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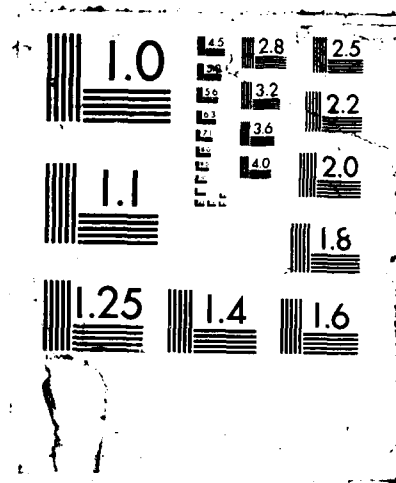
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CHEMICAL
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CONFIRMATION OF MULTIPLE
ORGANOFLUOROPHATE HYDROLYZING
ACTIVITIES IN THE PROTOZOAN
TETRAHYMENA THERMOPHILA

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October 1987

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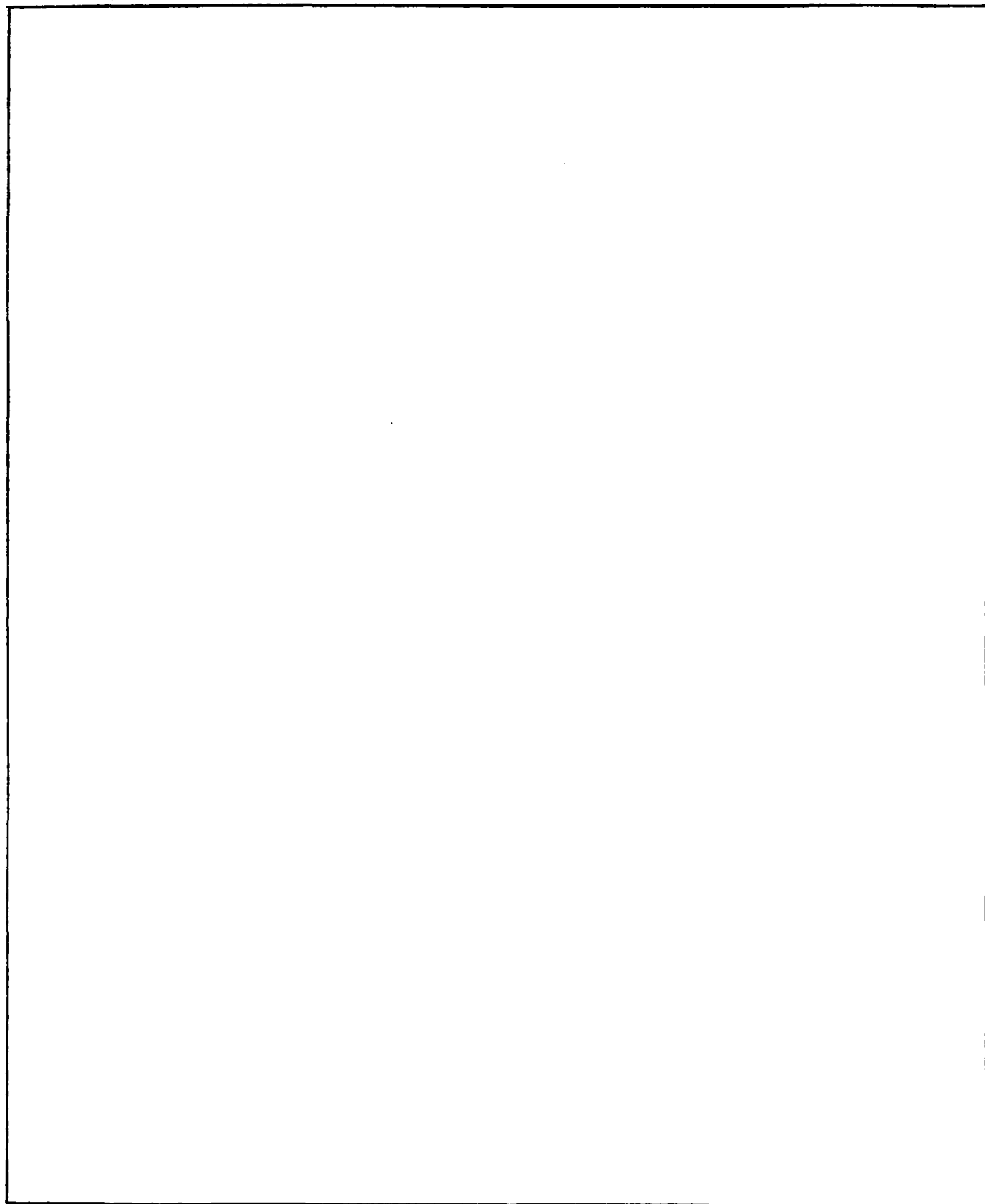
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PREFACE

The work described in this report was authorized under Project No. ETG2010, Aquatic Toxicology. This work was started in July 1986 and completed in April 1987.

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CONFIRMATION OF MULTIPLE ORGANOFLUOROPHATE HYDROLYZING ACTIVITIES IN THE PROTOZOAN TETRAHYMENA THERMOPHILA

1. INTRODUCTION

→ The term DFPase describes an enzyme capable of hydrolyzing an organofluoromonophosphate. Partially purified preparations of DFPase were subjected to flatbed thin-layer isoelectric focusing (IEF) in polyacrylamide gels with an ampholine carrier of pH 4-6.5. Until recently, the separation of DFPases was completed on molecular sizing columns (sephacryl S-300).¹ IEF provides information on the protein's isoelectric point. The isoelectric pH is the pH at which the net charge of the protein is zero.^{1,2} The use of IEF has confirmed the existence of multiple DFPases contained within the protozoan Tetrahymena thermophila. — — — — — →

2. METHODS AND MATERIALS

2.1 Sample Preparation.

Partially purified tetrahymena extract¹ was resuspended with distilled H₂O. The material was then centrifuged for 1 hr at a relative centrifugal force of 150,000 G at 4 °C. The supernatant was passed through a Pharmacia PD-10 column filled with sephadex G-25M to reduce the salt content. The supernatant was concentrated through evaporation by using an 8-cm long Spectrapor membrane tubing with a molecular weight cut off of 6,000-8,000D. The tubing was sealed at both ends and hung in a refrigerator at 4 °C for 24 hr.

2.2 Preparation of the Polyacrylamide Gel.

Using an ampholine carrier of pH 4-6.5, flatbed, thin-layer (0.5 mm) isoelectric focusing in a polyacrylamide gel was performed according to LKB Application Note 250⁴ and LKB Instruction Manual 2217.⁵

Two percent H₂PO₄ was used for the anode electrode solution and 2% ethylene diamine was used for the cathode electrode solution. Isoelectric focusing was run at 2,000 v 20 ma and 10 w using an LKB 2197 power supply. The concentrated tetrahymena samples were held in place on the gel with 5 x 10 mm filter pads. The maximum sample volume placed on the pads was 30 µl. Three markers were used to determine the pH gradient. The samples were run for 30 min. The pads were removed and the samples were run for an additional 4.5 hr.

After the run was completed, a sample path was removed from the gel and cut into 22 sections 0.5 cm wide. Proteins were eluted from the gel sections by placing them into 0.5 ml of Hoskin's buffer⁶ at 4 °C for 24 hr. The remaining portion of the gel was stained in a 0.5% solution of Coomassie blue R-250; the stained protein bands were analyzed on a laser densitometer.

2.3 DFP Assays.

The eluted proteins were assayed for activity according to methodology described earlier.⁶ Slight modifications were made by using 6 x -3M DFP solutions. The Orion 901 meter was calibrated to 1 and 50 µM of NaF.

3. RESULTS

Sixty-six protein bands were resolved by the laser densitometer. Four distinct areas of activity were isolated at slice numbers 8, 12, 15, and 18 (Figure 1). Slice 8 had a small amount of protein and could be an artifact. However, slices 12 and 15 had two bands of protein and slice 18 had three. (Figures 2 and 3). The approximate isoelectric points for slices 8, 12, 15, and 18 were 5.8, 5.2, 4.7, and 4.2 respectively (Figure 4). Slice 18 had the greatest amount of activity with slices 12 and 15 being approximately equal.

COMBINATION OF GELS 102186, 12986, AND 12987

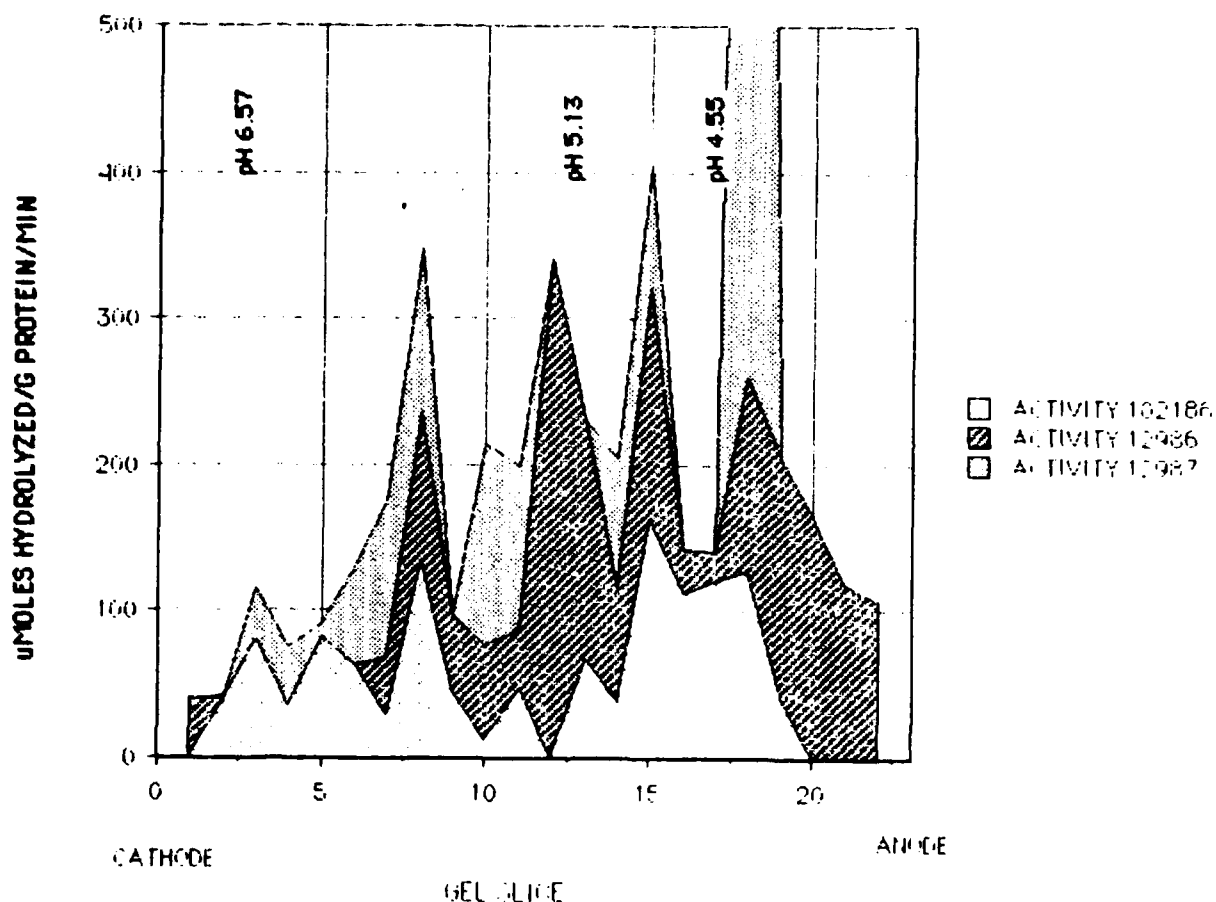


Figure 1. DFPase Activities of Partially Purified Preparations of *Tetrahymena thermophila*. The area graph combines the activities of the three runs to make the peaks of activity easier to see.

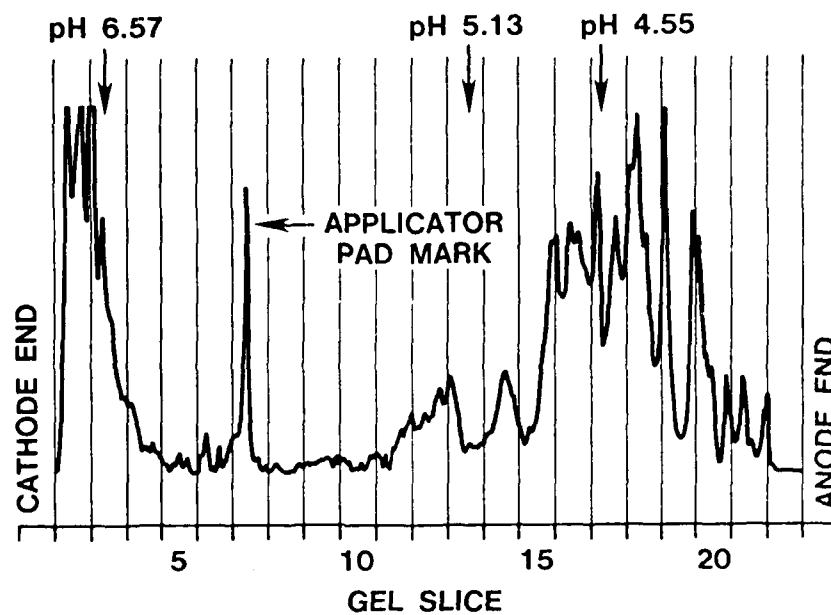


Figure 2. Laser Densitometer Scan of a Stained Gel After Tetrahymena Samples Were Run for 5 Hr

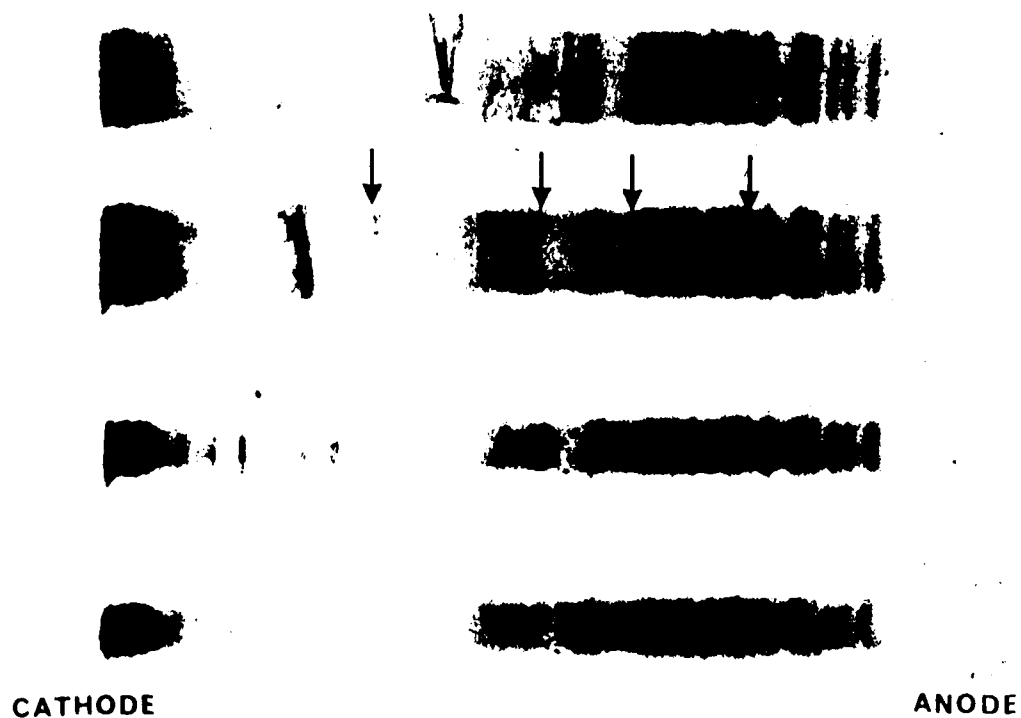


Figure 3. An Isoelectricfocusing Gel of Partially Purified Preparations of Tetrahymena Stained With a Solution of 0.5% Coomassie Blue R-250. The arrows indicate areas of high activity.

Gel Markers

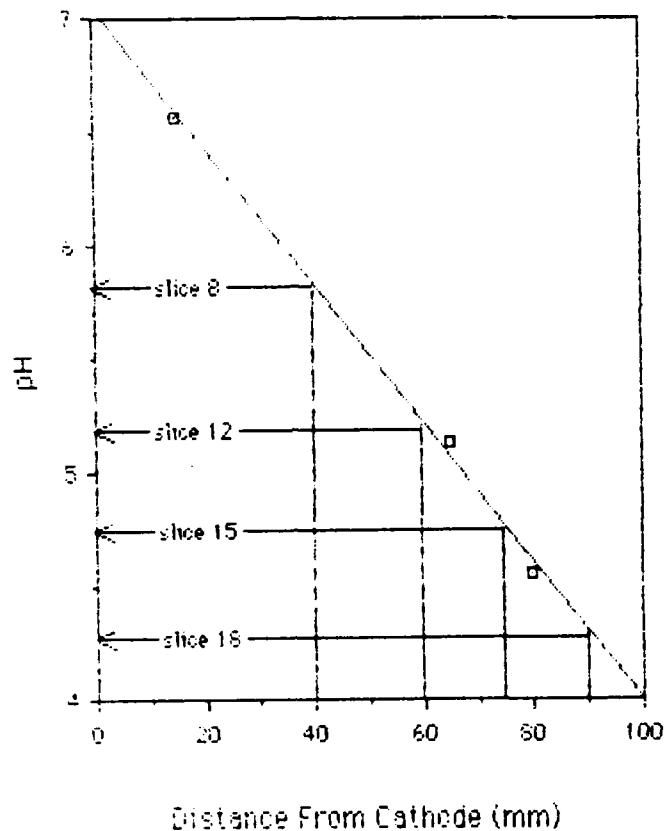


Figure 4. Beer-Lambert Plot of Isoelectric Markers and the Computation of Gel Slice Isoelectric Points

4. DISCUSSION

These experiments have confirmed the multiple DFPase entities as first discovered by sephacryl molecular sizing columns. Four areas of activity have been isolated (Figures 1 and 2). The activity in gel slice 8 may be an artifact. The amount of protein eluted from slice 8 was too small for reliable activity calculations. Slice 12, 15, and 18 were the major areas of activity.

The next stage of research is to run the partially purified preparations of DFPase on a preparative flatbed electrofocusing in a granulated gel. This method would allow applying a maximum sample size of 4.5 ml. These gels will separate such a large volume that the eluted protein bands would have a concentration large enough to be applied to molecular sizing columns.

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