Localization of Acetylcholinesterase Activity in the Protozoan, *Tetrahymena geleii* S. (18425)

GERALD R. SEAMAN.

From the Carter Physiology Laboratory, University of Texas Medical Branch, Galveston, Tex.

The presence of a specific acetylcholinesterase (AChE) in *Tetrahymena* has been demonstrated(1). It was shown that specific inhibition of the activity of the enzyme results in decreased motility of the organism. The suggestion was offered that the fibrillar structure in the pellicle of the ciliate serves as a primitive conducting mechanism analogous to nerve fibers, and as such, is dependent upon AChE activity for function. If this be true, all of the AChE activity of the cell should be localized in the pellicle, in the same fashion that the total activity of this enzyme is localized in the sheath of nerve fibers(2).

Methods. Cells of *Tetrahymena geleii* S were grown in pure culture, and were harvested and washed as described previously(1). The resulting cell concentrate was homogenized in 0.25 M sucrose with a Potter-Elvehjem grinder. The homogenate was centrifuged at 600 X *g* for 10 minutes. The supernatant, fraction S_1, was removed and the sediment washed twice by rehomogenizing, each time with 0.25 M sucrose, and centrifuging at 600 X *g* for 10 minutes. The supernatants were removed and added to fraction S_1. At this point, only nuclei, pellicular fragments and a few unbroken cells are contained in the sediment. Phase contrast examination (bright contrast) of this fraction reveals none of the cytoplasmic inclusions described for *Tetrahymena*(3). A force of 600 X *g* is obviously not sufficiently great to separate the small cytoplasmic particles; they are retained in fraction S_1. The residue was then suspended in 0.25 M citric acid and allowed to stand for 15 minutes. The mixture was then centrifuged at 600 X *g* for 10 minutes. The nuclei remain in the supernatant while pellicular material is sedimented. The supernatant was removed and was labeled fraction N. The sediment was resuspended by homogenizing with a pinch of quartz in a buffer consisting of 0.05 M Na_2HPO_4, 0.1 M NaCl, 0.02 M $MgCl_2$ (pH 7.0). This procedure brings the AChE into solution(4). The mixture was then centrifuged at 1600 X *g* for 20 minutes to remove all particulate matter. The supernatant, fraction S_2, contained no microscopically visible particles. The entire fractionation procedure was carried out at 4°C. AChE activity of each fraction was estimated by measuring the rate of removal of added acetylcholine (ACh). ACh was determined by the colorimetric method of Hestrin(5). Aliquots of the original homogenate were dried to constant weight at 80°C and the enzymatic activity was expressed as Q_{AChE}, μg ACh split/hour/mg dry weight of original homogenate.

Results and discussion. Table I shows that all the AChE in *Tetrahymena* is contained in fraction S_2 (the pellicular fraction). No significant esterase activity was obtained with the first supernatant, S_1, or the residue, fraction R.

TABLE I. Distribution of AChE Activity in Centrifugal Fractions of *T. geleii* S. Figures in parentheses indicate range obtained in 7 determinations. Figures in *italics* mean of values obtained.

Q_{AChE}	Fraction
(.067-.082) *.078*	Entire homogenate
(.002-.006) *.003*	S_1
(.043-.076) *.072*	S_2
(.001-.003) *.003*	N
(.000-.003) *.002*	R

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These results thus lend support to the hypothesis(1) that conduction along the fibrillar structure which connects the base of individual cilium in the pellicle of the organism is similar to conduction along nerve fibers, and is dependent upon AChE activity for function(6).

Attempts were made to localize, by histochemical methods, the AChE activity of *Tetrahymena*. It was thought that such procedures would visualize a pattern on the pellicle which would be identical with the pattern of the fibrillar system as revealed by the silver technic(7). Cells were therefore treated according to the procedures for histochemical localization of AChE as described by Gomori(8) and as by Koelle(9). The stain however, in all cases, diffused to a great extent and became located in cytoplasmic granules, which actually contained no AChE activity (Table I).

Summary. AChE activity in the ciliate *Tetrahymena geleii* S is localized in the fibrillar system of the pellicle. Upon differential centrifugation, a fraction was obtained which contained only enzymatic activity from the pellicle. This fraction contained the entire amount of AChE activity of the cell.

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Implantation of Ureters into Completely Isolated Loops of Small Intestine.* (18426)

WALTER L. MERSHEIMER, ALBERT J. KOLARSICK, AND HENRY KAMMANDEL.
(Introduced by Israel S. Kleiner.)

From the Department of Surgery, New York Medical College and Flower and Fifth Avenue Hospitals.

The problem of constructing an artificial urinary bladder has become increasingly more urgent because of the performance of large numbers of radical operations, particularly pelvic viscerectomy.

Reports have appeared concerning the use of partially or completely isolated loops of small intestine to serve as a urinary reservoir (1-4). Our method of performing ureteroileal-cutaneous implantation has not previously been reported and our investigation would indicate that it is superior to the older technics.

The procedure we have successfully performed is as follows: With the dog under intravenous sodium pentothal anesthesia, a left rectus muscle splitting incision is used to enter the abdomen. A loop of terminal ileum approximately 10 cm in length is isolated and the continuity of the small intestine is restored by end to end anastomosis. The proximal end of the isolated ileal loop is closed by the Parker-Kerr technic. The ureters are transected close to the bladder and the distal ends ligated. The ureters are implanted through the same seromuscular tunnel in the antimesenteric border of the isolated loop of the intestine. The distal open end of the

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