NONLETHAL DECILIATION OF *TETRAHYMENA* BY A LOCAL ANESTHETIC AND ITS UTILITY AS A TOOL FOR STUDYING CILIA REGENERATION

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INTRODUCTION

Several reports have described the removal of cilia or flagella from protozoan cells under conditions sufficiently mild to preserve cell viability and permit the subsequent analysis of the locomotor organelles (10–12). The most useful procedures for ciliates involve the sequestration of divalent cations by a chelating agent, followed by the addition of relatively high concentrations of calcium ions (11). This technique requires meticulously controlled conditions and, in our hands, produces fragile cells whose integrity is difficult to preserve. We report here an alternative and more convenient method for the nonlethal

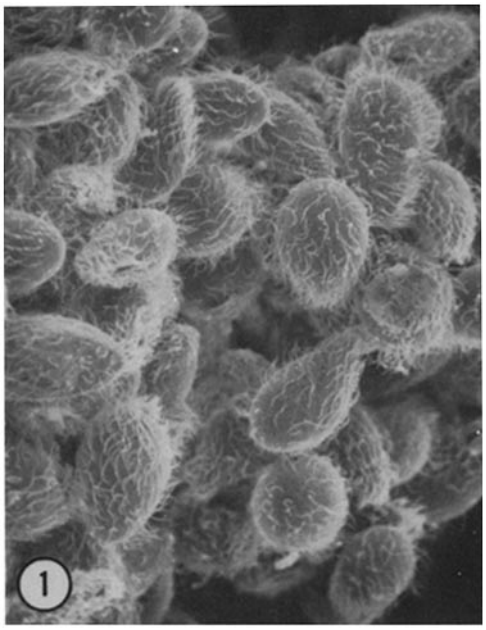

FIGURE 1 Normal cells before dibucaine treatment. × 1,250.

deciliation of *Tetrahymena pyriformis* using the local anesthetic dibucaine.

MATERIALS AND METHODS

Unless otherwise stated, *T. pyriformis* strain WH-14[1] was grown to a cell density of 3–5 × 10^5 cells as previously described (14). Cultures to be deciliated were centrifuged for 5 min at 1,500 rpm (365 *g*) in the GSA rotor of a Sorvall centrifuge (Ivan Sorvall, Inc., Newtown, Conn.) at room temperature. The cells were resuspended in 10 ml of their own medium; and 0.55 ml 25 mM dibucaine HCl (Nupercaine, Ciba Pharmaceutical Company, Division of Ciba-Geigy Corporation, Summit, N. J.), was added with mixing. Cilia were almost completely detached within 3–5 min, particularly if the process was assisted by drawing the suspension in and out of a large bore (0.4–0.6 mm inside diameter aperture) pipette several times. When all cell motility was lost, as determined by phase-contrast microscopy, 20 ml of fresh medium was added to the deciliated cell suspension, and it was recentrifuged at 1,500 rpm for 5 min. Practically every cilium remaining attached was lost during resuspension of the cell pellet in 180 ml of fresh medium.

The radioisotope studies utilized procedures previously described for [^{14}C]palmitate (14) and [^{3}H]-leucine (3). [1-^{14}C]Palmitate (54 mCi/mmol) was purchased from New England Nuclear, Boston, Mass., and L-[4,5-^{3}H]leucine (50–60 Ci/mmol) was obtained from the Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y. Dibucaine HCl was kindly provided by Dr. E. Grob of the Ciba Pharmaceutical Company. Electron microscopy was performed using a model 1000 A.M.R. scanning electron microscope, made available through the courtesy of Dr. Garry Cole, University of Texas. The cells were prepared for observation by a critical-point drying method followed by deposition of gold/palladium under vacuum (7).

RESULTS AND DISCUSSION

Dibucaine was tested in growth medium at concentrations of 0.75, 1.0, 1.1, 1.3, 2.3, and 6.0 mM. Although some deciliation was observed at all concentrations, 1.3 mM was the lowest concentration that produced a thorough and almost immediate loss of cilia. Oral cilia were often lost as a unit. Figs. 1 and 2 illustrate typical cells before and immediately after deciliation, respectively. The use of 2.3 or 6.0 mM dibucaine resulted in efficient deciliation but a low level of cell recovery.

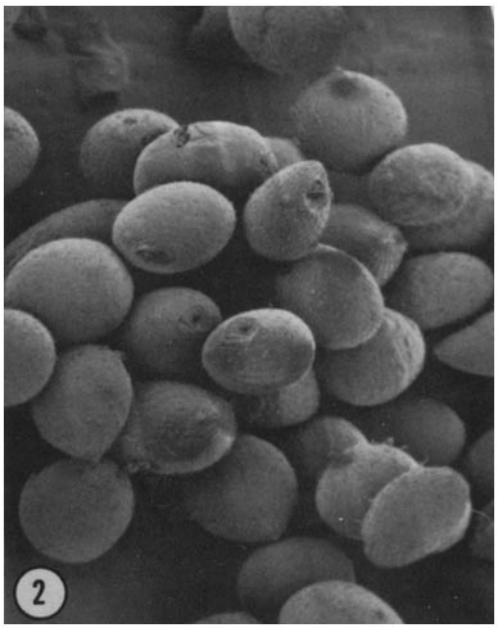

FIGURE 2 Cells immediately after dibucaine deciliation. × 1,250.

[1] This strain of *Tetrahymena*, used in much of our previous work, had been identified for us as strain E. However, recent microscope examination and isozyme analysis by Dr. Dennis Borden of Northwestern University indicate that the strain should properly be designated WH-14.

When resuspended in fresh medium, the denuded cells showed evidence of cilia regeneration in less than 30 min. By 30 min after deciliation, the cells could be seen by scanning electron microscopy to fall into two rather easily distinguishable categories (Fig. 3). Most cells conformed to the pattern described by Rosenbaum and Carlson (11) in that the somatic cilia regenerated asynchronously and in a seemingly random pattern. However, nearly 20% of the cells contained uniformly short cilia being formed synchronously. These latter cells were appreciably smaller than average in length (476 cells chosen at random averaged 68 μm; 85 cells bearing uniform cilia averaged 46 μm). Because of their smaller size, it is reasonable to speculate that the cells on which completely synchronous cilia regrowth was observed had recently divided.

Oral cilia and somatic cilia were regenerated simultaneously, but the oral cilia appeared to grow faster. Cells were observed by scanning electron microscopy at 30, 60, and 90 min after deciliation. The normal complement of cilia was regained during the period between the last two observations. Motility measurements, using the technique of Rosenbaum and Carlson (11), produced a recovery curve similar to that reported by the quoted authors and placed the time of full recovery as 90 min postdeciliation. This is less than half the generation time under our growth conditions. The cells could be deciliated again after partial or complete recovery, making it possible to analyze a population of cilia all synthesized within a brief period of time. After the second deciliation, the cells showed a reduction in viability; no attempts have been made to optimize survival under these conditions.

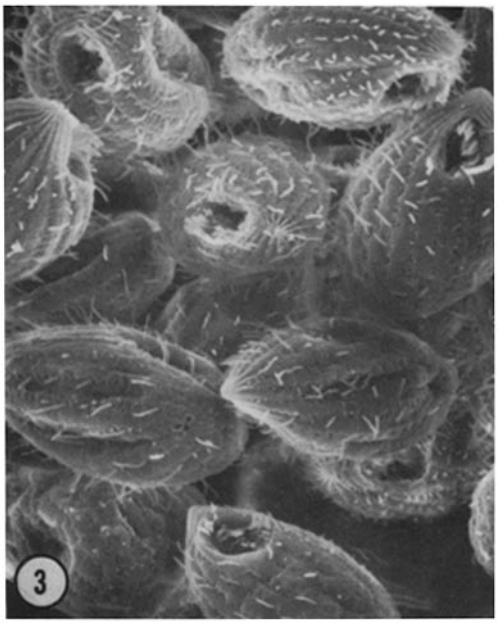

FIGURE 3 Cells 30 min after dibucaine deciliation. × 2,800.

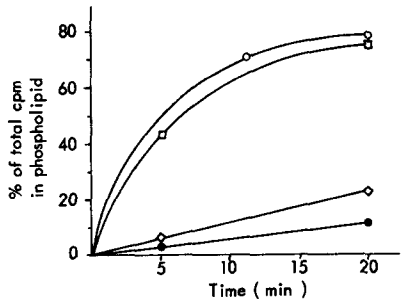

FIGURE 4 The rate at which 0.6 μCi [^{14}C]palmitate was incorporated into phospholipids of 50-ml cell suspension incubated in the absence (○) of dibucaine, or beginning 5 (□), 30 (◇), or 60 (●) min after the addition of dibucaine to a final concentration of 1.1 mM. Lipids were extracted from 10-ml aliquots by the Bligh and Dyer (1) procedure and were analyzed by thin-layer chromatography (14).

As indicated in Fig. 4, a brief dibucaine treatment had little influence upon the incorporation of [^{14}C]palmitic acid into membrane phospholipids. However, pretreatment of the cells for 30 or 60 min did sharply reduce their ability to form phospholipids from added palmitate. Thin-layer chromatography (8) revealed that the distribution of radioactivity among phospholipids of cells given [^{14}C]palmitate after a 5-min dibucaine treatment was similar to the distribution in control cells. Cells maintained for as long as 40 min in dibucaine recovered their ability to incorporate [^{14}C]palmitate into phospholipids within an hour after being resuspended in fresh medium.

The effect of dibucaine upon the incorporation of [^{3}H]leucine into protein is illustrated in Fig. 5. Cells deciliated and incubated in the presence of 1.3 mM dibucaine showed very little protein synthesis. Deciliated cells centrifuged and resuspended in fresh medium after a total of approximately 10 min of exposure to dibucaine recovered to the degree indicated in the figure or, in some cases, to an even higher degree. The ability of *Tetrahymena* to resume incorporation of

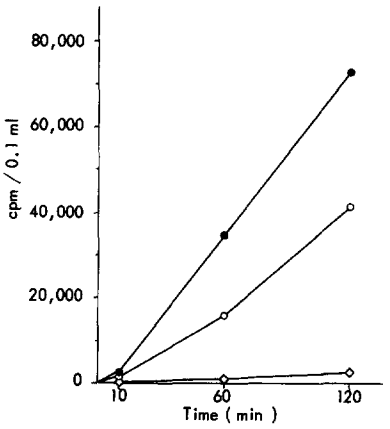

FIGURE 5 The rate of [^{3}H]leucine incorporation into proteins of control cells (●), cells deciliated in 1.3 mM dibucaine (◇), or cells deciliated in dibucaine, centrifuged, and resuspended in fresh medium (○). Each sample received 12.5 μCi [^{3}H]leucine/ml and, for the latter two curves, the radioisotope was added approximately 10 min after dibucaine addition. Each point represents the average of three determinations.

lipid and protein precursors shortly after deciliation will permit us to utilize this technique in studying membrane synthesis during cilia regeneration.

The mechanism of dibucaine action on membranes has been studied extensively (see review by Papahadjopoulos [9]). It and other related local anesthetics are thought to act on membranes by displacing bound calcium ions (5). This property suggests that dibucaine-induced deciliation may function in much the same manner as previously described calcium shock techniques(11). The following observations support this hypothesis.

Tetrahymena resuspended in the calcium-free inorganic medium of Hamburger and Zeuthen (6) were only partially deciliated by 1.2 mM dibucaine. In inorganic medium from which the normal constituent $MgSO_4$ had been replaced by 10 mM diNa EDTA, the same dibucaine concentration caused no deciliation whatsoever, but in inorganic medium supplemented with 1 mM $CaCl_2$, deciliation was even more rapid and complete than that observed in growth medium. Thus it would appear that the action of dibucaine may be to transport free or membrane-bound calcium ions inside the cell, thereby elevating the Ca^{++} concentration near the sensitive basal region of the cilia.

In conjunction with our studies of membrane biosynthesis, it is sometimes desirable to retain detached cilia and an associated fraction of soluble proteins for protein analyses (13). A satisfactory way to achieve this and avoid problems of contamination by proteose peptone from the growth medium is to deciliate with dibucaine in the inorganic medium (6) supplemented with 1 mM $CaCl_2$ and 0.25 M sucrose to prevent swelling and possible disruption of the cilia. Under these conditions there is no cell disruption, and exceptionally homogeneous cilia can be obtained. However, not all the cilia are recovered because a part of them become enmeshed in a copious gel which sediments at slightly higher centrifugal force than needed to pellet deciliated cells. It appears that this gel may be the synchronously extruded contents of the cells' mucocysts.

Everhart has reported that the calcium shock deciliation procedure is not equally effective with all strains of *Tetrahymena* (4). We also observed strain differences with dibucaine. Interestingly, 3.5 mM dibucaine, over twice the concentration used for strain WH-14, was needed to effectively deciliate strain W. Even then, a few cilia remained in place. Almost all cells recovered.

In contrast, cells of strain GL, although susceptible to deciliation by 3.0 mM dibucaine, swelled greatly and showed low viability. It is likely that further trials could establish more suitable conditions for deciliating this strain. The experiments show that use of dibucaine with various *Tetrahymena* strains requires that optimum conditions be devised in each case.

The cilia and flagella of many animal and plant cells can be detached at their basal regions by various natural and artificial stimuli (2). It is likely that local anesthetics could prove useful in studying the mechanisms of detachment and regeneration in species other than the one described here.

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