in different gonococcus fractions do not parallel toxicity.

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Received May 7, 1957. P.S.E.B.M., 1957, v95.

## Cholinesterases in Human and Ruminant Central Nervous Tissue.* (23324)

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In comparison to acetylcholinesterase (AChE), the role of butyrylcholinesterase (BuChE) in central nervous tissue has been relatively unexplored. This seems to be due to (a) lack of knowledge of the physiological substrate for BuChE, (b) presence of this enzyme in the glial tissue rather than in the axoplasm(1) which would rule out any function for this enzyme in nerve impulse propagation, and (c) its apparent absence from ruminant central nervous tissue, making it difficult to assess the fundamental function of the enzyme. Its reported absence from ruminant tissue has been based on the observation of Mendel, Mundell and Rudney(2), that benzoyl choline is a specific substrate for BuChE, and no benzoyl choline hydrolysis is evident when tissues from this species are assayed with this substrate(3). Recently, Hardwick(4) reported isolation of an enzyme from bovine plasma that possessed properties of a BuChE, except for its inability to hydrolyze benzoyl choline. This investigation was undertaken to determine whether a BuChE could be demonstrated to be present in bovine brain tissue. If this could be established it might be possible to view the role of this enzyme in brain tissue in a new perspective.

*Methods.* Because of observations that BuChE activity is higher in the white matter of central nervous tissue than in the gray(5), this part of the brain was used for assay. The brains were kept frozen at −15°C until ready for use, and then allowed to thaw, at which time the desired samples were dissected out, a 1:5 homogenate in 0.023 M $NaHCO_3$-0.154 M NaCl prepared, and aliquots ranging between 0.5 and 1 ml were used in the determinations. Activity was measured in the rotary Warburg apparatus by a modification of the method of Ammon(6), and rates were calculated from the initial velocity, although in most cases the velocities simulated a zero-order reaction during the test period which was usually 60 minutes. Differentiation of the 2 cholinesterases was made possible by the use of a specific acetylcholinesterase inhibitor, mytelase† (N′, N′,-bis-[2-diethylamino ethyl] oxamide bis-2-chlorobenzyl chloride)(7). Reaction mixtures with the final concentration of reactants in the vessel are given in the tables. In all cases the substrate was tipped in from the side arm after 5 minutes equilibration. All results are the averages of 4 experiments.

*Results.* The Q values, expressed as μmols of substrate hydrolyzed/g tissue/hr, for human, bovine and sheep central nervous tissue white matter, are shown in Table I. With acetylcholine as a substrate, hydrolysis occurs in the 3 species to varying extents, with human white matter having the highest activity, and bovine white matter the least. To assess the contribution of each of the 2 enzymes, the tissue was then assayed in the presence of $5 \times 10^{-7}$ M mytelase. It can be

* This work was aided by grant from Nat. Inst. of Neurological Diseases and Blindness, N.I.H.

† We are indebted to Winthrop Laboratories for the generous gift of this compound.

TABLE I. Cholinesterases in White Matter of Human, Bovine and Sheep Brain.

| Substrate | Human Q* | Human % inhibition | Bovine Q | Bovine % inhibition | Sheep Q | Sheep % inhibition |
|---|---|---|---|---|---|---|
| AcCh • I, $1 \times 10^{-2}$ M | 24.2 | | 11.7 | | 16.5 | |
| " + inhibitor† | 13.3 | 45.0 | 3.65 | 68.8 | 5.35 | 67.6 |
| BuCh • Cl, $1 \times 10^{-2}$ M | 33.4 | | 8.7 | | 16.5 | |
| " + inhibitor† | 32.2 | 3.6 | 8.1 | 7.0 | 15.7 | 5.0 |
| BzCh • Cl, $1 \times 10^{-2}$ M | 8.6 | | .0 | | .0 | |

* Q = $\mu$M substrate hydrolyzed/g wet tissue/hr. † Mytelase, $5 \times 10^{-7}$ M.

Reaction mixture: Substrate, $1 \times 10^{-2}$ M; buffer, 0.023 M $NaHCO_3$–0.154 M NaCl, pH 7.4; atm., 95% $N_2$–5% $CO_2$; homogenate equivalent to 200 mg wet tissue; total vol, 2 ml.

seen that the degree of inhibition for human, bovine and sheep white matter was 45.0%, 68.8% and 67.6% respectively. The residual activity for bovine brain was about 27% of the human, and in the case of the sheep brain was 40% of the human value. It can be seen that at this substrate concentration, somewhat less than half of the human enzymatic activity is due to AChE, while in the ruminant tissue, about ⅔ of the activity is due to this enzyme.

Using butyrylcholine as a substrate, which at the concentration of AChE, here encountered, is a specific BuChE substrate, an appreciable hydrolysis by the brain white matter from the 3 species is observed, which is only slightly inhibited by mytelase, although the activities in the ruminant tissues are lower than in human tissue. The ratios of the inhibited butyrylcholine:inhibited acetylcholine values for the human, bovine and sheep tissues are 2.2, 2.4 and 2.9 respectively, which is in agreement with the observed ratios of activity for these 2 substrates in serum, where the BuChE occurs(8). When benzoylcholine is used as a substrate, only the human white matter exhibits any activity for this substrate, whereas the ruminant tissue does not hydrolyze this compound.

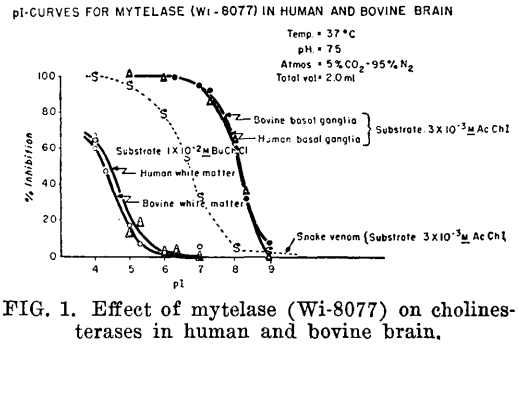


FIG. 1. Effect of mytelase (Wi-8077) on cholinesterases in human and bovine brain.

In Fig. 1, where the % inhibition is plotted against pI (negative of the logarithm of the inhibitor concentration), are shown the action of mytelase on human and bovine grey and white matter. In the basal ganglia, where AChE predominates, the inhibitor is completely effective in inhibiting hydrolysis of acetylcholine above a concentration of 1 x $10^{-7}$ M, with a $pI_{50}$‡ of 8.2. In white matter, using butyrylcholine as a substrate no inhibition occurs at a concentration of the inhibitor lower than 1 x $10^{-6}$ M, and a $pI_{50}$ of 4.4 is observed. This difference in inhibitory activity of 1:6300 to achieve separation in activities between the 2 enzymes makes this compound one of the most satisfactory anticholinesterase agents used for this purpose. Thus the data on inhibition previously obtained on serum and erythrocytes is applicable to central nervous tissue, and validates its use in separating the two enzymes in brain. Double inhibitor experiments using mytelase and 5 x $10^{-5}$ M eserine, abolished all enzymatic activity for acetyl- and butyrylcholine.

*Discussion.* It can be readily observed that there is present in ruminant white matter from the central nervous system, an enzyme which possesses all of the properties of a BuChE, except for its inability to hydrolyze benzoylcholine. It has a greater activity toward butyrylcholine than acetylcholine, and

‡ $pI_{50}$ = Negative of logarithm of inhibitor concentration required to produce 50% inhibition of the enzymatic system.

the ratio of the activities toward these 2 substrates is similar to the enzyme in human serum. In addition, its activity is completely inhibited by eserine. Other studies(9) have shown that the substrate optimum characteristics are similar to the BuChE in serum, showing increasing activity with higher substrate concentration. All of these observations leave little room for doubt that the enzyme being measured in ruminant brain tissue is the characteristic BuChE found in nervous tissue from non-ruminant species. That this enzyme from ruminant tissue is incapable of hydrolyzing benzoylcholine in contradistinction to the enzyme from non-ruminant sources is of interest and this differential characteristic may lead to studies on the nature of the binding sites on the 2 enzymes which cause such differences in substrate specificity.

If this enzyme is found to be present in other species where it has hitherto been presumed to be absent, then its ubiquity in nervous tissue would imply that it may serve some basic function in metabolism in that organ.

Finally, it must be stated that the use of the terms acetylcholinesterase and butyrylcholinesterase is occasioned not by any conviction as to nomenclature, but rather that the terms hitherto employed have outlived their usefulness, and that some standardization in terminology is required. In this work, the specificity of each enzyme for chain-length optimum has been used as a basis for identification.

*Summary.* Studies on the cholinesterase in white matter of human, bovine, and sheep brain indicate that there is present in ruminant white matter a butyrylcholinesterase with properties similar to enzyme in human tissue, except for its inability to hydrolyze benzoylcholine. However, the enzyme from these 2 sources gives ratios of hydrolysis for butyrylcholine : acetylcholine which are alike, and the activity is completely inhibited by eserine. Thus it would seem justifiable to attribute to the enzyme a fundamental role in nervous tissue function, if its presence in other species can be confirmed.

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Received May 7, 1957. P.S.E.B.M., 1957, v95.

## Influence of Cortisone and "Stress" upon Persistence and Multiplication of $MEF_1$ Virus in Hamsters.* (23325)

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It has been shown that under the influence of stress, the incidence of paralytic poliomyelitis in experimentally infected hamsters depends on endocrine reactivity to stress of individual animals(1). However, animals reacting to stress did not develop poliomyelitis when inoculated intraperitoneally unless proper inoculatory timing provided active virus during a specific stage of their endocrine stress-reaction(1). Observance of such timing requirements appeared to be particularly important for extraneural routes such as intraperitoneal, intramuscular, subcutaneous, etc., where the virus disappears within 3 days

* Aided by grant from Natl. Fn. for Infantile Paralysis.

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