The experimental data are summarized in the Table 1. It may be noted that nitrogen mustard and urethane produce a significant increase in 5-HIAA urinary excretion on the second day after injection. Chloral hydrate showed no effect. Therefore it seems that only nucleotoxic agents increase the excretion of 5-HIAA. In this respect there is no difference between the so-called radiomimetics and spindle poisons. Another spindle poison colchicin was shown by Marks and Sorgen[5] to produce degranulation of enterochromaffin cells, a result which is consistent with the present observations. It seems that these cells are also sensitive to both ionizing radiation and nucleotoxic agents. The time of maximal urinary excretion of 5-HIAA is, however, different in each case occurring on the first day after treatment for ionizing radiation, but on the second day for nucleotoxic agents.

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## Some properties of isozymes of brain acetylcholinesterase *

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It has been reported that the acetylcholinesterase of human brain consists of three isozymes when it is separated under the conditions described.[1] Since a vast literature exists on the biochemical properties and physiological function of this enzyme, it was thought that a comparison of some of the properties of the acetylcholinesterase isozymes might provide some insight into the nature of their differences. At the present time the nature and significance of multiple forms of a given enzyme are virtually unknown, and such a study could provide some data on this problem.

Starch-gel electrophoresis was performed according to Smithies.[2] After the starch-gel separation had been completed, one channel was cut from the gel and the enzyme localized by methods already described.[3] This was matched against the remaining gel, and the areas of enzymatic activity were dissected from the starch block.

Because of the proximity of the two fastest moving isozymes to each other, their isolation as individual components was not feasible, and they were treated as one band (band 2). Attempts at extracting the enzyme from the starch-gel by various techniques yielded recoveries of 10 to 20 per cent of the enzymatic activity. Consequently, the starch-gel was homogenized with an equal volume of distilled water to which 2% gum acacia was added to stabilize the enzyme. This was dialyzed overnight against 0·9% saline. No loss in enzymatic activity was noted during this procedure. Aliquots of the slurry were used as the enzyme source, and acetylcholinesterase activity was determined by a micro-titrimetric method[4] using acetylcholine iodide as a substrate in the presence of 0·01 M $MgCl_2$. No activity was discernible with butyrylcholine iodide as a substrate. All values were corrected for spontaneous hydrolysis. The determinations of the substrate optimum, Km values, as well as the inhibitor data were obtained at pH 7·5, and 37°. The Km values were obtained by treatment of the data according to Lineweaver and Burk[5], using the values on the low concentration side of the curve. The isozyme proximal to the origin is designated as band 1, and the distal isozymes as band 2. Human caudate nucleus and putamen were again used as an enzyme source. All values reported are the averages of three to six determinations.

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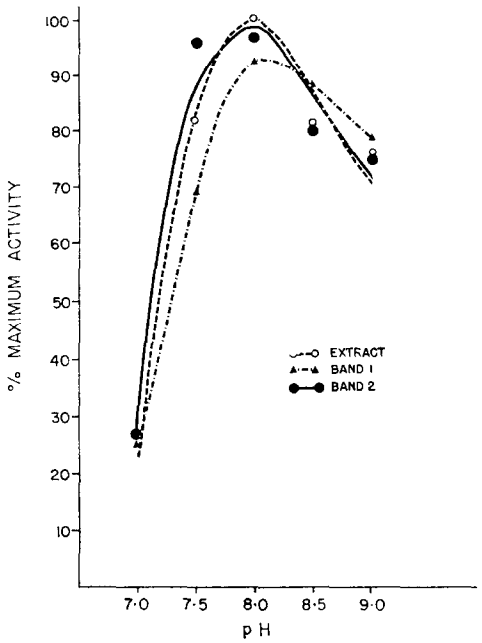


FIG. 1. Relative activity of acetylcholinesterase isozymes as a function of pH.

When activity is determined from pH 7·0 to 9·0 for the extract of the brain prior to the separation, and compared with that of bands 1 and 2, it can be seen (Fig. 1) that the three samples yield almost identical pH curves with the maximum activity at pH 8·0 to 8·5. This is in agreement with the results obtained on the purified enzyme from other sources.[6, 7]

The activity was then determined at various substrate concentrations for the two bands with the results shown in Fig. 2. It can be seen that both isozymes exhibit the typical bell-shaped curve for

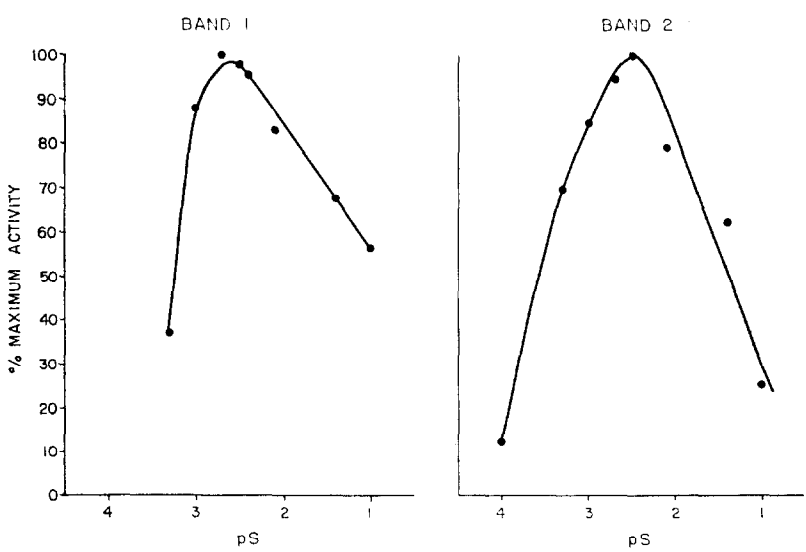


FIG. 2. pS-Activity curves for acetylcholinesterase isozymes.

acetylcholinesterase, with the activity being inhibited at higher concentrations.[8] No difference in substrate optimum was shown by the two bands; in both cases it was $3 \times 10^{-3}$ M.

The effect of three inhibitors on the isozymes was then determined. The compounds tested were the classical inhibitor physostigmine, a competitive inhibitor[9] Mytelase*, (N′,N′-*bis*(2-diethylamino ethyl)oxamide *bis*-2 chlorobenzyl chloride) and the tightly bound inhibitor DFEP (diisopropyl fluorophosphate). The $pI_{50}$ values obtained for the three compounds show no significant difference for the two bands (Table 1).

* Kindly donated by Winthrop-Stearns, Inc., New York, N.Y.

TABLE 1. ACETYLCHOLINESTERASE IN HUMAN BRAIN

| | Extract* | Band 1 | Band 2 |
|---|---|---|---|
| Km | $2{\cdot}2 \times 10^{-4}$ M | $1{\cdot}5 \times 10^{-4}$ M | $1{\cdot}3 \times 10^{-4}$ M |
| $pI_{50}$ (eserine) | | 7·4 | 7·2 |
| $pI_{50}$ (Mytelase) | | 8·0 | 7·8 |
| $pI_{50}$ (DFP) | | 5·9 | 5·8 |

* Prior to electrophoresis.

Km values obtained for the two bands on the low substrate concentrations of the curve again are not significantly different. However, the values are lower than that obtained from the original extract prior to electrophoresis. It is to be noted that an enzyme is found in caudate-putamen which hydrolyzes acetylthiocholine but which is not an acetylcholinesterase,[1] and probably is responsible for the higher value obtained in the crude extract as compared with the relatively purified bands. Another possibility is that the starch present in the assay medium may have some influence on the velocity of the reactions. The Km values reported here are smaller than those of acetylcholinesterases obtained from various sources,[10] and may reflect the absence of some esterase with higher Km values present in a crude homogenate which is removed by the electrophoretic technique. The low Km value obtained here also indicates the high affinity of the substrate for the enzyme.

In summary, the two acetylcholinesterase isozymes described here do not differ in pH optimum, substrate optimum, Km values, and $pI_{50}$ values for eserine, Mytelase, and DFP, and this would indicate no differences in a mechanism of action for both isozymes where such mechanism was dependent on the kinetic and inhibitor parameters studied. This does not preclude differences in enzyme structure since it has been shown for serum cholinesterase that release of sialic acid from the enzyme by the action of neuraminidase does not alter Km or some $pI_{50}$ values,[11] while it does alter its electrophoretic mobility.

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