The Effect of Reserpine on Growth and Catecholamine Content of *Tetrahymena*

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SUMMARY

Reserpine inhibits the growth of *Tetrahymena* and reduces the content of epinephrine and of norepinephrine of these cells. The growth inhibitory effect is partially reversed by glucose.

Recently Janakidevi *et al.* (1) reported that both epinephrine and norepinephrine were present in *Tetrahymena pyriformis* W. This observation prompted us to examine the possibility that these hormones could be depleted by reserpine. *Tetrahymena pyriformis* HSM were grown axenically at 25° in a medium consisting of 1% proteose-peptone and 0.05% liver extract in 0.02 M KH_2PO_4, pH 6.5. Reserpine phosphate (a gift of the Ciba Company) was dissolved in H_2O and sterilized by filtration. Cell counts were made with a Coulter Counter (Coulter Co., Hialeah, Florida), after suitable dilution with an appropriate salt solution. The growth of these protozoa was found to be exquisitely sensitive to reserpine, slight inhibition occurring at 1.2×10^{-5} M and complete cessation of growth occurring at 3.5×10^{-5} M (Fig. 1). Microscopic examination showed that cells treated with reserpine were smaller and more rounded than control cells and moved much less rapidly. Since one of the major functions of the catecholamines, in mammalian systems at least, is to control glycogenolysis via the adenyl cyclase system (2), an experiment was performed to test whether the addition of glucose to the medium would alter the growth inhibitory potency of reserpine. It can be seen in Fig. 1 that whereas 20 mM glucose has little if any effect on the inhibition of growth caused by 1.2×10^{-5} M reserpine, there is a significant reversal of the growth inhibition caused by higher concentrations of reserpine. Although these observations were consistent with the view that reserpine inhibited the growth of *Tetrahymena* by interfering with the hormonal regulation of metabolism, it was desirable to ascertain whether reserpine depleted the catecholamines from these protozoa, since in mammalian systems, a major effect of reserpine is to deplete catecholamines from storage sites within the cells (3). For this purpose, cells were grown in 6.5 l of medium containing 0.5 ml Dowex Corning Antifoam B per liter, with continuous stirring and with air bubbled through the culture. When the cells reached 260,000 per milliliter, about 4 l was collected by continuous flow centrifugation at 1100 *g*, washed with 200 ml of 0.5% NaCl, resuspended in 0.5% NaCl, and counted; a known number of cells were

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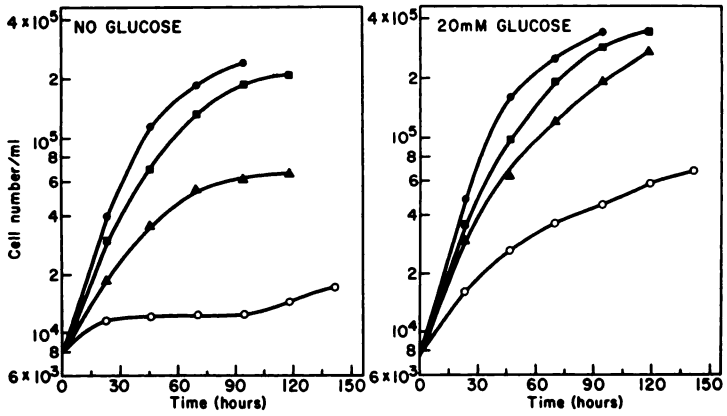

FIG. 1. *Effect of reserpine on the growth of Tetrahymena*

Cells were inoculated at zero time into fresh media from a logarithmically growing stock and reserpine was added immediately. The concentrations of reserpine used were: no reserpine, ●—●; 1.2×10^{-5} M, ■—■; 2.3×10^{-5} M, ▲—▲; 3.5×10^{-5} M, ○—○.

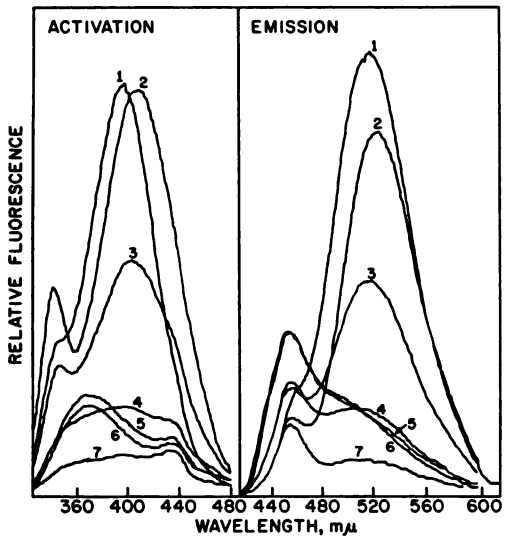

FIG. 2. *Fluorometric analysis of catecholamines in Tetrahymena*

Trihydroxyindoles were formed by the method of von Euler and Lishajko (4). Curve *1*, norepinephrine; *2*, epinephrine; *3*, extract of *Tetrahymena* grown in absence of reserpine; *4*, extract of *Tetrahymena* grown in presence of 2.1×10^{-5} M reserpine; *5*, faded blank of *4*; *6*, faded blank of *3*; *7*, faded blank of *1* and *2*. Activation wavelength for emission spectra was 390 mμ. Fluorescent wavelength for activation spectra was 520 mμ. Epinephrine and norepinephrine were present in the reaction mixture at a final concentration of 0.02 μg/ml.

suspended in ice cold 5% trichloroacetic acid and stored at —20°. To the remaining culture about 900 ml of fresh medium and freshly dissolved reserpine phosphate in 125 ml H_2O was added to yield 2.1×10^{-5} M. After 16 hours of exposure to this concentration of reserpine, the cells were collected and treated as described above. Aliquots of the trichloroacetic acid extracts were absorbed on alumina and assayed for catecholamines by the method of von Euler and Lishajko (4). Activation and emission spectra were obtained (Fig. 2). Treatment with reserpine reduced the norepinephrine from 0.45 to <0.05 μg/10^9 cells and reduced the epinephrine from 3.75 to 0.29 μg/10^9 cells. It should be noted that we find that the ratio of epinephrine to norepinephrine in *T. pyriformis* HSM is about 8:1 whereas Janakidevi *et al.* reported a ratio of about 1:2. Whether this difference is due to the different media used or is a strain difference remains to be determined. It is clear, however, that reserpine greatly reduced the content of both epinephrine and norepinephrine in *Tetrahymena*. Because *Tetrahymena* can be grown axenically in mass cultures in chemically defined media it may be a very useful system to study the role of the cate-

cholamine hormones in the regulation of metabolism and to study the detailed mechanism of action of reserpine.

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Formation of a Dead Code Triplet through Replacement of the Terminal Ribonucleoside in Guanylyl-Uridylyl-Uridine and Guanylyl-Uridylyl-Cytidine by 6-Azacytidine

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SUMMARY

The binding of valyl-tRNA to ribosomes *in vitro*, stimulated by guanylyl-uridylyl-uridine and guanylyl-uridylyl-cytidine is not effected by guanylyl-uridylyl-6-azacytidine. This finding indicates the high chemical specificity of the pyrimidine components of the codon.

Some 6-azapyrimidine derivatives belong among effective inhibitors of the metabolism of nucleic acid components (1). 6-Azauridine 5′-monophosphate (2) and 6-azacytidine 5′-monophosphate (3) are potent inhibitors of orotidylic acid decarboxylase, 6-azauridine 5′-diphosphate inhibits polynucleotide phosphorylase (4), and 6-azauridine 5′-triphosphate inhibits RNA polymerase (5). It was found in the course of the study of the biological effects of 6-azapyrimidine derivatives that 6-azacytidine 5′-diphosphate, in contrast with 6-azauridine 5′-diphosphate, can be incorporated *in vitro* into polyribonucleotides with the aid of polynucleotide phosphorylase (6).

It was considered to be important to establish the consequences for the genetic code of replacing a pyrimidine base by 6-azacytosine in the active code triplet. To this end we used a comparative study of the coding properties of trinucleotides in a cell-free system (7). Under the given conditions it was suitable from the experimental point of view to apply triplets of guanylyl-uridylyl-uridine and guanylyl-uridylyl-cytidine which code for valine (8, 9) and to compare their coding properties with those of a triplet containing the 6-aza analog of the pyrimidine base.

For preparing the trinucleotides we used the specific enzyme reaction of guanosine