LYSOSOMAL PHYSIOLOGY IN *TETRAHYMENA*

I. Effect of Glucose, Acetate, Pyruvate, and Carmine on Intracellular Content and Extracellular Release of Three Acid Hydrolases

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ABSTRACT

Log-phase *Tetrahymena* were washed and resuspended in a dilute salt solution supplemented with glucose, acetate, pyruvate, or carmine, as desired, and then incubated for 5 h. Intra- and extracellular activities of acid phosphatase, α-glucosidase, and ribonuclease were assayed. Extracellular activities were corrected for proteolytic degradation. The three nutritive substrates affected both the amount and pattern of extracellular enzyme release, but carmine had no effect. Intracellular activities declined early in the starvation period, but partially recovered with time, particularly α-glucosidase activity. Acetate reduced the decline in acid phosphatase activity; acetate and glucose enhanced the recovery of α-glucosidase activity; carmine had no effect on intracellular enzyme activities. Protein content changed little and was unaffected by the addition of substrates. Glycogen content increased during incubation; acetate and glucose enhanced the increase.

INTRODUCTION

Müller (1970, 1972) has reported that several acid hydrolases localized in the lysosomes of the ciliate *Tetrahymena pyriformis* are released from log-phase cells under normal culture conditions and when the cells are incubated in a dilute salt solution. The amount of enzyme activity released was substantial; after 5 h in a dilute salt solution 60–70% of the α-glucosidase, α-amylase, and β-*N*-acetylglucosaminidase, 30–40% of the deoxyribonuclease and acid phosphatase, about 15% of the ribonuclease, and a few percent of the proteinase had been released.

The mechanism of lysosomal enzyme release, and the factors controlling it, are obscure. The phenomenon, however, is not limited to *Tetrahymena*. Lysosomal enzymes are released from rabbit polymorphonuclear leucocytes treated with staphylococcal leucocidin (Woodin, 1962) and from human polymorphonuclear leucocytes during the uptake of indigestible particles (Weissmann et al. 1970), and lysosomal enzymes have been observed in perfusates of isolated bovine adrenal glands stimulated with carbachol (Schneider, 1968). Recently it has been proposed that inclusion-cell disease, a rare Hurler-like affliction, may result from a primary lysosomal defect involving exaggerated release of lysosomal enzymes (Wiesmann et al., 1971). Since *Tetrahymena* can form food vacuoles and autophagic vacuoles (Elliott and Clemmons, 1966) and since these ciliates are sensitive to a variety of drugs known to affect mammalian cells (Blum, 1967),

we felt that *Tetrahymena* might be a useful model for the study of lysosomal physiology. A comprehensive scheme for the intermediate metabolism of this protozoan has recently been constructed (Connett and Blum, 1972) which presents the possibility of relating lysosomal activities to metabolic events.

Three representative hydrolases were chosen for study: α-glucosidase, acid phosphatase, and ribonuclease. The time course of the change in intracellular content and extracellular release into a dilute salt solution was followed in control cells and in cells supplemented with acetate, pyruvate, or glucose. Cells were also supplemented with carmine particles, which simulate bacteria in size but can be considered as inert particles under the experimental conditions (Chapman-Andresen and Nilsson, 1968). The carmine particles stimulate *Tetrahymena* to form food vacuoles, a process which otherwise ceases when the protozoan is incubated in nonnutrient media (Chapman-Andresen and Nilsson, 1968). Since lysosomes are thought to be essential to the digestive functioning of food vacuoles, carmine might be expected to have a significant effect upon lysosomal distribution and thus upon the extracellular release of lysosomal enzymes.

Our investigations point to a physiological disjunction in the release of the three enzymes studied under various substrate conditions, and suggest the existence of multiple individually reactive pools of lysosomal enzymes. A preliminary report of these results has appeared (Rothstein and Blum, 1971).

MATERIALS AND METHODS

Growth and Harvesting of Cells, Experimental Procedure

Tetrahymena pyriformis, strain HSM, were grown at 25°C in a medium of 1% proteose peptone and 0.05% liver extract in 20 mM potassium phosphate adjusted to pH 6.5 with NaOH. Cells were grown with shaking to mid-log phase in 500-ml Erlenmeyer flasks containing 110–125 ml culture medium. The inoculum was always made from similarly grown log cultures.

Cells were collected at room temperature by centrifugation for 3 min at 200 *g*. The pellets were washed twice (200 *g* for 3 min; 200 *g* for 2 min) with a 1:100 dilution of the salt solution described by Wagner[1] (1956) and resuspended to a density of about 1.3×10^6 cells/ml. 12-ml portions were added to 1 liter Erlenmeyer flasks containing 4 ml dilute salt solution (control) or 4 ml of either 0.5 mg/ml carmine or 40 mM glucose, acetate, or pyruvate in the salt solution. Incubation time was marked from the addition of the cell suspension to the flasks. Aliquots for cell counts, glycogen, and protein determinations, and homogenization were taken at time zero from a control flask which was not incubated. Six flasks, three experimental and three control, were incubated in the dark without shaking at 25°C. One control and one experimental flask were collected after 1, 2.5, and 5 h of incubation.

At collection, aliquots for cell counts, glycogen and protein determinations, and homogenization were withdrawn from a given flask. The remainder of the cell suspension was centrifuged at 300 *g* for 3 min at 0°C, and the supernate was centrifuged again at 300 *g* for 5 min. The cell-free supernate was kept on ice until the enzyme assays were performed.

Samples for homogenization were placed in cellulose nitrate tubes on ice and treated with ultrasound twice for 30-s exposures separated by a 60 s lapse, using a Branson Model LS-75 ultrasonic generator at a setting of "7."

Assay Procedures

Cell counts were performed with a Coulter counter (Coulter Electronics, Inc., Fine Particle Group, Hialeah, Fla.), using a 100 μm aperture.

For protein assay about 9 ml of ice-cold 5% trichloroacetic acid were added to 1 ml of cell suspension of known cell count. The pellet obtained after brief centrifugation was assayed by the method of Lowry et al. (1951).

Pellets for glycogen assay by the phenol-sulfuric acid method of Dubois et al. (1956) were obtained from cells which had been suspended in ice-cold 95% ethanol for at least 1 h before centrifugation. Pellets from cells which had been incubated with glucose and their corresponding controls were washed once with 95% ethanol.

Enzyme assays were performed at 25.0 ± 0.1°C with substrates prepared in 0.1 M sodium acetate buffer, pH 5.0.

Reaction mixtures consisted of 3 ml of a diluted homogenate or supernatant sample, 3 ml of substrate, and either 0.1 ml of 2% Triton X-100 with

[1] The composition of Wagner's solution is, per liter: NaCl, 2.75 g; KCl, 149 mg, $MgSO_4 \cdot 7H_2O$, 246 mg; Na_2HPO_4, 1.37 g; KH_2PO_4, 320 mg. In several experiments there was no appreciable difference in enzyme release between cells suspended in this solution or in 10-fold or 100-fold dilutions.

homogenate samples, or 0.1 ml distilled water with supernate samples. It was established that there was no difference between assays performed on supernate samples using 0.1 ml H_2O or 0.1 ml Triton X-100.

The substrate solutions for the assay of acid phosphatase and α-glucosidase activities were 10 mM *p*-nitrophenyl phosphate and *p*-nitrophenyl-α-D-glucoside, respectively. At a minimum of four times during the assay, 1 ml samples of the reaction mixture were removed to an equal volume of ice-cold 0.5 M Tris base. These samples were kept in the cold and the absorbance at 410 nm was determined within 24 h. These assays are essentially the methods of Blum (1965) and Aronson and de Duve (1968).

The substrate solution for the assay of ribonuclease activity was 3 mg/ml yeast RNA purified according to the method of Kunitz (1940) as modified by Woodward (1944). 1 ml samples of the reaction mixture were removed to equal volumes of ice-cold 0.25% (wt/vol) uranyl acetate in 10% (wt/vol) perchloric acid. After a minimum of 1 h on ice the suspensions were centrifuged in the cold for 8–10 min at 900 *g*. The supernates were kept in the cold and the absorbance at 260 nm was determined within 24 h. This is essentially the method of de Duve et al. (1955).

The three enzyme assays were linear with respect to time and enzyme concentration. No significant changes in absorbance of samples to be read occurred with storage up to 48 h in the cold.

The slopes of the lines (time of assay incubation vs. absorbance) obtained for acid phosphatase and α-glucosidase activities were converted to units of micromoles *p*-nitrophenol released per hour, using a molar extinction coefficient of 1.62×10^4 (Blum, 1965), and normalized to 1×10^6 cells on the basis of the cell count recorded at the beginning of the experimental incubation period.

For ribonuclease the slopes were used directly to calculate units of activity, which represent a change at 260 nm of 1 absorbance unit per h, and normalized as above.

Intracellular activity was computed by subtraction of the value obtained for the supernate from that of the homogenate. No significant inhibition or augmentation of enzyme activity was found when supernate and homogenate samples were assayed together. Addition of acetate and pyruvate to supernate and homogenate samples from control cells did not change the activity of any of the three enzymes assayed. Glucose decreased the extracellular and intracellular α-glucosidase activities about 22% and 15%, respectively, but had no effect on ribonuclease or acid phosphatase.

Corrections Applied for Proteolytic Degradation

Previous studies have ignored the possibility that lysosomal proteases released into the medium might degrade the extracellular enzymes under study. Under the present growth and culture conditions, roughly 15% of the intracellular protease activity originally present was released into the dilute salt solution after 5 h incubation. To quantitate enzyme degradation occurring during the incubation period, cell-free supernate samples from each time point were divided at the time of collection. One portion was kept on ice, while the other was incubated at 25.0°C for 1–3 h, after which both portions were assayed. The rates of degradation for released enzyme activities were determined by comparing the activities found in the two assays.

Corrected rates of enzyme release were then computed for each interval. To do so, the average of the rates of enzyme degradation at the time points bracketing an interval was added to the rate of appearance of enzyme activity during that interval.

The corrected rates of enzyme release thus obtained were multiplied by the duration of the interval to yield the extracellular enzyme activity which would have been measured in the absence of proteolytic degradation.

The following set of data illustrates the process for acid phosphatase from control cells, where the corrections are small. Times are given in hours after the start of the incubation.

	Activity (U)
Flask 1, collected 1.0:	
supernate on ice	1.47
supernate incubated 1.29–4.19	1.36
Flask 2, collected 2.5:	
supernate on ice	2.77
supernate incubated 2.79–5.31	2.56
Flask 3, collected 5.0:	
supernate on ice	3.16
supernate incubated 5.29–6.38	2.99

Rate of appearance of enzyme activity (per hour)

$$0\text{–}1\text{ h}: \frac{(1.47 - 0)(1.0)}{(1.0 - 0)} = 1.47$$

$$1\text{–}2.5\text{ h}: \frac{(2.77 - 1.47)(1.0)}{(2.5 - 1.0)} = 0.86$$

$$2.5\text{–}5\text{ h}: \frac{(3.16 - 2.77)(1.0)}{(5.0 - 2.5)} = 0.16$$

Rate of enzyme degradation (per hour)

$$1\text{ h}: \frac{(1.47 - 1.36)(1.0)}{(4.19 - 1.29)} = 0.040$$

$$2.5\text{ h}: \frac{(2.77 - 2.56)(1.0)}{(5.31 - 2.79)} = 0.081$$

$$5\text{ h}: \frac{(3.16 - 2.99)(1.0)}{(6.38 - 5.29)} = 0.16$$

Corrected rate of enzyme release (per hour)

$$0\text{–}1.0\text{ h}: \quad 1.47 + \frac{(0.040 + 0)}{2} = 1.49$$

$$1.0\text{–}2.5\text{ h}: 0.864 + \frac{(0.081 + 0.040)}{2} = 0.93$$

$$2.5\text{–}5.0\text{ h}: 0.159 + \frac{(0.160 + 0.081)}{2} = 0.28$$

Enzyme activity in the absence of degradation

1 h: (1.49) (1) = 1.49

2.5 h: (0.93) (1.5) + (1.49) (1) = 2.88

5 h: (0.28) (2.5) + 0.93) (1.5) + (1.49) (1) = 3.58

Reagents

p-Nitrophenol phosphate, *p*-nitrophenol-α-D-glucoside, and torula yeast RNA, grade VI, were obtained from Sigma Chemical Co., St. Louis, Mo. Carmine and sodium pyruvate were obtained from K&K Laboratories, Inc., Plainview, N.Y. All other chemicals were reagent grade.

RESULTS

Preliminary experiments showed that there were no significant differences in the rates of release of the three enzymes studied for cell densities ranging from 0.3 to 1.2 × 10^6 cells/ml. There was no detectable cell lysis during the centrifugation to prepare the cell-free supernates, as indicated by resuspending and counting the pellets and by microscopic examination. None of the enzymes assayed were released in significant amounts during the preparation for incubation. Cell number increased from 5 to 25% during the 5 h incubation. This increase was characteristic of the cells used in a given experiment; there were no significant differences in percent increase between experimental and control groups. Cell division in the absence of nutrients has been reported before in *T. pyriformis* (Cameron and Terebey, 1967; Müller, 1972).

Intracellular and Extracellular Events in Control Cells

A typical time course of the variation of intracellular enzyme activity and of enzyme release during incubation of control cells in a dilute salt

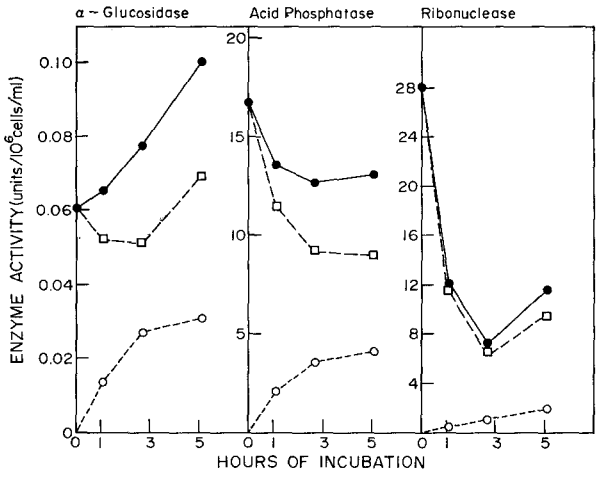

FIGURE 1 Variation of total, intracellular, and extracellular enzyme activities during incubation of *Tetrahymena* in a dilute salt solution. Logarithmically growing cells (~3 × 10^5 cells/ml) were harvested and resuspended at a density of about 10^6 cells/ml in a dilute salt solution. Activities were assayed at the times shown on the abscissa. Units of activity are micromoles per hour per 10^6 cells for α-glucosidase and acid phosphatase and Δabsorbance $_{260}$ per hour per 10^6 cells for ribonuclease. o---o, activity measured in cell-free supernates, i.e., extracellular activity. ●——●, total activity of whole homogenates, i.e., extracellular plus intracellular activities. □-----□, intracellular activity, obtained by subtracting extracellular activity from total activity.

solution is shown in Fig. 1. Intracellular acid phosphatase, ribonuclease, and α-glucosidase activities declined rapidly at first. Subsequently the α-glucosidase and ribonuclease activities rose, whereas acid phosphatase activity either rose slightly or continued to decline at a much slower rate. The increase in α-glucosidase activity during the latter half of the incubation carried it above its original intracellular level. After 5 h, typical intracellular values, as a percent of their initial values, were: acid phosphatase, 56%; ribonuclease, 36%; α-glucosidase, 112%.

Throughout the incubation each of these enzymes was released into the medium. The largest release occurred for α-glucosidase where an average of 52% of the initial activity was released. An average of 24% of the initial activity of acid phosphatase was released. Most of the α-glucosidase and acid phosphatase release occurred within the first 2.5 h. Ribonuclease activity appeared in the medium at a slow and steady rate, amounting to 9% of the initial intracellular content at the end of 5 h. These experiments were performed before it was realized that proteases might degrade the released hydrolases; since the values for extracellular release were not corrected for proteolytic degradation they are comparable to those reported by Müller (1970, 1972). At the end of 5 h the total activities (i.e., intracellular plus extracellular) averaged 165%, 80%, and 46% of the amounts initially present for α-glucosidase, acid phosphatase, and ribonuclease, respectively. Total protein content, however, was essentially constant throughout the incubation period, in agreement with the results of Müller (1970, 1972). The mean initial protein value for these logarithmically growing cells was 1.10 mg/10^6 cells, with a range from 0.93 to 1.28. After 1 h starvation the mean was higher by 6%, and after 5 h starvation, by 2%. This constancy of protein content was not altered by addition of any of the substrates described below.

Effects of Acetate, Pyruvate, and Glucose on Intracellular Enzymatic Activities

Fig. 2 shows the variation of intracellular enzyme activities during the incubation of cells in the dilute salt solution supplemented with acetate (A cells), pyruvate (P cells), or glucose (G cells), as compared to controls. The salient features of the displayed results were reproduced in duplicate experiments.

In cells supplemented with acetate or glucose, α-glucosidase activity decreased to the same level as that of control cells early in the incubation period, but then the activity of this enzyme increased more rapidly, so that at the end of the incubation it was 31% higher than the control for A cells and 53% higher for G cells. Acetate reduced the rate of decrease of intracellular acid phosphatase after the first hour, so that A cells had 41% more activity than control cells after 5 h.

There were no significant changes in ribonuclease in A cells nor in acid phosphatase or ribonuclease in G cells as compared to controls. P cells did not differ appreciably from controls in the pattern of variation of the three enzymes studied.

Effects of Acetate, Pyruvate, and Glucose on Release of Enzymes

Measurements of enzyme release were also made as part of the experiments shown in Fig. 2. These measurements, which were not corrected for proteolytic degradation, indicated that acetate and pyruvate significantly increased ribonuclease release as compared to that of control cells, while pyruvate also decreased the release of acid phosphatase and α-glucosidase. Glucose appeared to decrease the release of all three enzymes. These data served as a guide to subsequent experiments in which correction was made for proteolytic degradation.

Dickie and Liener (1962 *a*, *b*) have shown that *Tetrahymena* may release proteolytic enzymes under varying growth conditions, and we found that appreciable protease activity was released during incubation. Thus it seemed possible that these proteases could partially degrade the other enzymes being released. This is a factor which, to our knowledge, has not been considered in any previous studies on lysosomal enzyme release. We found that significant degradation of released enzymes occurred during incubation at room temperature, but not at 0°C. The amount of degradation varied for each of the three enzymes studied. A procedure to estimate and correct for this degradation was devised, as outlined in the section on Methods. This procedure was used in a set of experiments in which

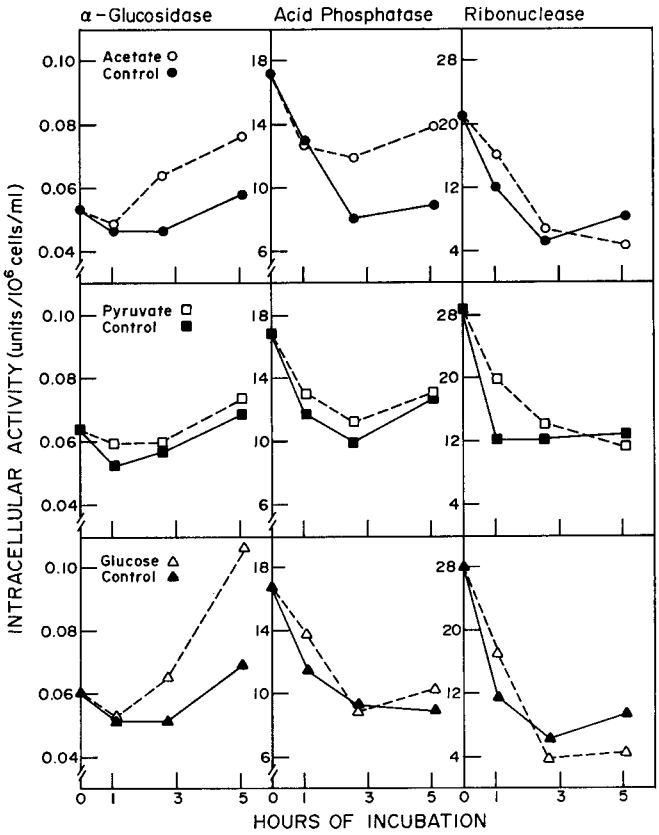

FIGURE 2 Effects of acetate, pyruvate, or glucose on intracellular lysosomal enzyme activities. Cells were harvested, resuspended in a dilute salt solution, and supplemented as indicated with 10 mM acetate (○-----○), pyruvate (□-----□), or glucose (△-----△). Separate rows show the results of three experiments. Solid lines, control cells; dotted lines, supplemented cells. For further details, see legend to Fig. 1. The variation of glycogen content during these experiments is shown in Fig. 5.

only extracellular activities were followed (Fig. 3). The corrections altered the values for activity present after 5 h incubation by 11–16% for acid phosphatase and 3–8% for α-glucosidase released from control cells, G cells, and cells which were ingesting carmine. For all other cases the correction at the end of 5 h was from 32 to 65% of the measured activity.

When A cells were compared with control cells, with proteolytic degradation corrected for, acid phosphatase and α-glucosidase release were similar during the first half of the incubation, after which release of these enzymes from A cells continued at a higher rate than for controls. Ribonuclease release, however, was much faster from the beginning of the incubation period onward in A cells.

Since in both P cells and G cells the rates of release of acid phosphatase and α-glucosidase appeared to be lower than in control cells (data not shown), P cells were compared with G cells directly to see whether there was a significant difference between the effects of pyruvate and glucose on extracellular release. α-Glucosidase release from G cells appeared slightly lower than that from P cells, and both rates were roughly constant during the incubation period. If account is taken of the inhibitory effect of glucose (see Methods) on α-glucosidase activity, then at 5 h the release from G cells and P cells is virtually the same. Acid phosphatase release from G cells paralleled that from P cells for the first half of the incubation period, after which release from G cells ceased while release from P cells continued unabated. Ribonuclease release from G cells began slowly and proceeded at a continually

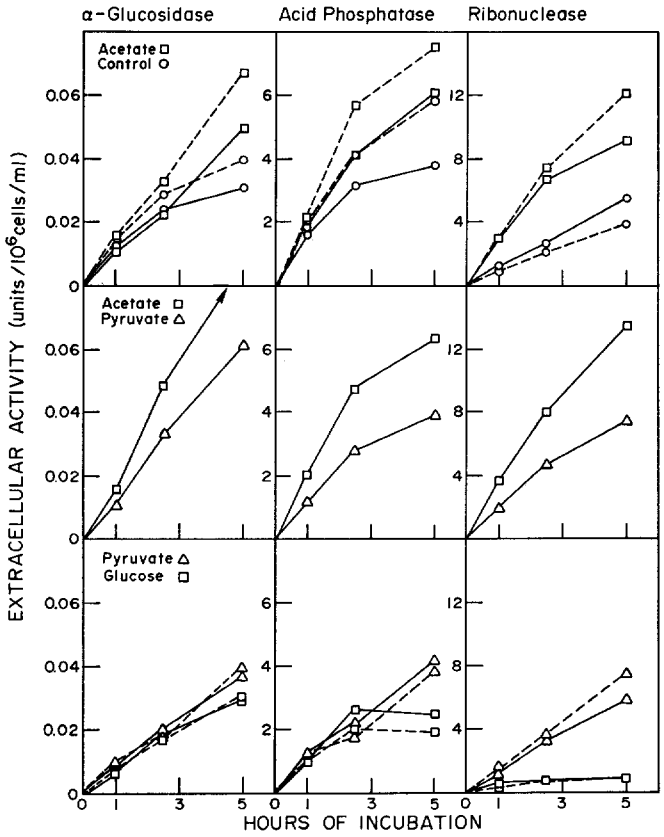

FIGURE 3 Effect of acetate, pyruvate, and glucose on time course of enzyme release from *Tetrahymena*. Cells were collected as described in the legend to Fig. 1. Substrates, when present, were 10 mM. The upper row shows two experiments (indicated by solid lines and by dashed lines) in which cells supplemented with acetate were compared to control cells. In a separate experiment, shown in the middle row, enzyme release from cells supplemented with acetate was compared to release from cells supplemented with pyruvate. The α-glucosidase activity reached 0.087 U/10^6 cells for A cells at 5 h, as indicated by the arrow. The lower row shows the results from two other experiments (again indicated by solid lines and by dashed lines) in which cells supplemented with pyruvate were compared to cells supplemented with glucose. In this figure, in contrast to Fig. 1, the extracellular activities shown were corrected for proteolytic degradation by the procedure described in Methods.

decreasing rate, in marked contrast to the more rapid and almost steady rate of release of this enzyme in P cells. Thus comparison of G cells with P cells revealed a different pattern of release for each of the three enzymes studied.

Earlier experiments (not shown) suggested that the patterns of enzyme release were similar for A cells and P cells although the rates of release were altered in comparison to control cells. When A cells were directly compared with P cells (Fig. 3) this was confirmed, i.e., there were no significant differences in the pattern of release, although more of each enzyme was released from A cells than from P cells.

Effect of Carmine Particles on Enzyme Release

The ingestion of carmine particles (confirmed by microscopic observation) had no effect on the time course of variation of intracellular enzyme content for any of the three enzymes studied (Fig. 4, upper row). There appeared to be little effect of carmine ingestion on extracellular release patterns (Fig. 4, middle row), but since small amounts of carmine remaining in the cell-free supernates could have interfered with the enzyme assays, the experiment was repeated with an additional procedure for the control

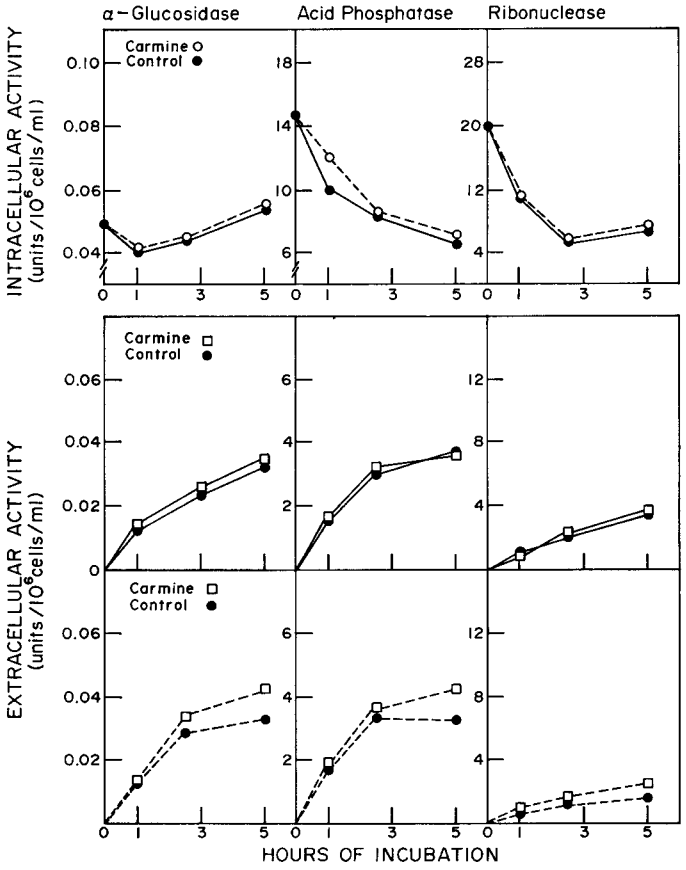

FIGURE 4 Effect of carmine on lysosomal enzyme activity in *Tetrahymena*. Cells were harvested and resuspended in a dilute salt solution as described in the legend to Fig. 1. Carmine, when present, was 0.13 mg/ml. Separate rows show different experiments. The upper row shows the variation of intracellular enzyme activity during incubation. The middle row shows enzyme released during incubation. The bottom row also shows enzyme release, but in this experiment carmine was added to the cell-free supernatant of control samples before they were assayed, as explained in the text. Extracellular enzyme activities were corrected for proteolytic degradation.

samples (Fig. 4, lower row). Just before enzyme assay, 0.13 mg/ml carmine was added to the cell-free supernates from the control cells. These supernates were then shaken and centrifuged at 300 *g* for 5 min at 0°C, thereby removing most of the carmine, after which they were assayed as usual. Again, there was no significant effect of carmine ingestion on enzyme release.

Effect of Acetate, Pyruvate, and Glucose on Glycogen Content

It has been shown above that the three substrates used affected the patterns of intracellular enzyme activities and extracellular release in different ways. It therefore seemed worthwhile to ascertain whether there were gross differences in metabolism between control cells and cells supplemented with acetate, pyruvate, or glucose. Glycogen content was chosen as an index of metabolic state. The glycogen content of control cells showed a steplike pattern of accumulation during incubation in the substrate-free salt solution (Fig. 5). Levy and Elliott (1968) had earlier shown that cell glycogen content increased during the first 8 h of starvation, and then declined. The increase in glycogen content occurs at the expense of intracellular lipids and amino acids (Wagner, 1956). Glycogen accumulation was markedly increased in G cells with loss of the steplike pattern. A similar but less pronounced

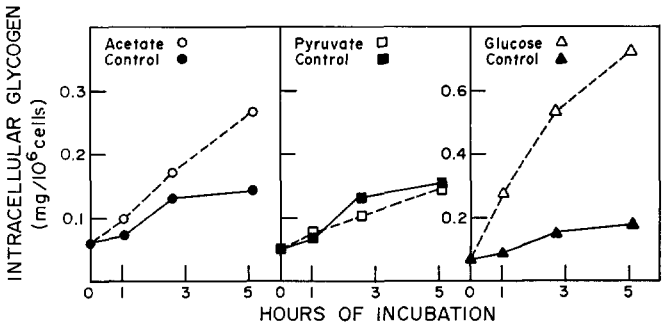

FIGURE 5 Variation of glycogen content of *Tetrahymena* during incubation in dilute salt solution. Glycogen content in milligrams per 10^6 cells is presented for each of the three experiments shown in Fig. 2.

effect was found in A cells, in agreement with the observations of Hogg and Wagner (1956) and of Levy and Elliott (1968). Incubation with pyruvate, however, had little effect on glycogen content at 5 h.

DISCUSSION

Tetrahymena starved for several hours show a number of striking cytoplasmic alterations. Food vacuole formation ceases immediately with the onset of starvation (Chapman-Andresen and Nilsson, 1968). Respiration drops to 30% of its value in logarithmically growing cells within the first hour of starvation, and thence to 20% in the next few hours (Hamburger and Zeuthen, 1971). Cytolysomes are observed within 1 h and their number increases with length of starvation (Nilsson, 1968). Within cytolysomes, cytoplasmic elements, including mitochondria, are degraded; mitochondrial degeneration has been shown to be unaffected when starved cells were supplemented with glucose or acetate (Levy and Elliott, 1968). The cellular content of Oil Red O-staining droplets, presumably triglycerides, reaches a peak after an hour of starvation and begins to decline after 4 h; in cells supplemented with acetate the peak is higher and occurs later (Levy and Elliott, 1968). Glycogen increases until about 8 h of starvation; this increase is higher in cells supplemented with glucose or acetate (Levy and Elliott, 1968). Cellular RNA is rapidly and significantly degraded, with losses amounting to about 30% in 3 h (Cline and Conner, 1966). These changes occur under the conditions in which lysosomal enzymes are released.

The imposition of step-down nutritional conditions on control cells results in marked changes in the intracellular content of lysosomal enzymes even as these enzymes are being released to the medium. Intracellular activity decreased for acid phosphatase and ribonuclease, but increased slightly for α-glucosidase. When the increase in α-glucosidase activity is considered in conjunction with its extracellular release, the total (intracellular plus extracellular) activity was 1.6 times its starting level after 5 h of incubation. Since protein content remained constant during the incubation, the increase in total α-glucosidase activity indicates either enzyme activation or a selective synthesis of this enzyme from preexisting pools of amino acids or from amino acids arising from protein turnover. Total activity of ribonuclease fell by more than half under our conditions. Müller (1972) found that ribonuclease activity increased about 135% and α-glucosidase activity decreased to about 75% of its initial value after a 5 h starvation period. The contrast between Müller's results and ours is presumably due to the differing growth conditions (Müller grew his cells in synthetic medium; we grew our cells in a proteose-peptone and liver extract broth), although it is also possible that the specific response to starvation differs in the two strains of *Tetrahymena* employed in these studies. The total activity of acid phosphatase changed little during the 5 h incubation, in agreement with Müller's findings (1972).

Levy and Elliott (1968) found that intracellular content of acid phosphatase decreased markedly after 24 h starvation while that of proteinase remained almost unchanged. Since there was a loss of about two-thirds of cell protein under these conditions, the specific activity of the proteinase more than doubled and that of acid phosphatase declined only slightly. Lloyd et al. (1971) found that the specific activity of acid phosphatase decreased about 60% while the specific activity of deoxyribonuclease remained the same after

16 h starvation. These studies, though not directly comparable with ours because of considerable loss of protein during the long period of starvation, indicate that changes in the proportions of intracellular lysosomal enzyme activities occur during starvation in *Tetrahymena*, a conclusion our data support. The present studies further show that supplementing the inorganic medium with glucose, acetate, or pyruvate can selectively influence intracellular lysosomal enzyme content; glucose and acetate increased the intracellular α-glucosidase content and acetate retarded the loss of intracellular acid phosphatase activity, although pyruvate scarcely affected intracellular enzyme activity changes.

The release of several lysosomal enzymes by *Tetrahymena* in different percentages of their intracellular content strongly suggests that release is occurring from a heterogeneous population of lysosomes. The existence of multiple pools of lysosomal enzymes in *Tetrahymena* is shown by the work of both Müller (1970, 1971, 1972), who found two peaks of lysosomal enzyme activity in isopycnic sedimentation studies, and Lloyd et al. (1971), who observed different density distributions for each of the three lysosomal enzymes they studied. The present study of the effect of metabolizable substrates on lysosomal enzyme release patterns provides evidence for the functional separation of release of the three enzymes studied. Large differences in the amount of ribonuclease released early in the incubation, when there was no difference in the release of the other two enzymes, were seen when A cells were compared with control cells and when P cells were compared with G cells (Fig. 3). These data allow the conclusion on purely physiological grounds that ribonuclease must be in a pool at least partially separate from the other two enzymes.

It is generally thought that in *Tetrahymena*, as in other cell types, lysosomes fuse with food vacuoles (Elliott and Clemmons, 1966) or, especially during starvation, with autophagic vacuoles to form cytolysomes (Levy and Elliott, 1968). Since carmine stimulates food vacuole formation and is itself indigestible, it would be expected to enhance the rate of extrusion of lysosomal enzymes were cytolysomes the intermediary for such release. In fact, carmine did not significantly alter lysosomal enzyme release. Müller (1972) attempted to influence release through induction of food vacuole formation and thereby of defecation, but was unable to alter the rate of release. He also found that suppression of contractile vacuole formation did not alter the rates of release, and suggested that the release of hydrolases by *Tetrahymena* should be regarded as a secretory process. Since release is accompanied by the reduction in amount of a subpopulation of lysosomes which equilibrates in the high density region of sucrose gradients (Müller, 1972), it is possible that this subpopulation does not ordinarily fuse with food vacuoles or, at least, with food vacuoles containing indigestible particles. Differences in lysosomal release from mouse macrophages during phagocytosis of indigestible as opposed to digestible particles have been observed (Weissmann et al., 1971). On the other hand, enzyme release may occur only from cytolysomes derived internally (i.e., autophagic vacuoles) rather than from food vacuoles.

Few studies of the metabolic control of lysosomal functions have been reported. Deter et al. (1967) showed that there was a marked increase in cytolysome formation in liver as a consequence of glucagon administration. The present study is the first, to our knowledge, to examine the effects of single metabolizable substrates on lysosomal enzyme release. The patterns of release from cells supplemented with acetate and pyruvate were similar, though more enzyme was released in the presence of acetate than pyruvate.

Tetrahymena oxidize glucose at a much lower rate than pyruvate or acetate (Connett et al., 1972). The difference in pattern of lysosomal enzyme release from G cells as compared to A cells or P cells may result in part from the lower rate of oxidation of glucose, but could also indicate that lysosomal release is controlled by two (or more) metabolic signals—one from acetyl CoA or metabolites derived from it, the other from glucose or derivatives thereof. Much more work will be necessary in order to comprehend the factors controlling lysosomal release. The present work demonstrates that this process can be altered by the nature of the substrate being utilized and thus invites further studies on the relations between metabolic state and lysosomal function.

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