEDIA WITH COPPER AND ZINC AT ROOM TEMPERATURE.
Fig. 4: The volume change of *B. epiderm*is*ma inter*med*ium grown in different buffers with varying pH and molarity.