Effect of Adrenergically Reactive Drugs on Peroxisomal Enzymes in *Tetrahymena*

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SUMMARY

The effects of several adrenergically reactive drugs and of triiodothyronine on the activities of five enzymes of *Tetrahymena* have been studied. The isocitrate dehydrogenase and malate dehydrogenase activities were not altered by exposure to any of the drugs tested. Of three peroxisomal enzymes studied, D-amino acid oxidase activity was not affected, catalase activity was decreased by high concentrations of reserpine but either not changed or slightly increased by the other adrenergic drugs, and isocitrate lyase activity was strongly decreased by those drugs which depleted cell glycogen and slightly increased by aminophylline, which increased cell glycogen content. Thus one effect of the adrenergically reactive drugs in *Tetrahymena* is to alter the activity of the glyoxylate bypass pathway.

INTRODUCTION

It has been shown that the ciliated protozoan *Tetrahymena pyriformis* contains epinephrine, norepinephrine, and serotonin (1, 2), and that reserpine inhibits the growth of this organism and depletes the cellular catecholamines (3). The growth of these cells is inhibited by phenothiazines (4) and by Segontin, desipramine, tranylcypromine, and aminophylline (5). Reserpine, tranylcypromine, and segontin deplete the cell glycogen content, but aminophylline increases it. The growth rate of *Tetrahymena* is also inhibited by the β-adrenergic blocking agent propanolol and by the α-adrenergic blocking agent dibenzyline. Dichloroisoproterenol and triiodothyronine, at concentrations that have little effect on growth, inhibit the net synthesis of glycogen. On the basis of these observations, it has been suggested that *Tetrahymena* possesses an intracellular adrenergic metabolic regulation system (5).

Tetrahymena resemble liver cells in that they have a high capacity for gluconeogenesis (6) and contain peroxisomes. These organelles, found so far only in liver, kidney, *Tetrahymena* (7), and *Acanthamoeba* (8), contain catalase and oxidases such as D-amino acid oxidase, DL-α-hydroxybutyrate oxidase, and L-lactate oxidase (7). Their physiological function is unknown, but it has been suggested that they play a role in gluconeogenesis (7). Such a role is made more probable by the demonstration that isocitrate lyase and malate synthetase are localized in the peroxisomes of *Tetrahymena* (9). These enzymes circumvent the oxidative decarboxylation steps of the Krebs cycle and permit the net formation of malate from acetyl-CoA, thus permitting gluconeogenesis from endogenous lipid (10) as well as from other endogenous or exogenous sources of acetyl-CoA (11–13). The malate so formed probably enters the glycolytic pathway after conversion to oxalacetate by malate dehydrogenase and conversion of the oxalacetate to phosphoenolpyruvate by phosphoenolpyruvate carboxykinase (14). It is known that growth of *Tetrahymena* in glucose leads to the loss of gluconeogenic capacity (15), the loss of both phosphenolpyruvate carboxykinase activity and malate

dehydrogenase activity (14), thus further substantiating the view that these enzymes play a key role in gluconeogenesis in *Tetrahymena*.

It is well established that, in metazoa, serotonin and the catecholamines are important regulators of glycogen metabolism and of gluconeogenesis (16). In view of the facts presented above, it was of interest to inquire whether the adrenergic metabolic control system of *Tetrahymena* influenced the level of any of the peroxisomal enzymes or of malic dehydrogenase. We have also examined the effect of adrenergically reactive drugs[1] on the level of isocitrate dehydrogenase, the only other enzyme known to utilize isocitrate as a substrate in *Tetrahymena*. In addition to the drugs mentioned above, we have shown that Ro-4-1284, a tetrabenazine-like drug, inhibits the growth of *Tetrahymena* and depletes the cell glycogen. In this paper we report that certain adrenergically reactive drugs and triiodothyronine alter the levels of isocitrate lyase in *Tetrahymena* in a way which correlates with the effect of these drugs on the glycogen content.

MATERIALS AND METHODS

Organism and culture conditions. Tetrahymena pyriformis, strain HSM, were grown axenically in a medium consisting of 1% proteose peptone and 0.05% liver extract in 0.02 *M* potassium phosphate at pH 6.5. Experiments were begun by transferring a stock culture and fresh medium to 1-liter Erlenmeyer flasks with Morton closure tops so that the desired initial cell number was achieved and the volume was 40 ml. To each flask was then added a sterile aqueous solution of the appropriate drug (see reference 5) and water so that the total amount of water in each flask was the same. The final volumes in these experiments usually ranged between 42 and 44 ml, thus assuring a high surface to volume ratio. Two control and four experimental flasks were prepared for each experiment. The cultures were then incubated at 25° without shaking for about 18 hr and counted, and sonic lysates were prepared. Cells were counted with a Coulter counter (Coulter Co., Hialeah, Florida).

Preparation of sonic lysates. After exposure to the drug, a count was taken and the culture was chilled in ice for several minutes. All subsequent steps were done at about 0°. The cultures were centrifuged for 5 min at 500 *g* and the culture medium was discarded. The cells were then resuspended in a buffer consisting of 0.25 M sucrose, 0.0667 M phosphate, pH 7.5, except for experiments in which D-amino acid oxidase activity was to be measured, where the buffer consisted of 0.25 M sucrose and 0.05 M pyrophosphate, pH 8.3. The cells were again centrifuged and washed once again in the buffer solution. After the final centrifugation the cells were resuspended in 5–10 ml of the buffer solution and treated with ultrasound twice for 30 seconds using a model LS-75 Branson Sonifier at setting number 5.

Enzyme assays. The protein content of the sonic lysate was measured by the method of Lowry *et al.* (17). Since the cell density of the washed cell suspension was also measured just prior to treatment with ultrasound, it was possible to compute the amount of protein per 10^6 cells as well as the specific activity of each enzyme assayed.

Glycogen was measured by the method of Dubois *et al.* (18). Since the assay was performed on saline-washed cells, total glucose residues of the cell were actually measured. As discussed elsewhere (5), most of these residues are in the form of glycogen in cells grown in the medium used in these experiments.

Isocitrate dehydrogenase, isocitrate lyase, and malate dehydrogenase were assayed in a silica cuvette of 1-cm path length in a Gilford model 2000 Multiple Sample Absorbance Recorder equipped with Beckman Model DU optics. The sample chamber was maintained at 30° by water circulating through thermospacers, and all reagents and the sonicate to be used were brought to 30° just prior to use.

[1] By adrenergically reactive drugs we mean drugs that are known to affect the metabolism, storage, or action of the catecholamines.

The reaction mixture for isocitrate dehydrogenase assay consisted of 0.2 ml each of the sodium salt of DL-isocitrate, 0.05 M; NADP, 1 mM; $MnCl_2$, 1 mM; and sodium phosphate, pH 8.0, 0.3 M. The reaction was started by adding 0.3 ml of a suitable dilution of the sonicate (diluted with sucrose-phosphate buffer). The absorbance was measured at 340 mμ and increased linearly with time.

Isocitrate lyase activity was measured by the phenylhydrazine method of Kornberg (19). Fresh phenylhydrazine reagent was prepared for each group of three assays. The final reaction mixture contained 0.2 ml of the phenylhydrazine-Tris-Mg-EDTA reagent described by Kornberg, 0.2 ml of 0.0125 M reduced glutathione, and 0.4 ml of sonicate, diluted if necessary with sucrose-phosphate buffer. The reaction was initiated by adding 0.2 ml of 0.05 M sodium isocitrate. As described by Kornberg (19) the reaction (observed as an increase in absorbance at 324 mμ) proceeded at a slow rate for the first 100–200 sec, and then accelerated and became linear with time. The rate of reaction was computed from the linear portion of the curve.

The malate dehydrogenase activity of *Tetrahymena* has been measured by Levy and Scherbaum (11) and by Shrago *et al.* (14). Both authors used the initial rate of change of absorbance per unit time to compute the activity, and both authors commented on the large variability of the assay. In our initial efforts to assay this enzyme in sonic lysates of *Tetrahymena*, it was found that the change in absorbance was not linear with time, but instead obeyed first-order kinetics (Fig. 1A). Furthermore, NADH itself tends to inhibit the reaction (Fig. 1B). This was true, though to different extents, with NADH that had been freshly weighed from desiccated vials as well as with NADH that was weighed from a larger quantity kept frozen. The cause of this inhibition has not been investigated. To achieve maximum reproducibility, the initial NADH concentration was always such that the absorbancy at 340 mμ was between 0.95 and 1.05. The reaction was started by adding NADH to a suitable dilution of the lysate in sucrose-phosphate buffer. The data were plotted on semilogarithmic paper and the first-order rate constant was computed from the initial linear slope.

Catalase was measured by the method of Baudhuin *et al.* (20). The assay was performed at 0°, using 0.1 ml of 2% (v/v)

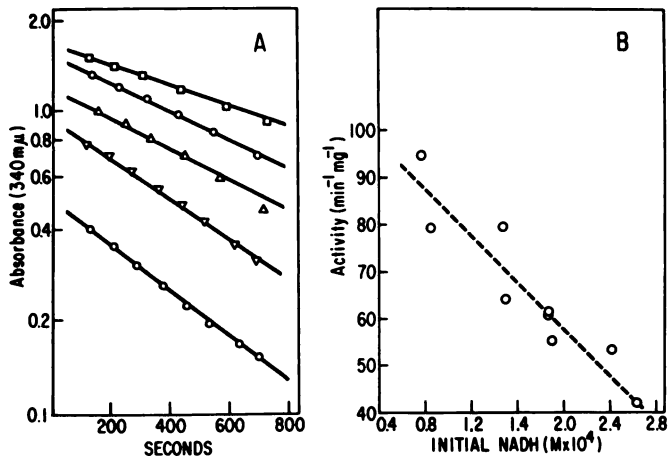

FIG. 1. *Assay of malate dehydrogenase activity*

A stock culture of *Tetrahymena* was collected and treated with ultrasound as described in the text. Part A shows that the enzyme obeys first-order kinetics and that the slope increases with decreasing initial concentration of NADH at constant protein concentration. Part B shows the rates computed from the data of part A and from other assays done at the same time. The initial concentrations of NADH from the uppermost line in part A down, respectively, were: 0.263 mM, 0.186 mM, 0.146 mM, and 0.0764 mM.

Triton X-100 and 0.2 ml of sonicate, diluted with sucrose-phosphate buffer if necessary, and was initiated by the addition of 5 ml of hydrogen peroxide solution (0.16 ml of 30% H_2O_2 per liter of 0.02 *M* imidazole, pH 7.0, containing 10% (w/v) of bovine serum albumin. The reaction was terminated by the addition of 3 ml of titanium sulfate reagent [0.85 g in 1 liter of 2 N H_2SO_4, prepared as described by Baudhuin *et al.* (20)]. The test tube was quickly warmed to room temperature, and the absorbance was measured at 410 mμ. Suitable standards and blanks were prepared. Although the catalase reaction is also first order, the rate constant was computed on the basis of common logarithms and for a volume of 50 ml, in accordance with the procedure of Baudhuin *et al.* (20). To convert the catalase units as reported in the Results section into true first-order constants, the catalase units should be multiplied by 21.7.

D-Amino acid oxidase activity was measured by the method of Verity *et al.* (21). Fluorescence was measured in a Farrand fluorometer, using a 389 mμ interference filter between the lamp and the cuvette and a 504 mμ interference filter between the cuvette and the photomultiplier. The reaction mixture consisted of 0.7 ml of sonic lysate prepared in sucrose-pyrophosphate buffer and 0.2 ml of 0.2 M D-alanine. Incubations were for 1 or 1.5 hr at 30°, and the reaction was terminated by the addition of 0.1 ml of 0.8 N trichloroacetic acid.

None of the enzyme activities were significantly inhibited if any of the drugs used in this study were added to the assay mixture.

Reagents and drugs. Chemical reagents were purchased from the following sources: bovine serum albumin, Armour; phenylhydrazine hydrochloride, Eastman Organic; titanium sulfate, Fisher Scientific Co.; Triton X-100, Rohm and Haas; orthophenylenediamine and dichloroisoproterenol, Aldrich; reduced glutathione, Fluka; NADH, sodium DL-isocitrate, D-alanine, oxalacetate, the ethylenediamine salt of aminophylline, and ethylene diamine, Sigma; NADP, Pabst. All other reagents were the highest purity commercially available.

The drugs used were gifts of the following pharmaceutical companies: desipramine, Geigy; Inderal (propanolol), Ayerst; Catron, Lakeside Laboratories; tranylcypromine and the sodium salt of 1-triiodothyronine, Smith, Kline, and French; Ro-4-1284 (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b-*H*-benzo[*a*]quinolizine), Hoffmann-La Roche; reserpine phosphate, Ciba; Segontin, Hoechst.

RESULTS

Effect of Ro-4-1284 on Growth and Glycogen Content of Tetrahymena

The effects of reserpine, tranylcypromine, Segontin, and desipramine on the growth and glycogen content of *Tetrahymena* have already been described (5). As background for the enzymatic results to be presented below, it is necessary to present the effects of Ro-4-1284 on the growth and glycogen content of *Tetrahymena* at this point. This drug is a benzoquinolizine derivative and is closely related to tetrabenazine. It causes sedation and lowers the serotonin and catecholamine content of the brain, and its effect is counteracted by monoamine oxidase inhibitors such as iproniazid (22). At 0.46 mM there is little inhibition of the growth of *Tetrahymena* for about 10 hr, but then growth ceases (Fig. 2). The growth-inhibitory effect of Ro-4-1284 is synergistic with the growth inhibitory effect of reserpine. A concentration of reserpine which by itself slightly inhibits the growth of *Tetrahymena* greatly enhances the growth-inhibitory effect of Ro-4-1284 (Fig. 2). It can also be seen in Fig. 2 that Ro-4-1284 decreases the glycogen content of *Tetrahymena*. Unlike the synergistic effect of a low concentration of reserpine on the growth inhibition by Ro-4-1284, reserpine did not further decrease the net rate of glycogen synthesis, but instead slightly increased it relative to that of cells treated with Ro-4-1284 alone. Part of the effect of reserpine plus Ro-4-1284 on the glycogen

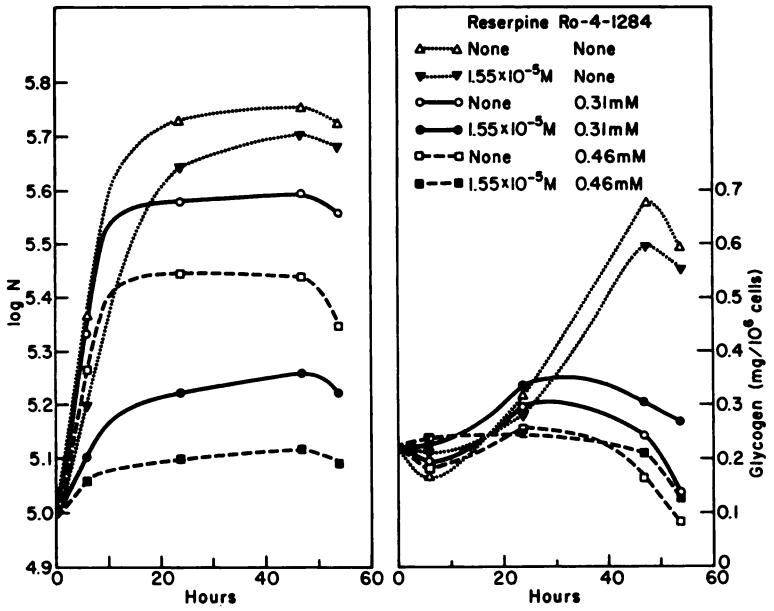

FIG. 2. *Effect of Ro-4-1284 and of reserpine on growth and glycogen content of Tetrahymena*

At zero time, 5 ml of water containing drugs at the indicated concentrations were added to 25 ml of cells in proteose–peptone–liver extract medium. The cultures were in 300-ml Erlenmeyer flasks and were incubated without shaking at 25°. At times shown on the abscissas, samples were taken for measurement of cell count and of glycogen content.

content of the cells may, however, have been a secondary result of the increased availability of oxygen as a result of the much lower cell density. It is clear, however, that although reserpine greatly enhanced the growth inhibitory effect of Ro-4-1284, it did not enhance the effect of Ro-4-1284 to inhibit net glycogen synthesis.

Effect of Adrenergically Reactive Drugs on Isocitrate Dehydrogenase of Tetrahymena

As shown in Tables 1 and 2, none of the drugs tested altered the isocitrate dehydrogenase activities of *Tetrahymena* with the possible exception of desipramine at the highest concentration tested, where a 20% loss of activity was observed. In data not shown here it was also established that triiodothyronine at 1.8×10^{-5} M did not affect the isocitrate dehydrogenase activity. At this concentration triiodothyronine generally causes a slight growth inhibition and an appreciable decrease in the level of cell glycogen (5).

Effect of Adrenergically Reactive Drugs on Catalase Activity of Tetrahymena

Exposure of *Tetrahymena* to desipramine, tranylcypromine, Segontin, or Ro-4-1284 for about 18 hours caused small increases in catalase activity (Tables 1 and 2). Aminophylline did not change the catalase activity (Table 2), but reserpine at high concentrations decreased the catalase activity by about 45% (Table 1). In an experiment not shown there was no effect of triiodothyronine, dichloroisoproterenol, or propanolol (at concentrations comparable to those used in Table 3) on the catalase activity of *Tetrahymena*.

Effect of Adrenergically Reactive Drugs on Isocitrate Lyase Activity of Tetrahymena

Segontin, desipramine, tranylcypromine, and Ro-4-1284 strongly decreased the isocitrate lyase activity of *Tetrahymena* (Tables 1 and 2). Reserpine had a relatively smaller effect on this enzyme activity, decreasing it by about 40% in contrast to

TABLE 1

Effects of reserpine, Segontin, desipramine, and tranylcypromine on isocitrate dehydrogenase, catalase, and isocitrate lyase activities of Tetrahymena

In each experiment two control and four experimental flasks were prepared. The drugs were added about 17 hr before the cells were collected. N_i and N_f are the cell densities at the time of addition of the drugs and at the time the cells were collected, respectively. The isocitrate lyase assays were performed in groups of three; i.e., in the reserpine experiment the sonic lysates which had activities of 1.39, 1.33, and 1.19 μmoles/hr·mg were assayed together, and about a half hour later the sonic lysates which had activities of 1.06, 0.83, and 1.27 μmoles/hr·mg were assayed, using a freshly prepared reaction mixture. For further details, see the section on methods.

Concentration (M × 10^4)	N_i (cells/ml × 10^{-3})	N_f (cells/ml × 10^{-3})	N_f/N_i	Isocitrate dehydrogenase (μmoles/min·mg)	Catalase (min^{-1} mg^{-1})	Isocitrate lyase (μmoles/hr·mg)	Protein (mg/10^6 cells)
			Reserpine				
None	17.4	119	6.8	0.40[a]	0.122	1.39	1.12
0.20	34.7	109	3.1	0.39[a]		1.33	1.17
0.27	52.1	109	2.1	—	0.125	1.19	1.24
0.34	69.5	102	1.5	0.36[a]	0.110	1.06	1.27
0.41	86.9	100	1.2	—	0.068	0.83	1.29
None	17.4	113	6.5	—	0.139	1.27	1.13
			Segontin				
None	13.8	214	15.5	0.37	0.118	1.34	1.38
0.071	27.6	198	7.2	0.36	0.133	0.97	1.55
0.11	41.4	191	4.6	0.32	0.145	0.73	1.71
0.14	55.2	200	3.6	0.39	0.132	0.50	1.78
0.18	69.0	187	2.7	0.35	0.114	0.40	1.75
None	13.8	181	13.1	0.39	0.108	1.14	1.47
			Desipramine				
None	21.3	221	10.4	0.40	0.108	1.24	1.28
0.36	21.3	163	7.7	0.41	0.120	0.93	1.22
0.50	28.4	165	5.8	0.43	0.120	0.81	1.25
0.65	42.7	134	3.1	0.42	0.118	0.44	1.39
0.79	56.9	110	1.9	0.34	0.100	0.41	1.46
None	21.3	208	9.8	0.40	0.106	1.17	1.51
			Tranylcypromine				
None	20.3	234	11.5	0.43	0.126	1.31	1.39
4.2	40.5	238	5.9	0.42	0.134	0.97	1.51
6.3	60.8	278	4.6	0.43	0.142	0.66	1.59
8.3	81.0	274	4.5	0.43	0.133	0.64	1.70
10.4	20.3	276	3.4	0.42	0.134	0.49	1.69
None		208	10.2	0.42	0.121	1.26	1.39

[a] Data on isocitrate dehydrogenase were obtained in a different experiment.

the 2- to 3-fold reductions caused by the above-mentioned drugs (Table 1). Aminophylline, even at concentrations which completely inhibited growth, slightly increased the isocitrate lyase activity (Table 2). In another experiment identical to that shown in Table 2, the increase in isocitrate lyase activity caused by aminophylline was about 20%. Dichloroisoproterenol and triiodothyronine, at concentrations where growth was only slightly inhibited, significantly reduced the isocitrate lyase activity (Table 3). When the cells were exposed to both drugs, there was a small further decrease in activity (Table 3). Propanolol at 1.53×10^{-4} M reduced the isocitrate lyase content

TABLE 2

Effects of aminophylline and of Ro-4-1284 on malate dehydrogenase catalase, and isocitrate lyase activities of Tetrahymena

Each flask in the aminophylline experiment contained 2.09 mM ethylenediamine. Cells were exposed to the drugs for about 18 hr. For further details, see legend to Table 1.

Concentration (M × 10^4)	N_i (cells/ml × 10^{-3})	N_f (cells/ml × 10^{-3})	N_f/N_i	Malate dehydrogenase (min^{-1} mg^{-1})	Catalase (min^{-1} mg^{-1})	Isocitrate lyase (μmoles/ hr·mg)	Protein (mg/10^6 cells)
			Aminophylline				
None	14.4	75.0	5.2	25.0	0.119	1.30	1.50
1.05	28.8	44.7	1.6	35.4	0.118	1.74	2.07
1.40	43.2	49.9	1.2	35.3	0.116	1.60	2.47
1.75	57.7	58.1	1.0	33.6	0.110	1.54	2.64
2.09	72.1	73.8	1.0	38.1	0.108	1.49	2.59
None	14.4	70.5	4.9	21.7	0.115	1.56	1.60
			Ro-4-1284				
None	17.1	236	13.8	31.4	0.108	1.99	1.46
0.21	25.6	262	10.2	37.0	0.120	1.03	1.45
0.27	34.2	261	7.6	38.8	0.120	0.82	1.44
0.32	42.7	250	5.8	36.9	0.116	0.58	1.23
0.38	51.2	248	4.8	39.9	0.116	0.50	1.30
None	17.1	223	13.1	34.1	0.102	1.68	1.46

of *Tetrahymena* almost 50% in the experiment shown in Table 3. In another experiment, 0.11 mM propanolol reduced the activity by 30%.

Since it is known that epinephrine can change the activity of muscle phosphorylase within a minute (23) it was of interest to ascertain the time course of the reduction of isocitrate lyase activity by one of these drugs. An experiment was therefore performed in which 20 ml of culture at a density of 220,000 cells/ml was transferred to each of a series of 500-ml Erlenmeyer flasks. Segontin (1.78×10^{-5} M) was added to the experimental flasks at zero time. Cells were collected from the control and experimental flasks and sonicated at 0.5, 2.5, and 6.0 hr after the addition of Segontin, and the isocitrate lyase activity was measured. The control activities decreased

TABLE 3

Effect of triiodothyronine, dichloroisoproterenol, and propanolol on D-*amino acid oxidase, malate dehydrogenase, and isocitrate lyase of Tetrahymena*

Cells were exposed to the drugs for about 22 hr. For further details, see legend to Table 1.

Drug M × 10^4	N_i (cells/ml × 10^{-3})	N_f (cells/ml × 10^{-3})	N_f/N_i	Malate dehydrogenase (min^{-1} mg^{-1})	D-Amino acid oxidase (μmoles/ hr·mg)	Isocitrate lyase (μmoles/ hr·mg)	Protein (mg/10^6 cells)
None	19.2	276	14.3	34.3	0.086	2.16	1.71
DCI, 1.36	19.2	259	13.5	34.7	0.085	1.75	1.66
DCI, 1.36 + T_3, 0.18	19.2	232	12.1	33.7	0.080	1.28	1.55
T_3, 0.18	19.2	256	13.3	30.1	0.072	1.42	1.58
Propanolol, 1.53	38.4	186	4.8	35.8	0.089	1.15	1.90
None	19.2	278	14.5	31.5	0.066	1.98	1.69

TABLE 4

Effect of reserpine, aminophylline, Segontin, and desipramine on D-*amino acid oxidase and malate dehydrogenase on Tetrahymena*

Cells were exposed to the drugs for about 18 hr. For further details, see legend to Table 1.

Expt. no.	Drug (M × 10^4)	N_i (cells/ml × 10^{-3})	N_f (cells/ml × 10^{-3})	N_f/N_i	Malate dehydrogenase (min^{-1} mg^{-1})	D-Amino acid oxidase (μmoles/hr·mg)	Protein (mg/10^6 cells)
I	none[a]	23.2	116	5.0	38.5	0.098	1.40
	aminophylline[a], 10.6	58.0	87	1.5	22.3	0.091	1.72
	aminophylline[a], 17.6	87.1	105	1.2	29.3	0.071	2.14
	reserpine	58.0	223	3.8	30.4	0.069	1.68
	reserpine	87.1	111	1.3	41.0	0.064	1.73
	none	23.2	278	12.0	32.6	0.076	1.66
II	none	21.1	224	10.6	22.0	0.099	1.59
	Segontin, 0.14	42.2	178	4.2	24.6	0.087	1.69
	Segontin, 0.18	63.4	172	2.7	28.3	0.099	1.66
	desipramine, 0.64	42.3	152	3.6	22.9	0.104	1.42
	desipramine, 0.79	63.4	144	2.3	30.9	0.100	1.45
	none	21.1	216	10.2	19.8	0.094	1.57

[a] The concentration of ethylenediamine was 1.76 mM in these cultures.

from 1.75 to 1.35 to 1.17 μmoles/hr·mg [presumably because of the increased aeration (13)] at these times while the isocitrate lyase activity of the Segontin-treated cells decreased from 1.75 to 1.29 to 0.85 μmoles/hr·mg at these times. Thus the decrease of isocitrate lyase activity caused by Segontin is not a fast process.

Effects of Adrenergically Reactive Drugs on Malate Dehydrogenase and D-*Amino Acid Oxidase Activities of Tetrahymena*

There were no significant changes in either of these two enzyme activities with any of the drugs tested (Tables 3 and 4).

Effect of Adrenergically Reactive Drugs on the Protein Content of Tetrahymena

Ro-4-1284, dichloroisoproterenol, triiodothyronine, desipramine, and reserpine did not alter the protein content of *Tetrahymena* (Tables 1–4). Tranylcypromine, Segontin, and propanolol increased the protein content by small but significant amounts, and aminophylline consistently increased the protein content by over 50% (Tables 2 and 4).

DISCUSSION

The work of Rogers (4), showing that growth and glucose metabolism of *Tetrahymena* are inhibited by phenothiazines, and the present finding that a tetrabenazine-like drug (Ro-4-1284) inhibits the growth and depletes the glycogen content of *Tetrahymena* further support the view (5) that these protozoa contain an intracellular metabolic control system with many features similar to those found in mammals. Since *Tetrahymena* contain both epinephrine and serotonin (1, 2) and since some of the drugs used in this study deplete the content of both amines in mammalian systems, it is not clear whether *Tetrahymena* have an adrenergic system only or both an adrenergic and a serotoninergic system. For brevity, we shall refer throughout this discussion to the adrenergic control system of *Tetrahymena* without, however, implying the absence of other control systems.

Although much remains to be learned about the control of gluconeogenesis in *Tetrahymena*, several important observations have recently been made. Levy and

Scherbaum have observed that the transfer of cells from aerobic to partially anaerobic conditions results in a doubling of the isocitrate lyase and malate synthetase activities within 3 hr, but no change in the activity of acetyl-CoA synthetase or in that of malate dehydrogenase (11). These authors have also noted that the change from aerobic to partially anaerobic conditions leads to an increased rate of gluconeogenesis within 3 hr (12). It was later shown that although the increase in isocitrate lyase activity can be partially or completely prevented by inhibition of protein synthesis by actinomycin D or by puromycin, these inhibitors do not prevent the increase in gluconeogenesis (24). Thus gluconeogenesis in *Tetrahymena* can be activated in the absence of enzyme synthesis. Hogg and Kornberg (10) had previously shown that in *Tetrahymena* with a high capacity for gluconeogenesis the isocitrate lyase was present in a particulate fraction [now known to be the peroxisomes (9)], but in cells with a low capacity for gluconeogenesis the isocitrate lyase was not bound to particles. Similarly, Harrop and Kornberg (25) showed that a strain of *Chlorella* contained an active isocitrate lyase under all conditions of growth but that the enzyme participated in the glyoxylate cycle only when it was bound to a particulate structure. For a number of reasons, therefore, it is probable that the peroxisomes are involved in gluconeogenesis, as first suggested by de Duve and Baudhuin (7).

The present experiments show that the isocitrate lyase activity of *Tetrahymena* is decreased by several adrenergically reactive drugs. The process is a slow one, and presumably represents a repression of synthesis and a loss of activity as a result of turnover, although it is also possible that enzyme inhibition occurs and is not relieved by sonication of the cells. Regardless of the exact mechanism of the loss of activity, a remarkable correlation exists between the effects of the adrenergic drugs on the glycogen content of *Tetrahymena* and their effect on isocitrate lyase activity. Thus reserpine, which tends to deplete cell glycogen to a smaller extent than Segontin or desipramine, causes a smaller inhibition of isocitrate lyase activity than do Segontin or desipramine, while aminophylline, which increases the glycogen content of *Tetrahymena* (5) does not decrease the isocitrate lyase activity. Triiodothyronine and dichloroisoproterenol individually depress the isocitrate lyase, and together depress it slightly more. Individually they depress the net rate of glycogen synthesis and together they depress it more.

It is important to stress that these effects are not caused by growth inhibition or by changes in aeration. Thus aminophylline at concentrations that completely inhibited growth caused a slight activation of isocitrate lyase activity, while dichloroisoproterenol and triiodothyronine, which scarcely inhibit growth, decreased the isocitrate lyase activity by almost half. Furthermore, ethylenediamine itself in a few experiments caused an appreciable inhibition of growth but did not affect the isocitrate lyase activity. Although the inhibition of growth does not of itself cause a decrease in the level of isocitrate lyase activity, the data generally show a correlation between the reduction of isocitrate levels and the reduction of growth rate for the drugs tested other than aminophylline. Presumably the inhibition of growth and the reduction of isocitrate lyase level are secondary to some other process which is altered by the adrenergically reactive drugs. Since Levy has shown that growth conditions are of crucial importance in determining the isocitrate lyase activity in *Tetrahymena* (13), these experiments were all done with very large surface to volume ratios, thus ensuring aerobic growth conditions. That the degree of aeration was not a determining variable in these experiments can be seen in several ways. First, in the experiments with tranylcypromine (Table 1) and Ro-4-1284 (Table 2) the cell density was slightly lower in the control flasks than in the flasks containing the drug, but the control cells had much higher isocitrate lyase activities. Second, cells treated with 7.9×10^{-5} M desipramine achieved a density of 110,000 cells/ml (Table 1) and had very low isocitrate lyase activities, while in the experiment with

reserpine (Table 1) control cells at the same density had much higher isocitrate lyase activities. Third, the experiments were generally designed so that flasks containing growth inhibiting drugs were inoculated at a higher cell density than the control flasks, and thus had a higher cell density during most of the growth period. To the extent that anaerobiasis played a role, therefore, it should have increased the isocitrate lyase activity in the flasks containing the drugs. This argument might be invalid if the drugs caused a large inhibition of respiratory rate. Preliminary experiments (J. P. Wexler, unpublished data), however, indicate that neither reserpine nor triiodothyronine appreciably alter the rate of oxygen consumption in short-term experiments.

It should also be pointed out that none of the drugs decreased the protein content of these cells, so that the large decreases in isocitrate lyase activity cannot be explained by a general loss of protein. An interesting observation is that aminophylline considerably increased the protein content while preventing cell division. Since the isocitrate lyase activity was only slightly increased (and, in any case, probably represents a very small fraction of cell protein) and aminophylline did not alter the activity of several other enzymes, it is clear that not all proteins increased, and that aminophylline therefore selectively increased the content of certain proteins. It would be of considerable interest to establish the identity of those proteins.

Since a plausible action of the adrenergic drugs might be to alter the balance between glycogen synthesis and glycogenolysis at the level of phosphorylase and glycogen synthetase, it might be argued that the effect of the drugs on isocitrate lyase activity was simply a result of an increased level of glycolytic pathway metabolites within the cell. It is known that glucose decreases the isocitrate lyase activity in static cultures of *Tetrahymena* (10, 13) and blocks gluconeogenesis (15). Shrago *et al.* (14) have shown that glucose decreases the phosphoenolpyruvate carboxykinase, malate dehydrogenase, and fructose diphosphatase activities of *Tetrahymena*. The present experiments show that the decrease in isocitrate lyase activity caused by the adrenergically active drugs is not equivalent to glucose repression, since the adrenergic drugs did not decrease the malate dehydrogenase activity.

Although the adrenergically reactive drugs altered the isocitrate lyase activity in a way which correlates with their effect on cell glycogen content, none of the drugs tested altered the D-amino acid oxidase activity. Reserpine at high concentrations decreased the catalase activity, but the other drugs tested either had no effect on catalase or slightly increased it. Thus the peroxisomal enzymes can vary separately, as had already been noted by Levy and Hunt (15) and by Blum and Wexler (26). The present results indicate that the adrenergic control system of *Tetrahymena* influences a peroxisomal enzyme which acts at the first step of gluconeogenesis from acetyl-CoA. Further experiments are required to ascertain whether the adrenergically reactive drugs also control the activity of glycogen synthetase or phosphorylase in *Tetrahymena*.

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REFERENCES

1. K. Janakidevi, V. C. Dewey and G. W. Kidder, *J. Biol. Chem.* **241**, 2576 (1966).
2. K. Janakidevi, V. C. Dewey and G. W. Kidder, *Arch. Biochem. Biophys.* **113**, 758 (1966).
3. J. J. Blum, N. Kirshner and J. Utley, *Mol. Pharmacol.* **2**, 606 (1966).
4. C. G. Rogers, *Can. J. Biochem.* **44**, 1493 (1966).
5. J. J. Blum, *Proc. Natl. Acad. Sci. U.S.* **58**, 81 (1967).
6. J. F. Ryley, *Biochem. J.* **52**, 483 (1952).
7. C. de Duve and P. M. Baudhuin, *Physiol. Rev.* **46**, 323 (1966).
8. M. Müller and K. M. Møller, *J. Protozool.* **14**, Suppl, 11 (1967).
9. M. Müller and J. F. Hogg, *Federation Proc.* **26**, 284 (1967).

10. J. F. Hogg and H. L. Kornberg, *Biochem. J.* **86**, 462 (1963).
11. M. R. Levy and O. H. Scherbaum, *Arch. Biochem. Biophys.* **109**, 116 (1965).
12. M. R. Levy and O. H. Scherbaum, *J. Gen. Microbiol.* **38**, 221 (1965).
13. M. R. Levy, *Comp. Biochem. Physiol.* **21**, 291 (1967).
14. E. Shrago, W. Brech and K. Templeton, *J. Biol. Chem.* **244**, 4060 (1967).
15. M. R. Levy and A. E. Hunt, *J. Cell Biol.* **34**, 911 (1967).
16. J. S. Hagen, *Pharmacol. Rev.* **19**, 367 (1967).
17. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
18. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.* **28**, 350 (1956).
19. H. L. Kornberg, "Régulations chez les microorganismes," p. 193. C.N.R.S., Paris.
20. P. Baudhuin, H. Beaufay, Y. Rahman-Li, O. Z. Sellinger, R. Wattiaux, P. Jacques and C. de Duve, *Biochem. J.* **92**, 179 (1964).
21. M. A. Verity, R. Gallagher and W. J. Brown, *Biochem. J.* **103**, 375 (1967).
22. A. Pletscher, A. Brossi and K. F. Gey, *Intern. Rev. Neurobiol.* **4**, 275 (1962).
23. E. W. Sutherland and G. A. Robison, *Pharmacol. Rev.* **18**, 145 (1966).
24. M. R. Levy, *J. Cell. Physiol.* **69**, 247 (1967).
25. L. C. Harrop and H. L. Kornberg, *Proc. Roy. Soc.* **B166**, 11 (1966).
26. J. J. Blum and J. P. Wexler, *Mol. Pharmacol.* **4**, 155 (1968).