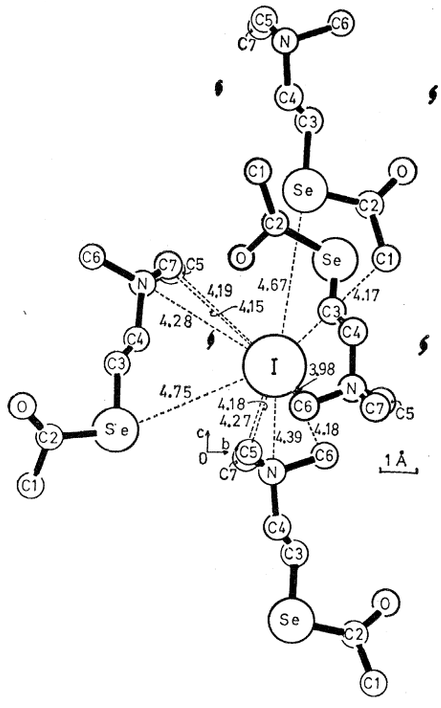

Fig. 2. The packing of the molecules about an iodine atom, as seen down the *a* axis. The dashed lines indicate the closest intermolecular contacts to the iodine atom.

drolysis of acetylcholine by acetylcholinesterase postulated by Wilson *et al.* (*7*). Similar reasoning can be readily extended to the present structure, especially in light of the similarity in the kinetic rates of hydrolysis of the two molecules in the presence of acetylcholinesterase.

The C—Se—C angle of 97° ± 1° agrees favorably with that found in dimethyl selenide, 98°. Within experimental error, all the other intramolecular bond distances and angles agree with their commonly accepted values.

The packing of the molecules about the iodine atom is shown in Fig. 2. All the intermolecular contacts about this atom are greater than the normal van der Waals distances between the respective atoms. Such packing is in agreement with the relatively large thermal motion found for the molecule; the average isotropic temperature factor for the molecule is 8 Å^2. There were no short intermolecular contacts found in the structure, which suggests that intermolecular forces are not responsible for the *trans* conformation.

In conclusion, the results tend to indicate that in order for a molecule to bind to the cholinergic receptor site of the electroplax preparation, it should be able to assume the *gauche* conformation. This requirement does not seem to be necessary for the hydrolysis of the molecule by acetylcholinesterase. The latter is also consistent with the findings of Wilson and Quan (*8*).

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Cilia Isolated from Tetrahymena after Membrane Stabilization by 1,5-Difluoro-2,4-Dinitrobenzene

Abstract. *The tendency for* Tetrahymena pyriformis *cells to lyse when they are centrifuged at speeds above 120g and then exposed to an ethanolic deciliating medium is inhibited by treatment with 1,5-difluoro-2,4-dinitrobenzene. This treatment facilitates greater yields and purer preparations of isolated cilia. Cilia from treated and untreated cells do not differ in some of their biochemical properties.*

A reliable technique for obtaining pure preparations of cilia isolated from the protozoan *Tetrahymena pyriformis* (*1*) has led to several studies on the biochemical properties of these organelles (*2*); the studies have revealed some insight into the possible mechanisms that underlie the function of the cilia. The protozoa used for the isolation of cilia must be harvested from their growth medium at a very low centrifugal force (120*g*) to prevent cell lysis during subsequent treatment with the ethanolic deciliating medium; any appreciable cell lysis results in ciliary preparations that are contaminated with cellular debris (*3*). At such a low centrifugal force, less than half of the cells can be harvested from the cultures; this is usually accommodated by processing large volumes of cell culture (8 liters or more) to obtain sufficient quantities of cilia (tens of milligrams) for biochemical analyses.

The report that erythrocytes do not lyse in distilled water after treatment with 1,5-difluoro-2,4-dinitrobenzene (DFF) (*4*) suggested that the difluoro compound might stabilize the *Tetrahymena* cell membrane in a manner analogous to the proposed strengthening by cross-links between membrane elements and the difluoride in erythrocytes (*4*, *5*). Experiments described here (i) illustrate the ability of DFF to prevent lysis of *Tetrahymena* during the procedure for isolating cilia and (ii) compare purity and some biochemical properties of cilia from untreated cells with those from cells treated with DFF.

Tetrahymena pyriformis, strain W, cultured in sucrose (0.8 percent) and proteose-peptone (1.2 percent) medium supplemented with iron for maximum growth (*6*), was exposed to 0.1 ml of 10 m*M* DFF per 50 ml of cell culture for 1 hour at 4°C with gentle agitation. Cells were harvested immediately thereafter by centrifugation (4°C) at either 120*g* or 360*g* and washed, and cilia were isolated by the method of Watson *et al.* (*1*). Control cells were treated in a similar manner and harvested at 120*g*, but pretreatment with DFF was omitted. Cilia and the supernatant above the ciliary sediments were assayed for (i) nitrogen by the microkjeldahl method, (ii) protein by a microbiuret method (*7*), and (iii) carbohydrate by the tryptophan-H_2SO_4 technique (*8*). Phosphatase activity of the isolated cilia was determined by incubating the cilia at 24°C in the presence of (final concentrations): 3 m*M* $MgCl_2$, 10 m*M* KCl, 0.1 percent sodium deoxycholate, and tris-HCl buffer (*p*H 7.2) with 1.2 m*M* adenosine-5′-triphosphate (ATP), or adenosine-5′-diphosphate (ADP) as substrate. The reaction was stopped after

Table 1. Comparison of the organic constituents of ciliary supernatants and some biochemical properties of cilia isolated from untreated cells (control) of *T. pyriformis* harvested at 120*g* (C_{120}) and from DFF-treated cells (experimental) harvested at 120*g* (E_{120}) and 360*g* (E_{360}). Values given for ciliary supernatants represent averages from three different experiments. Ciliary yield represents material obtained from 150 ml of cell culture in each experiment. Phosphatase activities of isolated cilia are given in micromoles of ortho-phosphorus per milligram of nitrogen per 10 minutes. *N* represents the number of experimental values. Micrograms of carbohydrate, nitrogen, and protein are given per milliliter of supernatant.

Cells	Carbohydrate (μg/ml)	Nitrogen (μg/ml)	Protein (μg/ml)	Ciliary yield (mg of protein)	Phosphatase activity		Carbohydrate (%)
					ATP	ADP	
C_{120}	29	6	383	1.52	5.2 ± 1.1 (*N*=10)	2.8 ± 0.8 (*N*=6)	4.8 ± 1.0 (*N*=6)
E_{120}	12	0	383	1.43			
E_{360}	18	0	383	2.86	4.8 ± 1.0 (*N*=4)	1.9 ± 0.9 (*N*=4)	3.7 ± 0.5 (*N*=6)

10 or 20 minutes by adding an equal volume (1 ml) of 3 percent perchloric acid; after an additional 20 to 30 minutes at 0 to 4°C, the precipitate was centrifuged out and the content of ortho-phosphorus in the supernatant was determined by the method of Lowry and Lopez (*9*). Kinase transphosphorylation activity in the cilia preparation was investigated by detecting the presence of ATP (and AMP) after cilia had been incubated in the above reaction mixture for 20 minutes with 1.2 m*M* ADP as substrate. The nucleotides were identified under ultraviolet light by thin-layer chromatography on cellulose plates; a mixture of tertiary amyl alcohol, formic acid, and water (3 : 2 : 1) was used as the solvent, as described by Randerath (*10*).

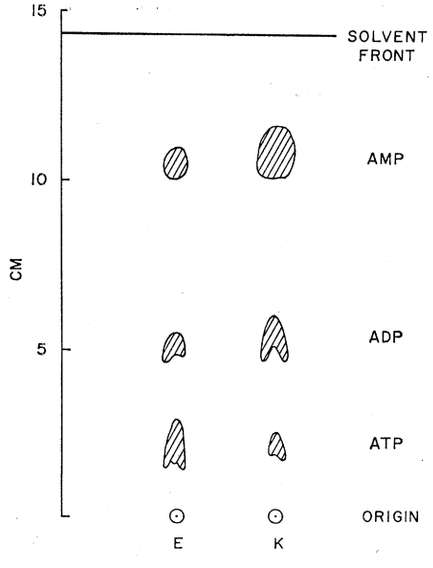

Fig. 1. Drawing of a thin-layer chromatogram of nucleotides obtained from a reaction mixture containing cilia and ADP (*E*) and knowns (*K*) that had been treated in a similar manner. The appearance of AMP (adenosine monophosphate) and ATP in the experimental (*E*) series indicates the presence of an adenylate kinase.

These results, for both DFF-treated and untreated control cells, are summarized in Table 1. Even when the DFF-treated cells are harvested at three times the centrifugal force applied to the control cells, the carbohydrate present in ciliary supernatants is reduced. Nitrogen contamination is insignificant in the controls and nonexistent in ciliary preparations pretreated with DFF. Nitrogen present in the ciliary supernatants is accounted for by the ethylenediaminetetraacetic acid (EDTA) in the deciliating medium. Values shown in Table 1 represent the corrected nitrogen values after subtracting the EDTA-nitrogen. Biuret-protein determinations of these ciliary supernatants do not vary between experimental and control preparations. Thus, the ciliary supernatant from DFF-treated cells contains less organic contaminants than the ciliary supernatant from untreated cells; nevertheless, the yield of ciliary protein is approximately doubled (Table 1) by harvesting the cells at three times the centrifugal force used for the control.

Phosphatase activity of cilia isolated from DFF-treated cells is not significantly different from that of cilia from untreated cells. Adenosine triphosphatase activity of both control and experimental preparations is very similar. The lower phosphatase activity observed when ADP was used as a substrate in ciliary preparations from DFF-treated cells, although not statistically significant, led to the investigation of adenylate kinase transphosphorylation activity. Kinase activity is known to be present in cilia from normal untreated cells (*11*). The presence of this enzyme was shown by thin-layer chromatography, which also shows that activity of the enzyme is uninhibited in cilia from cells treated with the difluoro compound (Fig. 1).

Although the carbohydrate content of cilia isolated from DFF-treated cells is slightly lower than that of cilia from untreated cells, this difference is also not statistically significant.

It has been shown that pretreatment of the cells of *T. pyriformis* with DFF will allow the isolation of cilia in greater quantities and in purer quality. Although there was no significant difference, in the biochemical properties studied, between cilia isolated from treated or untreated cells, some modifications of cilia are to be expected from this treatment, because of the reactivity of DFF with various protein side groups (*5*). Such alterations, however, may be limited to the ciliary sheath and leave the enzymes associated with the axoneme and matrix unaffected.

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