LYSOSOMAL PHYSIOLOGY IN *TETRAHYMENA*

III. Pharmacological Studies on Acid Hydrolase

Release and the Ingestion and

Egestion of Dimethylbenzanthracene Particles

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ABSTRACT

The ingestion of ^{14}C-labeled 9,10-dimethyl-1,2-benzanthracene particles, the extracellular release of acid phosphatase, ribonuclease, and α-glucosidase, and the egestion of preingested dimethylbenzanthracene particles by *Tetrahymena* taken from logarithmically growing cultures and resuspended in a dilute salt solution were followed in the presence of several pharmacologic agents. Serotonin, caffeine, and, to a lesser extent, dibutyryl cyclic AMP increased the rate of particle ingestion, but did not alter the rate of release of the three acid hydrolases studied. Added catecholamines did not affect either particle ingestion or acid hydrolase release, but particle ingestion was inhibited by the catecholamine antagonists, dichloroisoproterenol, desmethylimipramine, reserpine, and phenoxybenzamine. These drugs also increased the release of acid phosphatase and ribonuclease in 5-h incubations. Desmethylimipramine acted within 1 h to increase acid hydrolase release, but the effect of dichloroisoproterenol developed more slowly and was secondary to a change in cellular content of the hydrolases. Desmethylimipramine increased the energy of activation for the release of acid phosphatase, while dichloroisoproterenol did not. Both of these drugs enhanced the egestion of preingested dimethylbenzanthracene particles, supporting the view that acid hydrolase release occurs through a cytoproct egestion mechanism. Particle ingestion was also inhibited by colchicine, vinblastine, and cytochalasin B, but these agents had no effect on acid hydrolase release, thus further differentiating the properties of the ingestion mechanism from those of the egestion mechanism. It appears that both microtubules and microfilaments play a role in the ingestion process and that this process may be controlled in part by a cyclic AMP-mediated serotoninergic and adrenergic system.

INTRODUCTION

Several acid hydrolases localized in the lysosomes of *Tetrahymena* are released to the extracellular medium during incubation of this ciliate in a dilute salt solution or in proteose-peptone medium (Mül- ler, 1970, 1972). The rate of release of these enzymes varies with the addition of glucose, acetate, or pyruvate (Rothstein and Blum, 1973). Studies on the distribution of lysosomal hydrolases

in sucrose density gradients of *Tetrahymena* homogenates showed that there were two populations of lysosomes and that only the population which sedimented to the higher density region of the gradient served as the source of secreted hydrolases (Müller, 1972). The existence of at least two populations of lysosomes was also deduced from the differential effects of glucose and pyruvate on the release of acid phosphatase and ribonuclease from starving *Tetrahymena* (Rothstein and Blum, 1973). Despite these reports, little is known about the mechanism of the release process or the role of lysosomes in either intracellular or extracellular digestion in this ciliate.

Tetrahymena contain catecholamines and serotonin (Janakidevi et al., 1966a, b) and their growth and glycogen content is affected by a number of drugs that interfere with catecholamines (Blum, 1967, 1969; Iwata et al., 1967). Since catecholamines have been shown to inhibit the release of acid hydrolases from liver lysosomes and polymorphonuclear leukocyte lysosomes in vitro (Ignarro et al., 1971; Ignarro and Colombo, 1973), it was of interest to examine the effects of these agents and of several catecholamine antagonists on the release of acid hydrolases from *Tetrahymena*. In this paper we report that several catecholamine antagonists enhanced the release of acid phosphatase and ribonuclease from *Tetrahymena*.

An intimate relationship exists between lysosomal function and intracellular digestion in *Tetrahymena* (Elliott and Clemmons, 1966; Blum and Rothstein, 1974). Food particles are ingested at the oral apparatus and, after investment with a membrane, enter the cytoplasm as food vacuoles. Digestion is thought to begin after fusion of the food vacuole with a lysosome. Undigested material is egested via the cytoproct and, presumably, acid hydrolases are also released via this route. If the catecholamine antagonists were acting to alter the rate of formation of food vacuoles, one might then observe a change in the rate of release of acid hydrolases in the absence of any effect of these drugs on the release mechanism. Thus, to interpret the effects of catecholamine antagonists on hydrolase release, it was essential to monitor the effects of these drugs on the ingestion process.

Although some attention has been paid to ingestion by *Tetrahymena* (Elliott and Clemmons, 1966; Chapman-Andresen and Nilsson, 1968; Nilsson, 1972; Ricketts, 1971 a, b, 1972; Rasmussen and Modeweg-Hansen, 1973), little is known about the physiology or pharmacology of food vacuole formation. Since it was known that *Tetra-*

hymena will ingest inert particles, it was convenient to use a radioactively labeled polybenzenoid hydrocarbon particle to assay the effect of drugs on the rate of ingestion. In this paper we report that the rate of ingestion of inert hydrocarbon particles by *Tetrahymena* was enhanced by serotonin, caffeine, and, to a lesser extent, dibutyryl cyclic AMP, and inhibited by a number of catecholamine antagonists. In addition, ingestion was inhibited by colchicine, vinblastine, and cytochalasin B, whereas neither the secretion of acid hydrolases nor the egestion of preingested particles was affected by these agents. Since the ingestion and egestion processes differ markedly in their pharmacologic sensitivities to serotonin, catecholamine antagonists, and agents that interfere with microtubule and microfilament function, it is possible to establish the effects of these agents on acid hydrolase release independently of their effects on food vacuole formation.

A preliminary report of this work has appeared (Rothstein, 1973).

MATERIALS AND METHODS

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 ($3-4 \times 10^5$ cells/ml) in 500-ml Erlenmeyer flasks containing 105–115 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 the salt solution described by Wagner (1956, for composition see Rothstein and Blum, 1973) supplemented with 1 mg/ml bovine serum albumin and resuspended to a density of about 1.3×10^6 cells/ml. Bovine serum albumin was used to retard proteolytic degradation of released acid hydrolases (Rothstein and Blum, 1973); it also served to facilitate the ingestion of dimethylbenzanthracene particles (Ricketts, 1971 a, 1972; Rasmussen, 1973). Cell counts were performed with a Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.) using a 100-μm aperture.

Procedure for Pharmacologic Studies of Acid Hydrolase Release

6-ml portions of washed and resuspended cells were added to 500-ml Erlenmeyer flasks containing 2 ml dilute salt solution with 1 mg/ml bovine serum albumin (control) or 2 ml of such a solution containing a given pharmacologic agent. Incubation time was marked from the addition of the resuspended cells to the flasks. Samples for cell counts were withdrawn and the flasks were incubated without shaking at 25°C in the dark since

in preliminary experiments shaking was found to disrupt cells in the presence of some of the drugs used in this study. Aeration without shaking was adequate, however, because of the shallow depth of the culture. After 5-h incubation, samples for cell counts were withdrawn and the flasks collected by centrifuging the cell suspensions at 300 g for 3 min at 0°C. The supernates were quickly decanted and centrifuged again at 300 g for 5 min. The cell-free supernates were then kept on ice until enzyme assays were performed.

Before collection, samples of the cell suspensions from each of the flasks were examined microscopically. None of the drugs in the concentrations reported herein had an adverse effect on cell viability as judged by the presence of cell motility and the absence of fine granulation of the cytoplasm, characteristic of nonviable *Tetrahymena*. None of the drugs produced gross morphologic alterations. Cell number in control flasks increased an average of 3.5% during the 5-h incubation. There were no significant differences in this increase between experimental and control flasks.

In experiments in which the intracellular content of acid hydrolases was measured, 12-ml portions of resuspended cells were added to 1-liter Erlenmeyer flasks containing 4 ml dilute salt solution with albumin (control) or 4 ml of such a solution containing a given pharmacologic agent. Samples for cell counts and homogenization were taken at time zero (from a control flask not incubated) and after 5-h incubation without shaking at 25°C. Homogenization was carried out as previously described (Rothstein and Blum, 1973).

Assay of Acid Hydrolases

Assays for acid phosphatase, α-glucosidase, and ribonuclease were performed as previously described (Rothstein and Blum, 1973). In experiments in which intracellular and extracellular acid hydrolase activities were determined, 0.1% Triton X-100 was present in both homogenate and supernate reaction mixtures. In experiments in which only extracellular enzyme activities were determined, Triton X-100 was omitted since it does not affect extracellular activity (Rothstein and Blum, 1973). Units of activity are micromoles of p-nitrophenol released per hour per 10^6 cells for acid phosphatase and α-glucosidase and Δ-A_{260}/h/10^6 cells for ribonuclease. Intracellular activities were computed by subtraction of the value obtained for the supernate from that of the corresponding homogenate as discussed elsewhere (Rothstein and Blum, 1973).

Preparation of Particles

9,10-dimethyl[9-^{14}C, 1,2]benzanthracene (50 μCi in 0.7 ml) and unlabeled 9,10-dimethyl-1,2-benzanthracene (about 12.8 mg/ml) were dissolved in dimethylsulfoxide and frozen. On the day of an experiment, dimethylbenzanthracene (DMBA) in dimethylsulfoxide was thawed and added slowly to about 11 ml dilute salt solution with albumin, yielding a fine dispersion of particles. The suspension (on ice) was treated with ultrasound twice for 30 s with a 60-s lapse, using a Bronson Model LS-75 ultrasonic generator at a setting of "5". The sonicated suspension was centrifuged at 300 g for 3 min at 0°C and then at 1000 g for 1.5 min, producing a small precipitate containing about 1.4% of the original amount of DMBA which was discarded. The supernate, free of heavier particles which would cosediment with cells, was used for the ingestion experiments. The supernate contained 316 μg/ml 9,10-dimethyl-1,2-benzanthracene and 0.158 μCi/ml in 2.6% (vol/vol) dimethylsulfoxide. It was diluted with an equal volume of a pharmacologic agent plus 2 vol of cell suspension so that cells were exposed to about 79 μg/ml DMBA in 0.66% dimethylsulfoxide. Microscopic examination indicated that the bulk of the particles were about 0.1 μm in diameter.

Procedure for Study of Pharmacologic Effects on Ingestion

2-ml portions of washed cells resuspended to a density of about 2×10^6 cells/ml were added to 125-ml Erlenmeyer flasks containing 1 ml of particles and 1 ml of a pharmacologic agent prepared in the dilute salt solution with albumin. Control flasks contained 1 ml of dilute salt solution with albumin instead of the pharmacologic agent. Incubation time was marked from the addition of the cell suspension to the flasks. The flasks were incubated at 25°C without shaking.

At 15 min and again at 45 min, aliquots of 1 ml were transferred from the flasks to 9-ml ice-cold dilute salt solution and immediately centrifuged at 300 g for 3 min at 0°C. Ice-cold temperatures were found to markedly reduce the release of acid hydrolases (Rothstein and Blum, 1974 a) and were presumed to be capable of similarly reducing the egestion of ingested particles as well as reducing the rate of further ingestion. The cell pellets contained ingested DMBA particles and the supernates contained uningested DMBA. At the end of each experiment, 0.3 ml samples of the cell suspensions were taken directly from the flasks for assay of total radioactivity so that ingestion could be expressed as a percent of total particles added.

Cell pellets and samples of cell suspensions were dissolved in 0.4 ml hyamine and counted in a Packard scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.) after addition of 15 ml [2,5-bis-2-(5-tert-butylbenzoxazolyl)thiophene-toluene]-ethanol (13:10 vol/vol) scintillation solution. Supernates containing uningested DMBA were dissolved directly in 15 ml scintillation solution and counted. Counts were corrected for quench using an external standard.

Studies on Egestion of Preingested Particles

12-ml portions of washed and resuspended log cells were added to 1-liter Erlenmeyer flasks containing 4 ml radioactive DMBA particles and incubated without shaking at 25°C in the dark. After 60–65-min incubation, cells had ingested about 55% of the particles and were collected by centrifugation at 200 g for 3 min at room temperature. The pellets were washed twice (200 g for 2 min) with dilute salt solution with albumin and resuspended to a density of 1.1–1.3 × 10⁶ cells/ml.

To measure egestion, 12-ml portions of these resuspended cells were then added to 1-liter Erlenmeyer flasks containing 4 ml dilute salt solution with albumin (control) or 4 ml of such a solution containing a given pharmacologic agent and incubated without shaking at room temperature. At appropriate intervals, 1.1–1.5-ml samples of cell suspensions were removed and centrifuged at 300 g for 3 min at 0°C. The supernates were recentrifuged and 0.2-ml samples of the cell-free supernates were removed and assayed for ¹⁴C. At the end of the incubation, samples were taken directly from the flasks for assay of the total amount of radioactivity present. DMBA in the supernate was expressed as a percent of the total DMBA present.

For studies involving sequential particle ingestion, 100-ml aliquots of logarithmically growing cells were placed in each of two 500-ml Erlenmeyer flasks. Experiments were begun by adding 3 ml unlabeled DMBA particles to flask 1 and 3 ml radioactive DMBA particles to flask 2. The two flasks were incubated with shaking at 25°C. After 30 min, small samples were withdrawn from each flask for cell counts and assay of total radioactivity, and the cells collected (separately), washed twice, and resuspended in 100 ml proteose peptone or 100 ml dilute salt solution, as specified, in 500-ml Erlenmeyer flasks.

3 ml radioactive particles were added to flask 1' and 3 ml unlabeled particles were added to flask 2', and the flasks incubated again with shaking at 25°C. After 30 min, small samples were withdrawn and the cells collected, washed with dilute salt solution, washed with dilute salt solution with albumin, and resuspended in about 68 ml dilute salt solution with albumin. At this time, cells of the first group contained about 50% of the radioactive DMBA particles they had been exposed to and cells of the second group contained 31%. Egestion was measured as described above with 21-ml portions of each suspension and either 3 ml dilute salt solution with albumin (control) or 3 ml of such a solution containing dichloroisoproterenol (DCI) or desmethylimipramine (DMI). Samples were prepared for assay of radioactivity and counted as described above.

Reagents

Chemicals were obtained from the following sources: 9,10-dimethyl[9-¹⁴C,1,2]benzanthracene, Radiochemi-

cal Centre, Amersham; dimethylsulfoxide, J. T. Baker Chemical Co., Phillipsburg, N. J.; 9,10-dimethyl-1,2-benzanthracene, 3',5'-cyclic monophosphoric acid, adenosine 5'-monophosphoric acid, 5-hydroxytryptamine (serotonin), isoproterenol, caffeine, and ascorbic acid, Sigma Chemical Co., St. Louis, Mo.; cytochalasin B, Imperial Chemical Ind., Inc., Cheshire, England; desmethylimipramine, U. S. V. Pharmaceutical Corp., Tuckahoe, N. Y.; phenoxybenzamine hydrochloride, Smith, Kline and French Laboratories, Philadelphia, Pa.; vinblastin sulfate, Eli Lilly and Co., Indianapolis, Ind.; reserpine phosphate, Ciba Pharmaceutical Co., Summit, N.J.; epinephrine bitartrate, Winthrop Labs., New York; colchicine, General Biochemicals, Chagrin Falls, Ohio; dichloroisoproterenol, Aldrich Chemical Co., Inc., Milwaukee, Wis.; and hydroxide of hyamine and 2,5-bis-2-(5-tert-butyl-benzoxazolyl)thiophene, Packard Instruments Co., Inc., Downers Grove, Ill. All other chemicals were reagent grade.

RESULTS

Effect of Pharmacologic Agents on Acid Hydrolase Release

Release of acid hydrolases from *Tetrahymena* is initially rapid but has significantly tapered off by the end of 5-h incubation (Müller, 1970; Rothstein and Blum, 1973). Acid hydrolase release after 5-h incubation in the presence of various pharmacologic agents is reported in Table 1 as a percent of acid hydrolase release from control cells. In seven experiments the amounts of acid phosphatase, ribonuclease, and α-glucosidase release from control cells ranged from 4.0 to 5.2, 6.3 to 8.4, and 0.038 to 0.065 U, respectively. In five experiments in which two control flasks were incubated, the mean error in activity released was not more than 1.5% for each of the three acid hydrolases measured.

Neither epinephrine, norepinephrine, isoproterenol, nor serotonin caused any significant change in acid hydrolase release at 5 h that could not be attributed to the small and variable effects of ascorbic acid, which was to prevent possible oxidation of these biogenic amines. Although exogenous catecholamines had no effect, DCI, a β-sympatholytic, and phenoxybenzamine, an α-sympatholytic, significantly altered acid hydrolase release. The effects of DCI were noticeable at .006 mM. At 0.1 mM, it affected each hydrolase differently: ribonuclease release was increased threefold, acid phosphatase was increased by 60%, and α-glucosidase was slightly decreased. Phenox-

TABLE I

Influence of Pharmacologic Agents on Release of Acid Hydrolases and Ingestion of Dimethylbenzanthracene Particles by Tetrahymena

Pharmacologic agent	Number of exp	Concentration	Control activity released after 5 h			Number of exp	Concentration	Particles ingested relative to control cells	
			P	R	α			15 min	45 min
		mM	%	%	%		*mM*	%	%
Serotonin	1‡	0.19	102	92	106	1§	0.019	141	109
	1‡	0.39	97	98	100	4‡	0.19	218 ± 40	114 ± 5
						3	0.19	134 ± 6	107 ± 9
						2	0.39	151–155	97–108
Dichloroisopro-terenol	1	0.0062	129	150	98	1	0.033	72	81
	1	0.025	174	235	86	2	0.10	56–58	46–52
	4	0.10	159 ± 4	299 ± 32	78 ± 7				
Desmethylimi-pramine	1	0.018	157	234	90	1	0.024	76	80
	4	0.071	189 ± 21	375 ± 58	94 ± 10	2	0.071	57–60	26–30
Reserpine	3	0.029	170 ± 11	217 ± 22	103 ± 3	3	0.029	80 ± 9	94 ± 7
Phenoxybenza-mine§	2	0.20	177–177	168–174	115–115	1	0.1	56	73
Caffeine	1	0.10	102	111	92	3	0.1	170 ± 39	120 ± 6
						3	2.0	312 ± 9	116 ± 6
Dibutyryl cyclic AMP	2	1.0	99–100	96–99	100–108	3	1.0	142 ± 34	116 ± 3
5'-AMP	1	1.0	96	97	94	4	1.0	188 ± 21	121 ± 4
Colchicine	2	1000*	92–102	88–98	77–96	1	1000*	73	70
Vinblastine	2	20*	93–106	92–106	75–84	1	0.8*	77	37
						2	20*	57–59	22–34
Cytochalasin B‖	1	9.5	121	105	74	1	0.95*	62	65
	1	12	119	113	85	2	9.5*	50–58	43–47

For the experiments on acid hydrolase release washed log cells were incubated for 5 h at about 10^6 cells/ml in dilute salt solution with albumin plus the indicated pharmacologic agent as described in the section on Materials and Methods. Cell-free supernates were assayed for acid phosphatase (P), ribonuclease (R), and α-glucosidase (α).

For the experiments on ingestion 2 ml washed cells at about 2 × 10^6 cells/ml were incubated with 1 ml of the indicated pharmacologic agent and 1 ml DMBA particles in dilute salt solution with albumin. Ingestion is expressed as percent of the DMBA ingested by control cells at the indicated times. For both the hydrolase release experiments and the particle ingestion experiments, the control values are set at 100%.

* The concentration units for these compounds are μg/ml.

‡, § Ascorbic acid (‡ = 50 μg/ml, § = 5 μg/ml) was present in the flasks with serotonin. This concentration of ascorbic acid had no reproducible effects on the release of any of the three hydrolases when tested against controls, but increased DMBA particle ingestion to 153% at 15 min for 50 μg/ml and to 127% at 15 min for 5 μg/ml, and had no effect on particle ingestion at 45 min for either concentration of ascorbic acid.

‖ Dimethylsulfoxide (at 1.2, 0.95, and 0.095% vol/vol) was present in the appropriate control flasks as well as in the flasks containing cytochalasin B. Dimethylsulfoxide at 0.95 and 1.2% increased the amount of each acid hydrolase released in 5 h to about 130% of control values; at 0.95% and 0.095% it increased DMBA particle ingestion to about 190% and 130%, respectively, of control values at 15 min, and had no effect at 45 min.

For experiments with $n \geq 3$, the data shown is the mean ± standard deviation.

ybenzamine was insoluble in the dilute salt solution with albumin and so was dissolved in unsupplemented dilute salt solution. Acid hydrolase release in the control flasks (0.75 mg/ml albumin) for experiments with phenoxybenzamine was the same as in the usual control flasks with 1 mg/ml albumin. At 0.2 mM, phenoxybenzamine increased the release of both acid phosphatase and

ribonuclease by about 75%, but it had no significant effect on α-glucosidase release.

Reserpine, known to deplete the catecholamine stores of *Tetrahymena* (Blum et al., 1966), approximately doubled the release of acid phosphatase and of ribonuclease without affecting α-glucosidase release. DMI was the most potent stimulator of acid hydrolase release examined. At .07 mM,

DMI increased ribonuclease release fourfold, and acid phosphatase release twofold, without altering the release of α-glucosidase. Thus each of the four catecholamine antagonists examined caused little or no alteration of α-glucosidase release but significantly increased the release of acid phosphatase and ribonuclease. Both DMI and DCI increased ribonuclease release to a much greater extent than acid phosphatase release.

Intracellular Content and Extracellular Release of Acid Hydrolases at 1 and 2.5 h

Each of the adrenergically reactive drugs used above increased the release of acid phosphatase and ribonuclease during a 5-h incubation. Since the release of some acid hydrolases from *Tetrahymena* is roughly proportional to their cellular contents as that content changes with culture age (Rothstein and Blum, 1974 *a*), it appeared possible that one or more of these drugs acted to increase the rate of synthesis and/or decrease the rate of degradation of the two acid hydrolases, producing an increase in their rate of release as a consequence of an increased intracellular content. Some support for this view was obtained from experiments with cycloheximide, known to inhibit protein synthesis in *Tetrahymena*. Cycloheximide (10 μg/ml), added at the beginning of the 5-h incubation period, had little effect on acid phosphatase release but decreased the release of ribonuclease and α-glucosidase by about 40%. This suggested that a portion of the acid hydrolases released are newly synthesized during the 5-h incubation, although a direct effect of cycloheximide on the release process cannot be ruled out. Since DCI and DMI had the largest effects on acid hydrolase release, they were chosen for further study at shorter incubation periods where changes in synthesis would, of necessity, play a smaller role.

At 1 h of incubation with DMI, release of each of the acid hydrolases was increased considerably over that of control cells, and to a different extent for each enzyme (Table II, exp 1 and 3). The initial enhancement of release did not persist for α-glucosidase, whose extracellular level at 5 h was slightly below that of control cells (cf. Table I). The release of the three hydrolases did not appear to be secondary to a change in their intracellular content. For α-glucosidase, total (intra- plus extracellular) activity did not differ from control values; thus released α-glucosidase accounted quantitatively for that which was lost intracellularly. For acid phosphatase, the intracellular activity

increased slightly, suggesting that some of the acid phosphatase secreted into the medium had been replaced by newly synthesized enzyme at 1 h. For ribonuclease, the intracellular activity was slightly increased over control values and total activity was moderately increased. The increased intracellular activity, however, was clearly not sufficient to produce a secondary rise in ribonuclease release, since for both control and DMI-treated cells, intracellular activities at 1 h were considerably below the values at the start of the incubation. DMI thus appears to "spare" ribonuclease from the degradative loss which occurs under these "step-down" nutritional conditions (Rothstein and Blum, 1973), partly by enhancing its rate of release.

The results with DCI were entirely different (Table II, exp 2, 3, and 4). At 1 h of incubation, acid hydrolase release was essentially the same as that of control cells. The intracellular activities of ribonuclease and acid phosphatase, however, were significantly increased, and this was reflected in a parallel rise in the total activities of these two enzymes. The intracellular level of ribonuclease at 1 h was similar to that of control cells at the beginning of the incubation; thus DCI prevented the degradative loss of ribonuclease noted above.

Between 1 and 2.5 h of incubation, DCI caused the release of much more ribonuclease and acid phosphatase than was released by control cells. At 2.5 h the intracellular levels of all three hydrolases were close to those of control cells, but the total activities of ribonuclease and acid phosphatase were increased, with most of the increase accounted for by released enzyme. Thus, in contrast to the rapid initial effect of DMI, the increased release of ribonuclease and acid phosphatase caused by DCI appears to be due in part to the establishment of a new balance between enzyme synthesis and degradation, favoring increased intracellular levels of these two acid hydrolases. It is shown elsewhere (Rothstein and Blum, 1974 *b*) that the additional intracellular activities of these two hydrolases appear in the higher density populations of hydrolase-containing particles after centrifugation of cell homogenates on sucrose density gradients.

Measurement of Ingestion by Tetrahymena

It was possible that the increased hydrolase release caused by catecholamine antagonists depended upon a change in the rate of ingestion. If the rate of food vacuole formation were stimu-

Variation of Total, Intracellular, and Extracellular Acid Hydrolase Activities during Incubation of Tetrahymena with DMI and DCI

	Total activity			Intracellular activity			Extracellular activity		
	P	R	α	P	R	α	P	R	α
Experiment 1									
Control, 0 h	15.8	64.5	0.0864	15.8	64.5	0.0864			
Control, 1 h	16.0	42.2	0.0847	15.2	39.2	0.0799	0.788	2.99	0.00475
DMI 7.1 × 10⁻⁵ M, 1 h	17.4	58.6	0.0837	14.9	44.9	0.0721	2.46	13.7	0.0116
DMI, 1 h as a percent of control, 1 h	109%	139%	99%	98%	114%	90%	312%	458%	245%
Experiment 2									
Control, 0 h	20.2	54.2	0.0803	20.2	54.2	0.0803			
Control, 1 h	18.2	30.2	0.0794	17.1	27.1	0.0730	1.13	3.01	0.00636
DCI 1 × 10⁻⁴ M, 1 h	23.7	56.2	0.0795	22.8	52.8	0.0742	0.0905	3.43	0.00534
DCI, 1 h as a percent of control, 1 h	130%	186%	100%	133%	194%	102%	80%	114%	84%
Experiment 3									
Control, 0 h	17.3	63.5	0.0702	17.3	63.5	0.0702			
Control, 1 h	16.2	35.0	0.0712	15.3	32.0	0.0650	0.913	3.02	0.00624
DMI 7.1 × 10⁻⁵ M, 1 h	19.7	52.4	0.0687	16.7	37.7	0.0555	3.01	14.6	0.0132
DCI 1 × 10⁻⁴ M, 1 h	19.4	61.9	0.0704	18.5	58.3	0.0642	0.983	3.59	0.00625
DMI, 1 h as a percent of control, 1 h	122%	150%	96%	109%	118%	85%	329%	484%	212%
DCI, 1 h as a percent of control, 1 h	120%	177%	99%	121%	182%	99%	108%	119%	100%
Experiment 4									
Control, 0 h	15.6	53.8	0.0733	15.6	53.8	0.0733			
Control, 1 h	12.4	14.5	0.101	8.90	8.26	0.0678	3.47	6.24	0.0332
DCI 8 × 10⁻⁵ M, 2.5 h	18.2	31.2	0.0923	12.1	9.67	0.0672	6.19	21.5	0.0251
DCI 2 × 10⁻⁵ M, 2.5 h	17.0	24.1	0.0875	9.97	6.33	0.0574	7.03	17.8	0.0300
DCI 8 × 10⁻⁵ M, 2.5 h as a percent of control, 2.5 h	148%	215%	91%	135%	117%	99%	178%	345%	76%
DCI 2 × 10⁻⁵ M, 2.5 h as a percent of control, 2.5 h	137%	166%	87%	112%	77%	85%	202%	285%	91%

Washed log cells were suspended at about 10^6 cells/ml in dilute salt solution with 1 mg/ml bovine serum albumin (control) or such a solution with added DMI or DCI at the specified concentrations. Cell suspensions were incubated for 1 or 2.5 h as indicated. Assays for acid phosphatase (P), ribonuclease (R), and α-glucosidase (α) were performed on whole homogenates (total activity) before and after incubation, and on cell-free supernates after incubation (extracellular activity). Intracellular activity is the difference between extracellular activity and total activity. Activities for cells with DMI and DCI are also expressed as a percent of activities for control cells, after incubation.

lated, the rate at which food vacuoles fuse with lysosomes might be similarly increased, and this in turn could increase the rate at which phagolysome contents, including acid hydrolases, would be egested via the cytoproct. The effects of drugs on the ingestion process were investigated by measuring the uptake of ¹⁴C-labeled 9,10-dimethylbenz-anthracene, an insoluble polybenzenoid hydrocarbon which was sonicated to yield a relatively uniform suspension of small particles. Since the cells were incubated with 79 μg DMBA particles/ ml, the particle diameter is about 0.1 μm, and the particle density is approximately 1 gm/ml; there were about 2 × 10¹¹ particles/ml or about 2 × 10⁵ particles/cell.

Preliminary experiments showed that less than 4% of the DMBA was found in cell pellets from samples taken within 1 min of the start of the incubation, an amount which could be entirely in the extracellular space of the pellet. In 11 experiments control cells had ingested 12.7 ± 2.7% of the total particles at 15 min and 48.4 ± 7.9% at 45

min. Fig. 1 shows the results of an experiment in which ingestion was followed for 3 h. The initial slow rate of ingestion may have been due to the presence of dimethylsulfoxide or the prior centrifugation of the cells, as Nilsson (1972, 1973) has reported that the ability of *Tetrahymena* to form food vacuoles is reduced immediately after a change in the suspending medium. Ingestion of DMBA then accelerated and did not begin to taper off until after 1 h, a maximum being reached at about 1.5 h. The maximum amount of particle accumulation was probably determined by a combination of factors such as the rate of egestion, the availability of membrane (Nilsson, 1972; Werb and Cohn, 1972; Ricketts, 1973), and the supply of lysosomes (Ricketts, 1971 *b*, 1973).

These results are at variance with others (Nilsson, 1972; Chapman-Andresen and Nilsson, 1968) where ingestion of carmine particles by *Tetrahymena* occurred predominantly during the first few minutes of incubation and had all but ceased after 20–30 min, or under certain conditions, was periodic (Ricketts, 1971 *b*). It is not known whether this difference in the time sequence of ingestion is due to the different particles ingested, the presence of dimethylsulfoxide, or other factors such as growth conditions or strain differences.

Recovery of DMBA was computed by adding the percent of the total radioactivity ingested to the percent uningested as determined from supernate samples. For control cells, recovery ranged from
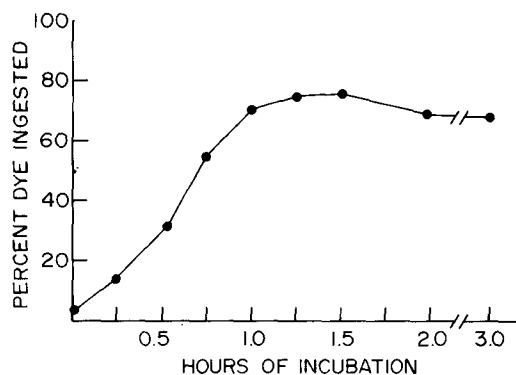

FIGURE 1   Time course of ingestion of dimethylbenzanthracene particles. Logarithmically growing cells were harvested and resuspended at a density of about 1.3 × 10⁶ cells/ml and treated as described in the section on Materials and Methods except that 12 ml of these cells were added to 4 ml of particles in a 1-liter Erlenmeyer flask.

96 to 102% at both 15 and 45 min in five experiments. In 2 exp with 0.1 mM DCI, a potent inhibitor of ingestion, recovery was 100 ± 1% at both 15 and 45 min. In one experiment with 2 mM caffeine, a potent stimulator of ingestion, over 96% of the DMBA was recovered at both 15 and 45 min. Thus there was no loss of label from the system.

Effects of Pharmacologic Agents on Ingestion

The effects of various pharmacologic agents on the ingestion of DMBA particles are presented in Table I. The error in measurement of the particles ingested after 15 min in duplicate control flasks was 2.4–6.5% in eight experiments and 16.7% in two others (mean = 6.6%). The mean error in measurements after 45 min of incubation was 3.5%. Duplicate control flasks were handled first and last in each experiment to allow for the effects of order of preparation.

Since many of the drugs which were used in these experiments have marked effects on the metabolism of glycogen, the experiments reported here were done by adding the drug and particles together to the cells and confining measurements to 15- and 45-min exposure. Presumably some ingestion occurs before a drug achieves its maximal effect so that the effects of drugs which inhibit ingestion are more apparent at 45 min; however, any effects observed are likely to reflect direct effects on the mechanism of ingestion rather than indirect effects consequent to long term alterations in metabolism. Because the amount of DMBA ingested never exceeded 77% of the total DMBA added under any of the experimental conditions, the effects of drugs which enhance ingestion are usually more apparent at 15 min than at 45 min.

Although serotonin at 0.39 mM increased the initial rate of particle ingestion by 50%, there was no effect of added epinephrine or norepinephrine on uptake. The effect of serotonin was enhanced when it was added in conjunction with ascorbic acid, but this was due to a direct effect of ascorbic acid on ingestion. The finding that ingestion is enhanced by ascorbic acid emphasizes the sensitivity of ingestion to chemical signals from the environment and suggests that reducing compounds may be involved in initiating feeding.

Although there were no effects of added catecholamines on particle ingestion, the catecholamine antagonists which increased acid hydrolase

release substantially inhibited particle ingestion. Both DMI and DCI caused noticeable inhibition at less than 0.1 mM. Reserpine moderately inhibited ingestion within 15 min at a concentration known to affect the glycogen metabolism of *Tetrahymena* in longer exposures (Blum, 1967). Measurements of the effect of phenoxybenzamine were complicated by the insolubility of this drug mentioned above. Phenoxybenzamine dissolved in water inhibited particle ingestion when compared to an appropriate control, although this control showed increased ingestion over the usual control cells. Varying the concentration of albumin between 0 and 1 mg/ml in control experiments caused no significant change in the rate of ingestion. Phenoxybenzamine in water also inhibited ingestion in an experiment in which cells and DMBA were prepared in dilute salt solution and the albumin was added separately. In this case too, the control cells ingested more particles than the usual control cells. It is possible that distilled water added to sonicated DMBA particles enhanced their ingestibility. Although this anomaly remains unresolved, it is clear that phenoxybenzamine inhibits ingestion of DMBA particles.

Several agents were used to examine the possible involvement of cyclic AMP in food vacuole formation (Table I). Dibutyryl cyclic AMP enhanced ingestion at 15 min, but 5'-AMP at the same concentration caused an even stronger stimulation. Caffeine, which inhibits the cyclic AMP phosphodiesterase of *Tetrahymena* (Blum, 1970) markedly increased particle ingestion. When used at 0.1 mM in conjunction with 1.0 mM dibutyryl cyclic AMP, the effects of the two agents were not synergistic; the effects of caffeine with serotonin were also not synergistic (data not shown). Neither dibutyryl cyclic AMP, 5'-AMP, nor caffeine had any effect on acid hydrolase release.

Microtubules and possibly microfilaments are present in the oral apparatus of *Tetrahymena* and other ciliates (Elliott and Clemmons, 1966; Tucker, 1972; Allen, 1973). Both colchicine and vinblastine, agents which interfere with the organization and function of microtubules (Malawista and Bensch, 1967; Bensch and Malawista, 1968, 1969), inhibited ingestion. Vinblastine was particularly effective in that ingestion virtually ceased after 15 min at 100 μg/ml and was inhibited with as little as 0.8 μg/ml. Cytochalasin B is thought to act by interfering with the function of microfilaments (Wessells et al., 1971). Cytochalasin B dissolved in

dimethylsulfoxide inhibited particle ingestion when compared to appropriate dimethylsulfoxide controls, which ingested particles somewhat more rapidly than the usual control cells. Since all flasks (including controls) contained 0.66% (vol/vol) dimethylsulfoxide from the DMBA particle preparation, control cell ingestion here may be stimulated over that observed by other investigators (Nilsson, 1972; Chapman-Andresen and Nilsson, 1968). Nilsson (1973) has independently reported that colchicine and cytochalasin B inhibit the ingestion of carmine particles by *Tetrahymena*. Neither colchicine nor vinblastine altered the rate of acid hydrolase release except for a slight decrease in α-glucosidase release with vinblastine. Cytochalasin B had no significant effect on acid hydrolase release when compared with appropriate dimethylsulfoxide controls, although this solvent increased the release of each of the three acid hydrolases by about one-third as compared to the usual control cells.

Pharmacologic Effects on Egestion of Preingested Particles

Although the experiments so far described are essential to an understanding of the factors governing release of particular acid hydrolases, and provide much new material on the pharmacologic sensitivity of the ingestion process, detailed interpretation of the nature of the release mechanism *per se* is rendered difficult because of possible changes in the synthesis and degradation of released enzymes. To study the extrusion process without these complications, and to compare the pharmacologic reactivity of acid hydrolase release with the egestion of particulate material, experiments were performed on cells which had ingested labeled DMBA particles for 1 h, as described in the section on Materials and Methods. In these experiments about 11% of the DMBA was in the extracellular supernate when measurements of egestion began, as a result of incomplete washing and of some egestion occurring before the first measurement was made (see Fig. 2). Measurements of egestion beyond about 45 min were not entirely reproducible, possibly as a result of reingestion of egested particles. In two experiments (Fig. 2, exp IV and V) cells were incubated with added unlabeled DMBA (12.7 μg/ml, plus 0.21% dimethylsulfoxide) to reduce the probability of reingestion of egested radioactive particles. Eges-
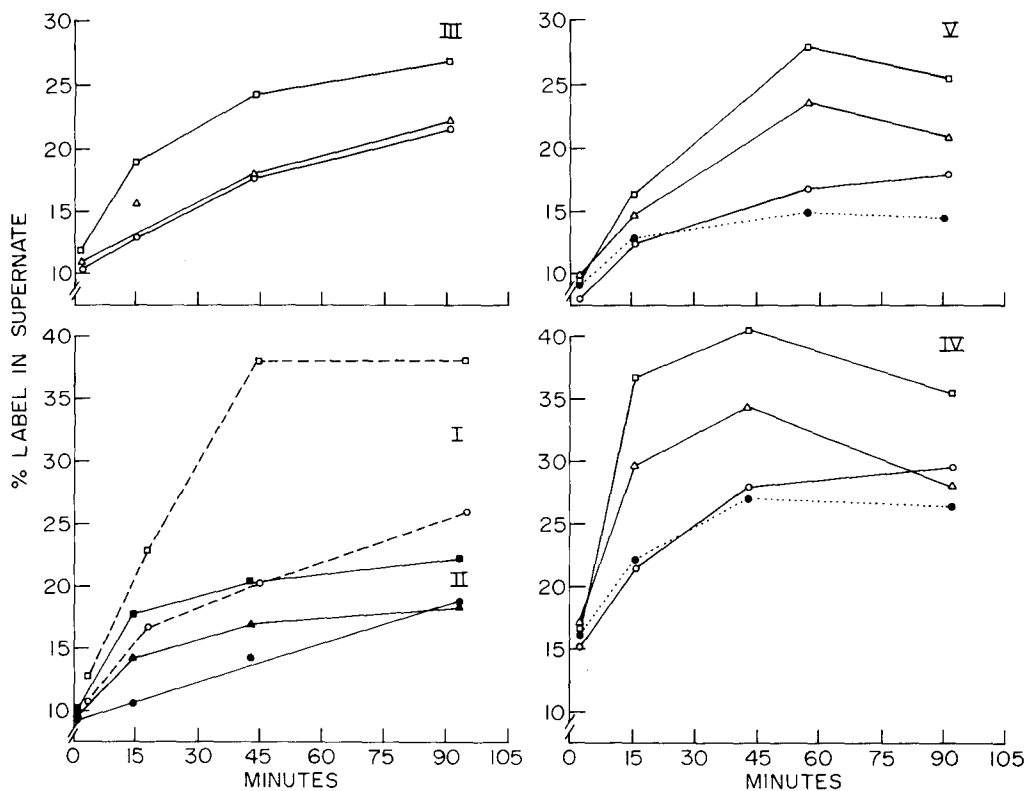
FIGURE 2   Effect of DMI and of DCI on egestion of preingested dimethylbenzanthracene particles. Washed log cells were incubated with 9,10-dimethyl[9-^{14}C, 1,2]benzanthracene for about 1 h as described in the section on Materials and Methods except for exp IV and V in which 6 ml of [^{14}C]DMBA were added directly to logarithmically growing cultures, so that particles were ingested with digestible substrates from the growth medium. Cells were then washed and resuspended with DMI (7.1 × 10^{-5} M; □, ■) or DCI (1 × 10^{-4} M; △, ▲) or in dilute salt solution alone (○, ●) and the radioactivity egested into the medium was measured in cell-free supernates obtained at the times indicated on the abscissa. Two experiments are shown in the lower left panel; exp I, dashed lines, and exp II, solid lines. In exp IV and V the dotted lines show the egestion of [^{14}C]DMBA in control cells which were supplemented with 12.7 mg/ml of unlabeled DMBA pa..icles. The concentration of [^{14}C]DMBA in mg/ml, its specific activity in µCi/ml, and the dimethylsulfoxide concentration in volume percent for the particle preparations used in each experiment were, respectively: exp I, 0.20 mg/ml, 1.52 µCi/ml in 4.3% dimethylsulfoxide; exp II and III, 0.20 mg/ml, 0.26 µCi/ml in 1.9% dimethylsulfoxide; exp IV and V, 0.40 mg/ml, 0.40 µCi/ml in 3.5% dimethylsulfoxide.

tion by these cells was not, however, different from control cell egestion. In two other experiments (data not shown), duplicate control flasks were incubated in order to obtain an estimate of the reliability of measurements of egestion. In both experiments the difference in amount egested between the two controls was less than 1.5%.

Cells incubated with DMI egested 2-3 times as much DMBA as control cells after 15 and 45 min (Fig. 2, exp I–III). Cells incubated with DCI also egested more particles than control cells, but the

effect was not as great as with DMI (Fig. 2, exp II and III). Neither colchicine nor vinblastine caused any significant change in the egestion of particles (data not shown). Cytochalasin B, compared to an appropriate dimethylsulfoxide control, produced a minor and probably insignificant decrement in egestion after 50 min (data not shown).

Egestion of Sequentially Ingested Particles

Although electron microscope studies of *Tetrahymena* (Elliott and Clemmons, 1966) do not

indicate the presence of a tract which could serve to convey vacuoles in an ordered fashion from the oral apparatus to the cytoproct, orderly vacuolar movements have been observed in other cells (Freed and Lebowitz, 1970) and Allen (1973) has observed a band of microtubules which appears to convey membrane fragments from the cytoproct back to the oral apparatus in *Paramecium*. If there were a mechanism for the orderly progression of vacuoles from mouth to cytoproct, the effects of drugs on the rate of release of acid hydrolases and of preingested particles might result from changes in the rate of conveyance of primary or secondary lysosomes or food vacuoles rather than changes at the cytoproct *per se*. Experiments using sequentially ingested radioactive and unlabeled DMBA particles were performed as described in the section on Materials and Methods to evaluate this possibility. Cells were divided into two portions, one fed unlabeled particles followed by radioactive particles (group 1), the other fed radioactive particles followed by unlabeled particles (group 2) in proteose peptone (Fig. 3, lower panel). If there were an orderly progression of food vacuoles between the oral apparatus and cytoproct, one would expect a higher proportion of radioactive particles to be egested at early times from group 2 than from group 1 cells. There was, however, no significant difference in egestion between the two groups, even when DCI or DMI was added to increase the rates of egestion. An experiment was also done in which cells were fed particles in proteose peptone followed by particles in dilute salt solution (Fig. 3, upper panel). Here again there was no significant difference in egestion between the two groups. The failure to find any indication of an orderly progression of vacuoles from mouth to cytoproct is consistent with earlier findings suggesting that release of acid phosphatase and α-glucosidase is a random process (Rothstein and Blum, 1973, 1974 a).

Effect of DMI on the Thermal Dependence of Acid Phosphatase Release

Increasing temperature increases the amount of acid phosphatase and α-glucosidase released in a 1-h incubation in accordance with the Arrhenius equation (Rothstein and Blum, 1974 a). The release of ribonuclease under identical conditions did not obey Arrhenius kinetics. The ability of DMI to stimulate the initial rate of release of acid

phosphatase (and ribonuclease) suggested that this drug might act by changing the activation energy for the rate-limiting step of the extrusion process. It was therefore of interest to compare the effect of temperature on acid phosphatase release from control cells and from cells treated with DMI. In
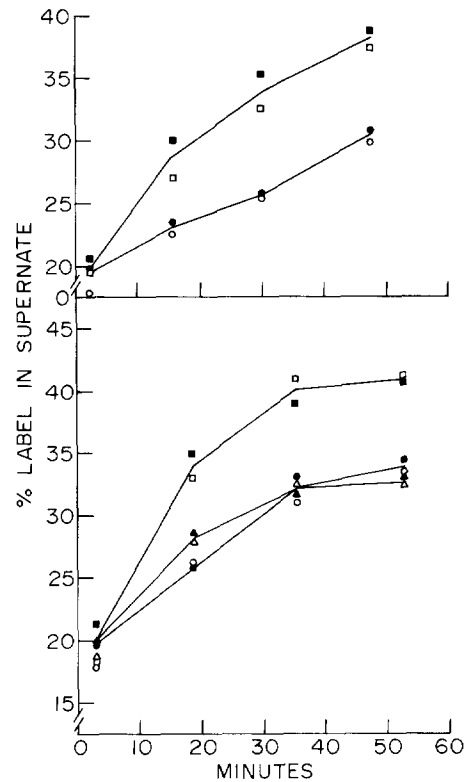

FIGURE 3 Egestion of sequentially ingested dimethyl benzanthracene particles. Logarithmically growing cells were incubated sequentially for 2 half-hour periods with ¹⁴C-labeled or unlabeled DMBA particles as described in detail in the section on Materials and Methods. Cells were washed and resuspended with 3.6×10^{-5} M DMI (□, ■) or with 5×10^{-5} M DCI (△, ▲) or in dilute salt solution containing albumin without any drug (○, ●), and the radioactivity released was measured in cell-free supernates obtained at the indicated times. Lower panel: group 1 (open symbols) received unlabeled particles in proteose peptone followed by labeled particles in proteose peptone; group 2 (solid symbols) received labeled particles in proteose peptone followed by unlabeled particles in proteose peptone. Upper panel: group 1 (open symbols) received unlabeled particles in proteose peptone followed by labeled particles in dilute salt solution; group 2 (solid symbols) received labeled particles in proteose peptone followed by unlabeled particles in dilute salt solution.

three experiments such as that shown in Fig. 4, activation energies of 12.3 ± 0.29, 11.3 ± 0.33, and 11.0 ± 0.31 kcal/mol were obtained for control cells (cf. Rothstein and Blum, 1974 a) and 16.6 ± 0.26, 17.0 ± 0.20, and 15.3 ± 0.13 kcal/mol for cells treated with DMI. These values are significantly different at <0.005% confidence level. Thus DMI, contrary to what might have been expected, increased the activation energy required for the rate-limiting step of acid hydrolase release. DCI, which did not affect acid hydrolase release at 1 h, did not cause any significant change in activation energy for acid phosphatase release. The effect of changing temperature on the rate of ingestion of DMBA particles was complex and did not conform to the Arrhenius equation (data not shown) in agreement with the results obtained by Nilsson (1972) on the effects of temperature on the uptake of carmine particles.

DISCUSSION

Much evidence has accumulated suggesting that *Tetrahymena* possesses a primitive adrenergic control system regulating metabolism. Thus Blum et al. (1966) found that reserpine inhibited the growth and decreased the catecholamine content of these ciliates, and that a number of adrenergically
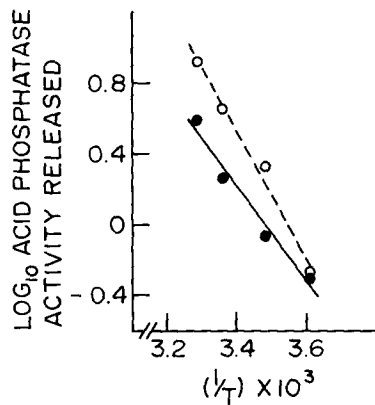

FIGURE 4 Effect of DMI on the activation energy of acid phosphatase release. Logarithmically growing cells were washed and incubated at about 1 × 10⁶ cells/ml for 1 h in dilute salt solution supplemented with 1 mg/ml bovine serum albumin. Solid lines, control cells; dashed lines, cells with 7.1 × 10⁻⁵ M DMI. The log of the acid phosphatase activity released, normalized to 1 × 10⁶ cells/ml on the basis of initial cell counts, is plotted as a function of the reciprocal of the absolute temperature of incubation. Lines were determined by linear regression analysis.

reactive drugs including DCI, DMI, propanolol, and reserpine inhibited growth or reduced the glycogen content, and altered the activity of several enzymes involved in gluconeogenesis (Blum, 1967, 1968, 1970; Iwata et al., 1967). Cyclic AMP, 5'-AMP, and caffeine also have been shown to have metabolic effects on this ciliate (Blum, 1967; Porter and Blum, 1973). More recently Rosensweig and Kindler (1972) have reported that the adenyl cyclase of *Tetrahymena* is activated in vitro by epinephrine and serotonin. Activation of the cyclase by epinephrine was prevented by the β-sympatholytic agent propanolol, although propanolol did not interfere with activation by serotonin. Voichick et al. (1973) have shown that cyclic AMP content of *Tetrahymena* is inversely correlated with gluconeogenic capacity, thus providing a link between changes in cyclic AMP content and some of the metabolic effects observed by Blum (1967, 1970). Until the present work, however, there was no evidence for any direct effect of added catecholamines or serotonin on any physiological process in *Tetrahymena*.

The present work shows that the ingestion of DMBA particles by *Tetrahymena* is enhanced by serotonin. In view of the in vitro properties of *Tetrahymena* adenyl cyclase (Rosensweig and Kindler, 1972), and the ability of serotonin to stimulate cyclic AMP synthesis elsewhere (Mansour et al., 1960; Stone and Mansour, 1967), it is reasonable to suppose that serotonin may act by activating the cyclase. This accords well with the strong enhancement of ingestion caused by caffeine which inhibits the cyclic AMP phosphodiesterase of *Tetrahymena* (Blum, 1970), and with the inhibition of ingestion by DCI which, like propanolol, is a potent β-sympatholytic agent that presumably acts by preventing the activation of adenyl cyclase by endogeneous epinephrine in a manner similar to its effects on other systems (Sutherland et al., 1968; Nakano et al., 1969). Consistent with this are the effects of reserpine, which depletes the catecholamine stores of *Tetrahymena* (Blum et al., 1966) and of DMI which inhibits the uptake of catecholamines by nerve membranes (Maxwell et al., 1970) and has been reported to interfere with the actions of cyclic AMP (Nakano and Ishii, 1970).

The possibility that ingestion in *Tetrahymena* is controlled in part by a serotoninergic and adrenergic system whose effects are mediated by cyclic AMP is, of course, also consistent with the observation that dibutyryl cyclic AMP enhanced parti-

cle ingestion. It should be noted that a number of workers have implicated cyclic AMP in the regulation of ingestion by polymorphonuclear leukocytes (Park et al., 1971; Manganiello et al., 1971; Bourne et al., 1971; Seyberth et al., 1973).

Although this possibility is attractive it should not be accepted uncritically. It is not clear, for example, why the effects of serotonin plus caffeine or dibutyryl cyclic AMP plus caffeine are not synergistic. One must also assume that exogeneous epinephrine cannot reach an intracellular receptor whereas exogeneous serotonin can.[1] The effect of phenoxybenzamine is also surprising since *Tetrahymena* adenyl cyclase was insensitive to the α-sympatholytic agent phentolamine (Rosensweig and Kindler, 1972), although α-sympatholytic agents do affect the growth and glycogen metabolism of *Tetrahymena* (Blum, 1967, 1969; Iwata et al., 1967). Finally the effect of 5'-AMP could indicate that the increase in ingestion produced by dibutyryl cyclic AMP was nonspecific. However, 5'-AMP has been shown to inhibit glyconeogenesis in *Tetrahymena* (Raugi et al., 1973). If this effect were mediated by a change in cyclic AMP content, then the work of Voichick et al. (1973) would indicate that the cyclic AMP level had risen, which would be consistent with the observed increase in the rate of particle ingestion. Direct measurements of cyclic AMP content are needed to examine this and, indeed, to provide further evidence concerning the possibility that ingestion in *Tetrahymena* is controlled by endogeneous biogenic amines through the action of cyclic AMP.

Although serotonin, caffeine, and dibutyryl cyclic AMP increased ingestion of DMBA, the release of three acid hydrolases was insensitive to these compounds and to catecholamines. However, four adrenergically reactive drugs—DCI, phenoxybenzamine, reserpine, and DMI—substantially increased the release of acid phosphatase and ribonuclease after 5-h incubation. This might suggest that an adrenergic system is involved in the regulation of acid hydrolase release. Further consideration puts this suggestion in a less favorable light. DCI had no effect on acid hydrolase release after 1-h incubation, in contrast to its rapid inhibition of ingestion, while increased hydrolase release at later times appeared to be secondary to a

change in the intracellular content of acid phosphatase and ribonuclease. Thus its primary effect does not seem to concern lysosomes or the release of their contents so much as the synthesis and degradation of acid hydrolases. Since reserpine and phenoxybenzamine were only examined at 5 h, their effects may also have been secondary to a change in cellular acid hydrolase content. DMI, on the other hand, increased the release of all three acid hydrolases after only 1-h of incubation, and it also enhanced the release of preingested particles during short term incubation. This rapid effect does not necessarily imply that it acts by interfering with an adrenergic regulatory system. It is known that impiramine (a congener of DMI) binds to phospholipid membranes and inhibits the calcium-sensitive ATPase of the sarcoplasmic reticulum (Balzer et al., 1968 *a, b*). The effect of DMI could, therefore, be a nonspecific one on the lysosomal or cytoproct membrane. This might help explain the unexpected finding that although DMI increased the activation energy for acid hydrolase release, it also increased the rate of release. Since caffeine and dibutyryl cyclic AMP had no effect on acid hydrolase release, there is no compelling reason to believe that egestion is controlled by a cyclic AMP-mediated adrenergic system despite the effects of several catecholamine antagonists. On the other hand, the rapid effects of DMI on acid hydrolase release and particle egestion at fairly low concentrations ($\sim 10^{-5}$ M) make it premature to rule out participation of an adrenergic system in the egestion process and further work will be required to clarify this point.

Earlier work, discussed above, was tentatively interpreted as being consistent with a random extrusion of the contents of primary lysosomes or phagolysomes. Although the site of acid hydrolase release was uncertain, it was thought by Müller (1970) and by us (Rothstein and Blum, 1973) that this most likely occurred via the cytoproct. The finding that DMI enhanced the rate of egestion of preingested particles, which certainly occurs at the cytoproct, and enhanced the rate of release of the three acid hydrolases examined strongly supports this view. The present work also supports the view that egestion occurs as a result of the random arrival of primary lysosomes or phagolysomes at the cytoproct since radioactive and unlabeled particles ingested sequentially were not egested sequentially. Thus food vacuole contents appear to enter a pool from which egestion occurs in a

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[1] It may be that the serotonin receptor is in the oral apparatus and hence accessible to exogenous serotonin whereas the epinephrine receptor is not.

random fashion, consistent with the view that there is no channelled route which might impose temporal order on ingestion, digestion, and egestion. It should be noted that within the time scale of this experiment (about 1.5 h) some food vacuoles may have fused with lysosomes and then subsequently fused with newly formed food vacuoles thus randomizing their contents. Other evidence weighing against a cellular digestive "tract" comes from the absence of any effect of colchicine, vinblastine, or cytochalasin B on acid hydrolase release and particle egestion. These drugs significantly inhibited particle ingestion, indicating a direct role for microtubules and microfilaments in the ingestion process, although it must be emphasized that these drugs have effects beyond the microtubular and microfilament system (Creasey and Markiw, 1964; Wilson et al., 1970; Sirakora et al., 1971; Mizel and Wilson, 1972; Stadler and Franke, 1972; Fyfe and Goldman, 1973; Kletzien and Perdue, 1973; Lieberman et al., 1973). Since colchicine and vinblastine-sensitive microtubules have been implicated in orderly vacuolar movements (Freed and Lebowitz, 1970), the effects of these drugs indicate that such structures do not extend beyond the oral apparatus to the cytoproct, and point to a significant difference in the ingestion and egestion mechanisms.

Although the catecholamine antagonists used in this study were known to alter glycogen metabolism and inhibit growth, there was no apparent relation between their effects on metabolism and on growth. It has been reported, however, that *Tetrahymena* do not grow well in particle-free proteose-peptone medium (Rasmussen, 1973; Rasmussen and Modeweg-Hansen, 1973) and that particles induce food vacuole formation in *Tetrahymena* which have ceased such activity (Chapman-Andresen and Nilsson, 1968). Thus the inhibition of growth caused by some of the drugs examined herein may be due in part to their inhibition of ingestion.

Müller (1970, 1972), Lloyd et al. (1971), and Rothstein and Blum (1973, 1974 a) have noted that the acid hydrolases of *Tetrahymena* are contained in at least two classes of lysosomes. Whatever the mechanism by which DMI enhanced the release of acid hydrolases, its effect indicates that there may be three populations of lysosomes. After 1 h of incubation, DMI stimulated the release of each acid hydrolase to a different degree: about 4.5 times for RNAse; 3.2 times for acid phosphatase,

and 2.3 times for α-glucosidase. Further work will be necessary to correlate any differences in membrane properties related to extrusion with differences in acid hydrolase content of the heterogenous population of lysosomes.

Müller (1972) has suggested that acid hydrolase release be regarded as a normal secretory function in *Tetrahymena*. On the basis of studies of the utilization of exogeneous particulate polyglucans, Reynolds (1969) proposed a dual intra- and extracellular digestive mechanism in this ciliate. The results reported here, indicating a possible role for biogenic amines in the control of ingestion and egestion, raise the possibility that the intra- and extracellular digestive functions in *Tetrahymena* are in part subject to adrenergic and serotoninergic regulation. The ability to follow particle ingestion and egestion as well as the secretion of acid hydrolases make this ciliate attractive for further experimentation on all phases of the digestive system at this level of its evolutionary development.

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