sociation of DNA synthesis and mitosis are correct, they suggest that DNA synthesis is a requirement, independent of mitosis, for certain sequences in the differentiation of tubules. The evidence does not indicate whether such a requirement would involve doubling of the DNA content, synthesis at specific loci of the genome (*13*), or metabolic turnover of DNA (*14*).

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Crystal and Molecular Structure of Acetylselenocholine Iodide

Abstract. *The structure of acetylselenocholine iodide has been determined by x-ray crystallographic analysis. The molecule is in the* trans *conformation about the C—C bond of the choline residue. This conformation appears to explain the molecule's inability to give a positive cholinergic response when added to an electroplax preparation.*

Recent studies (*1*) with an isolated single cell electroplax preparation showed that acetylcholine and acetylselenocholine (ASeCh) have markedly different pharmacological activities. The seleno derivative was found to be inactive in a very wide concentration range on this cholinergic receptor site. Though a striking difference in the reactivity exists for these compounds on this cholinergic receptor, the two molecules are hydrolyzed at similar rates by acetylcholinesterase (*2*). In an effort to obtain structural information about ASeCh and also the structural requirements of the active sites of acetylcholinesterase and the electroplax preparation, the crystal structure of ASeCh iodide was investigated.

Single crystals of the compound were supplied by H. G. Mautner (Yale University). We measured the following crystallographic data: $\mathbf{a} = 7.923 \pm 0.006$ Å; $\mathbf{b} = 11.83 \pm 0.01$ Å; $\mathbf{c} = 13.22 \pm 0.01$ Å; space group, $P\,2_12_12_1$; ρ (measured by flotation) = 1.801 g/cm^3; ρ (calculated assuming four molecules in the unit sell) = 1.802 g/cm^3.

Three-dimensional intensity data were measured with a General Electric XRD-5 diffractometer by the stationary counter-stationary crystal technique with Mo$K\alpha$ radiation. Balanced filters were used. The intensities were corrected for the Lorentz and polarization factors. No absorption corrections were made. In total, 570 reflections had peak intensities greater than twice their calculated standard deviations [errors of the S.D.'s computed by method of Evans (*3*)]. The coordinates of the atoms comprising the molecule were readily obtained from three-dimensional Patterson and Fourier syntheses. The positional and thermal parameters of the molecule were refined by block diagonal least squares analysis. The final R value (the usual discrepancy index) was 0.055 for the observed data.

In discussing the pertinent features of the molecule it is most interesting to make a comparison with the very recently reported structure of acetylcholine bromide (*4*). The most dramatic difference between the two molecules is the conformation about the C—C bond of the choline moiety. The torsion angle (ϕ_{cc} defined as the dihedral angle between the projection of the C—N$^+$ and the C—Se or C—O bonds) is *gauche* in acetylcholine, that is, in the neighborhood of 60°, and *trans* in the present structure, 179° (see Fig. 1). These represent the two possible staggered conformations possible about the C—C bond. Sundaralingam (*5*) has indicated that in N$^+$—C—C—O systems the *gauche* conformation is always the preferred one. The reason for the *trans* conformation in this structure is not as yet completely clear, but it is most probably due to the larger van der Waals radius of the selenium atom, 2.0 Å as compared to oxygen's 1.4 Å. Presently, work on the thio-derivative is being carried out to determine the influence of the van der Waals radius on the conformation.

It is interesting to note that a staggered conformation exists about the C(4)—N bond. This conformation was also observed in acetylcholine.

The C(2)—Se and C(3)—Se bond lengths are 1.82 Å and 2.18 Å (± 0.05 Å), respectively. These distances deviate significantly from the C—Se distance of 1.977 Å found in the electron diffraction study on dimethyl selenide (*6*). In acetylcholine it was also observed that the bond lengths about the ester oxygen deviated in the same manner from the commonly accepted C—O single bond length. It was noted in the acetylcholine report that the partial double bond character in the carboxyl carbon to oxygen bond is consistent with the mechanism of hy-

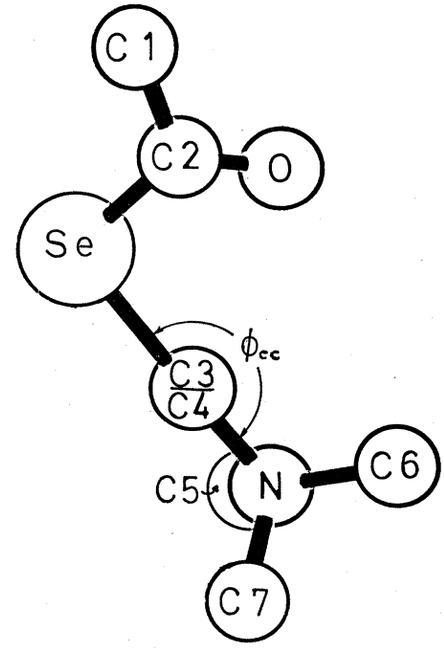

Fig. 1. Projection of molecule down the C(3)—C(4) bond.

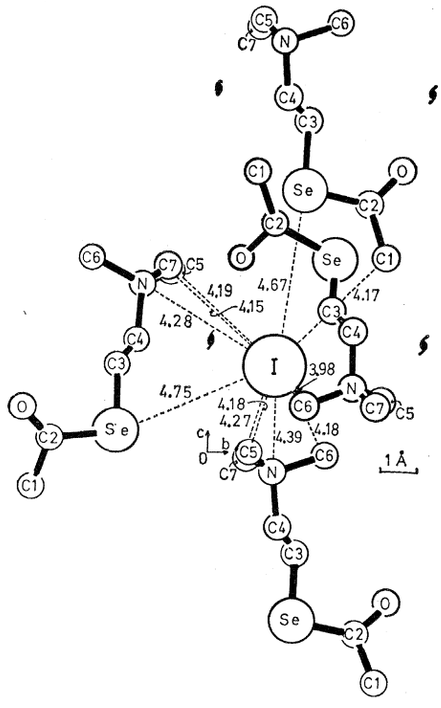

Fig. 2. The packing of the molecules about an iodine atom, as seen down the *a* axis. The dashed lines indicate the closest intermolecular contacts to the iodine atom.

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