Comments and Communications

Distinction between Acetylcholine-Esterase and Other Choline Ester-splitting Enzymes

During the past few years, choline ester-splitting enzymes have been studied by many investigators. One of the problems involved has been the question of the existence of an esterase specific for acetylcholine—a question of considerable interest in view of the important physiological function of this ester. Much information has been obtained and the facts are, with a few exceptions, in good agreement. However, the picture may appear confusing, due mainly to the lack of a consistent classification and terminology. It is the purpose of this note to propose a classification and a terminology which may contribute to clarifying the situation.

The question of the existence of an esterase specific for acetylcholine was first raised by Stedman, Stedman and Easson (*Biochem J.* 1932, **26**, 2056). These investigators thought that the enzyme which they prepared from horse serum was specific for choline esters, although not for acetylcholine, since it split butyrylcholine at a higher rate than acetylcholine. Later experiments, however, have shown that the esterases present in some sera are not specific for choline esters, although they split them faster than noncholine esters (VAHLQUIST, B. *Skand. Arch. Physiol.*, 1935, **72**, 133; and GLICK D. *J. biol. Chem.*, 1938, **125**, 729; 1939, **130**, 527; 1941, **137**, 357). Alles and Hawes found that the esterase in red blood cells differs markedly from the serum esterase (*J. biol. Chem.*, 1940, **133**, 375). The former is inhibited by high concentrations of acetylcholine in contrast to the latter; it hydrolyzes acetyl-β-methylcholine, which is not split by serum esterase (Glick, *op. cit.*). Richter and Croft (*Biochem J.*, 1942, **36**, 745) confirmed these observations and found the red cell esterase highly specific for acetylcholine. Brain esterase was found to be similar to the red cell esterase (ZELLER, E. A. and BISSEGGER, A. *Helv. chim. Acta*, 1943, **26**, 1619; NACHMANSOHN, D. and ROTHENBERG, M. A. *J. biol. Chem.*, 1945, **158**, 653). These two esterases do not split benzoylcholine (MENDEL, B., MUNDEL, D. B., and RUDNEY, H., *Biochem. J.*, 1943, **37**, 473). Testing a variety of esters and esterases of different tissues, Nachmansohn and Rothenberg showed that the esterase in all conductive tissue, nerve and muscle, and in erythrocytes has a number of well-defined properties. In addition to a well-defined optimum acetylcholine concentration, described previously, they found that the enzyme splits propionylcholine at the same or at a lower rate than acetylcholine, whereas butyrylcholine was shown to be split at a very low rate or not at all. At a concentration optimal for acetylcholine, noncholine esters were split at a very low rate or not at all. The observations included a virtually pure esterase preparation obtained from the electric tissue of *Electrophorus electricus*. In striking contrast to this type of esterase, esterases of other tissues, like that of serum, split propionylcholine at a higher rate than acetylcholine and butyrylcholine at a higher rate than propionylcholine.

The importance of studying the enzyme activity as function of substrate concentration for all esters has been emphasized by K. B. Augustinsson (*Acta. physiol. Scand.*, 1948, **15**, Suppl. 52). Testing the activity as function of substrate concentration, he found that the esterases from conductive tissue and erythrocytes have the same optimum substrate concentration for propionylcholine and butyrylcholine, whereas, e.g., for acetyl-β-methylcholine they have a different optimum (*J. biol. Chem.*, in press). Noncholine esters, like triacetin, show entirely different activity-substrate concentration relationships. At very high substrate concentrations, hydrolysis rate may be even higher than with acetylcholine.

From all these investigations it is obvious that there

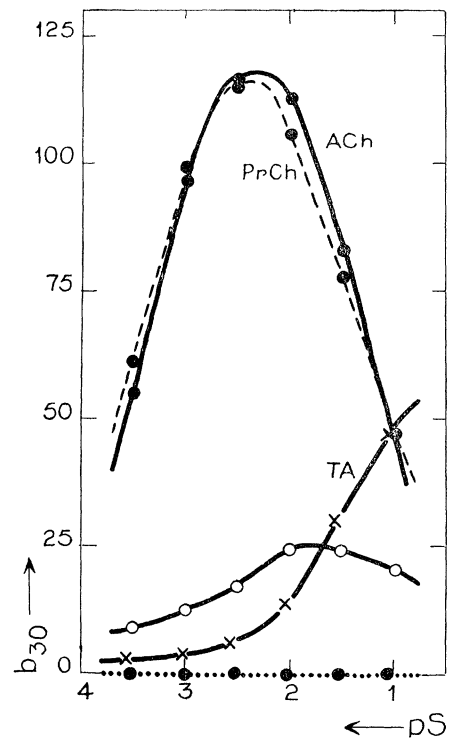

FIG. 1. *Acetylcholine-esterase.* Source: electric tissue of *Electrophorus electricus*. Activity-pS curves for the enzymatic hydrolysis of various esters.

- ●------● Acetylcholine (ACh);
- ●.....● butyrylcholine (BuCh);
- ●----● propionylcholine (PrCh);
- ○——○ DL-acetyl-β-methylcholine;
- ×——× triacetin (TA).

is a type of esterase which is highly specific for acetylcholine, although this specificity is relative and not absolute. Whereas the facts accumulated present a fairly clear picture, no agreement exists concerning the terms

used for the different types of choline ester-splitting enzymes. Zeller distinguishes the enzymes according to the material where they were first found and described. Nachmansohn and Rothenberg referred to the enzyme in conductive tissue and erythrocytes as a cholinesterase specific for acetylcholine, but a term distinguishing this type from other choline ester-splitting enzymes appears desirable. Augustinsson defined as cholinesterase all those esterases which, at substrate concentration optimum

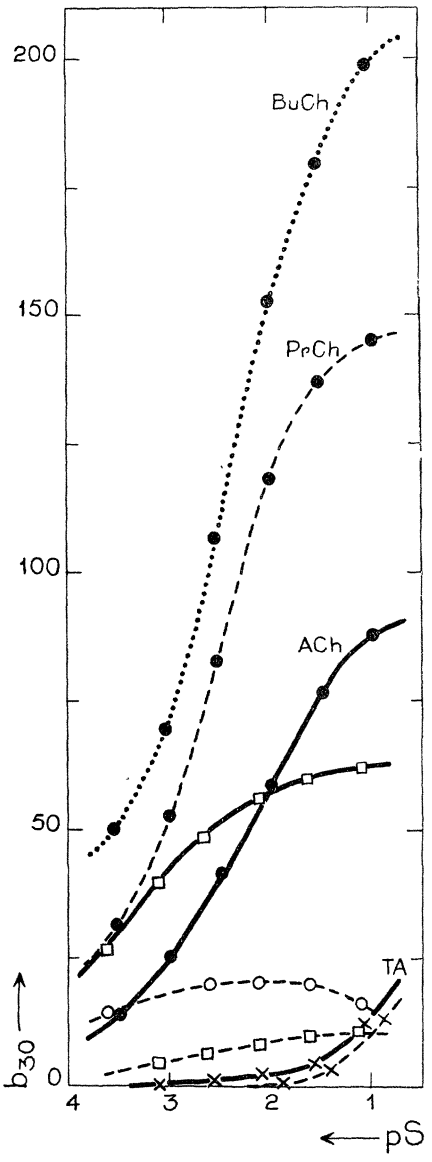

FIG. 2. *Cholinesterase.* Source: human serum. Activity -pS curves for the enzymatic hydrolysis of various esters. Symbols the same as in Fig. 1.

for acetylcholine, split this ester at a higher rate than any other ester. Since he included in this classification the serum esterase, this definition must be revised.

Mendel and his associates introduced the terms "true" and "pseudo" cholinesterase. In contrast to the finding of other investigators, Mendel and Rudney found an optimum rate of acetylcholine hydrolysis by brain esterase to be below 0.0002 M substrate concentration. This optimum rate, at an extremely low acetylcholine concentration, they consider to be the decisive difference of "true" as compared with the "pseudo" cholinesterase, for which they found an optimum of above 0.02 M. These data were based on inadequate techniques (NACHMANSOHN, D. and ROTHENBERG, M. A. *J. biol. Chem.*, 1945, **158**, 653). The experiments were not carried out under optimal conditions, especially in regard to the salt concentrations (AUGUSTINSSON, K. B. *Acta physiol. Scand.*, 1948, **15**, suppl. 52). Also, the expressions "true" and "pseudo" enzyme have met general opposition by enzyme chemists (GLICK, D. *Science*, 1945, **102**, 100).

Since there exists a type of esterase with well-defined properties and highly specific for acetylcholine, we suggest the use of the term acetylcholine-esterase (ACh-esterase) for this enzyme. Its most outstanding characteristics may be summarized as follows: It has a high affinity to acetylcholine, that is, the Michaelis constant is small. The turnover number is high. No other ester is hydrolyzed at a higher rate. The enzyme splits propionylcholine at the same or at a lower rate, butyrylcholine, at a low rate or not at all. Noncholine esters are not split or at a low rate. The enzyme has a low affinity to these latter esters. If the enzyme activity is plotted against log M acetylcholine concentration, a bell-shaped curve is obtained, consistent with the theory of Haldane about inhibition of enzyme by excess of substrate (see Fig. 1). The ACh-esterase occurs in conductive tissue (nerve and muscle) and in erythrocytes. Esterases with similar characteristics occur in a few special cases, like snake venom and *Helix* blood (AUGUSTINSSON, K. B. *Acta physiol. Scand.*, 1948, **15**, suppl. 52), although these enzymes have a few distinct features as compared with the ACh-esterase of conductive tissue.

The properties of acetylcholine-esterase make it possible to distinguish this enzyme from other esterases. There are other esterases, e.g., in some sera, and in the pancreas, which split choline esters at a higher rate than noncholine esters. According to the usual enzymological terminology, these esterases may be called cholinesterases, as was originally proposed by Stedman for the serum esterase. Their physiological substrate is at present unknown and so the name seems at least temporarily appropriate. These esterases as compared with acetylcholine-esterase have a high Michaelis constant for choline esters. They are not inhibited by high concentrations of acetylcholine. Hydrolysis rate increases with increasing length of the acyl chain (from acetyl to *n*-butyryl) (Fig. 2).

It is hoped that the classification and terminology proposed may help to avoid further confusion. The term ACh-esterase, moreover, will associate this type of enzyme with its physiological substrate, at least in nerve and muscle tissue, where a function of acetylcholine appears fairly well established.

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