SPECIAL ARTICLES

THE CHOLINESTERASES IN THE LIGHT OF RECENT FINDINGS

WHEN Alles and Hawes,[1] continuing the work of Galehr and Plattner,[2] observed that the cholinesterase of human red blood cells was far more active at low than at high concentrations of acetylcholine and that the reverse was true for the cholinesterase of human serum, they were led to believe that erythrocytes contained one type of enzyme, "cell cholinesterase," while the cholinesterase found in serum represented another type, "serum cholinesterase."

Of the above experiments those concerning the enzyme in red blood cells were interpreted correctly by the authors; their assumption, however, that the cholinesterase in serum is homogeneous and represents the "serum type" enzyme proved to be incorrect, since a serum devoid of "cell cholinesterase" has as yet not been found. Serum of man, it is true, contains predominantly "serum cholinesterase" and only comparatively small amounts of "cell cholinesterase," but fairly large quantities of "cell cholinesterase" are present in the sera of dog, cat, guinea pig, rabbit and rat.[3,4] Had Alles and Hawes examined the sera of ruminants (ox, sheep),[3] they would have found that the only cholinesterase contained therein is, according to their nomenclature, "cell cholinesterase"—a reductio ad absurdum of a classification of cholinesterases based on their locale.

Our work, which finally resulted in a definite differentiation between two cholinesterases, was prompted by the question whether a specific enzyme or a non-specific esterase hydrolyzes acetylcholine in the animal body. Since this question could only be answered by closely examining the enzyme in all its peculiarities, purification became a matter of necessity. Highly purified enzyme preparations were obtained,[5,6,7] and it was the difference in the properties of these preparations which made it clear beyond doubt that two distinct cholinesterases exist in the animal body: a specific or true cholinesterase, hydrolyzing choline esters exclusively, and a non-specific or pseudo-cholinesterase capable of hydrolyzing a variety of esters, including those of choline.[5]

These experiments, which revealed the intrinsic properties—specificity and non-specificity—characteristic of the two cholinesterases throughout the animal kingdom, at the same time disclosed another difference between the two enzymes: the true cholinesterase, in contrast to the non-specific enzyme, was found to display a far greater activity at low than at higher concentrations of acetylcholine. This peculiarity of the specific enzyme, though conspicuous in mammals, fades, however, as we descend the evolutionary scale and is no longer encountered in the true cholinesterase present in primitive forms of life, *e.g.*, in Planaria.[8] Decreasing activity with rising substrate concentrations should therefore not be considered an inherent property of the specific enzyme.

Furthermore, we have recently found[9] that the activity-substrate concentration curve of the true cholinesterase can be changed at will and even reversed by adding to the enzyme solution certain organic colloids capable of reversing the electric charge of the enzyme particles—a fact which might have some bearing on the theory of nerve impulse transmission. Protamines, for example, carrying a strong positive charge are able to change the activity-substrate concentration relationship of the true cholinesterase in mammalian brain to such an extent that the enzyme, now resembling the pseudo-cholinesterase, displays increasing activity with rising substrate concentrations (see Fig. 1). Subsequent addition of negatively

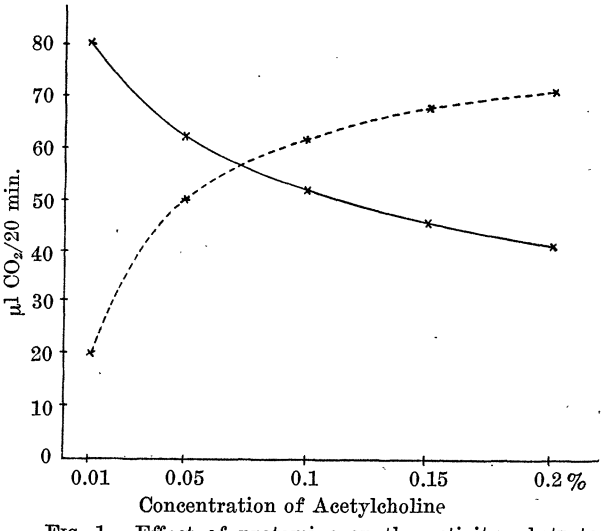

FIG. 1. Effect of protamine on the activity-substrate concentration curve of true cholinesterase (dog brain cortex). Activity determined manometrically by Warburg's method. 0.1 ml of a suspension of ground cortex (1 part in 3 parts of water) in 5.0 ml 0.025 M sodium bicarbonate saturated with 5 per cent. CO_2 in N_2

without ————,

with 1 mg. clupein sulphate .....

[1] G. A. Alles and R. C. Hawes, *Jour. Biol. Chem.*, 133: 375, 1940.

[2] O. Galehr and F. Plattner, *Pflüg. Arch. ges. Physiol.*, 218: 488, 1928.

[3] B. Mendel, D. B. Mundell and H. Rudney, *Biochem. Jour.*, 37: 473, 1943.

[4] D. B. Mundell, *Nature*, 153: 557, 1944.

[5] B. Mendel and H. Rudney, *Biochem. Jour.*, 37: 59, 1943.

[6] B. Mendel and D. B. Mundell, *Biochem. Jour.*, 37: 64, 1943.

[7] F. Strelitz, *Biochem. Jour.*, 38: 86, 1944.

[8] B. Mendel and R. B. Hawkins. To be published shortly.

[9] B. Mendel and H. Rudney, paper presented before Toronto Biochemical and Biophysical Society, April 15, 1943.

charged colloids, *e.g.*, gum acacia, to the protamine-treated true cholinesterase restores the original activity-substrate concentration relationship.

The change in the activity curve of the true cholinesterase, brought about by the addition of protamines, in no way affects the fundamental property of the enzyme, its specificity towards choline esters. This shows that the activity-substrate concentration relationship is but a secondary characteristic of the enzyme, determined by its physical environment, whereas the specificity of the enzyme, unchangeable irrespective of environmental conditions, is an inherent property of the true cholinesterase.

Thus, a classification of cholinesterases according to their locale or to their activity-substrate concentration relationship, though seemingly expedient, is at variance with the facts and will inevitably lead to confusion. Specificity alone, therefore, remains the true criterion for a differentiation of cholinesterases.

Alles and Hawes contend that "the findings of Glick on the behavior of the enzyme of the cat superior cervical ganglion, make the acceptance of 'pseudo-cholinesterase' as a suitable name for the serum enzyme seem inadvisable."[10] If the enzyme activity of the superior cervical ganglion were, in fact, due to pseudo-cholinesterase alone, such findings would indeed support the contention of Alles and Hawes. Our experiments, however, have shown that a mixture of both cholinesterases occurs in this ganglion, the true cholinesterase being present in considerable amounts in the ganglion of the cat and predominating in that of the dog.[11]

Further experiments performed by us on pseudo-cholinesterase have tended to minimize the importance of this enzyme. We have found that blood[3] and tissues[12] of ox and sheep do not contain any pseudo-cholinesterase. In rats, moreover, we have been able to reduce the activity of the pseudo-cholinesterase of serum and tissues considerably without effecting any noticeable physiological changes. An 80 per cent. reduction of the pseudo-cholinesterase level in the rat can be brought about by the oral administration of 5 g/Kg of tri-ortho-cresyl phosphate. This chemical, which is toxic to rabbits, was found by Hottinger and Bloch[13] to reduce their cholinesterase level; it does not, however, produce any symptoms in rats, whose true cholinesterase, according to our experiments,[12] is insensitive to tri-ortho-cresyl phosphate—a fact which may help to explain the absence of toxic effects in these animals.

Despite the above findings, the name pseudo-cholinesterase was not chosen to detract from the significance of the non-specific enzyme. The term cholinesterase was retained in order to provide continuity with the earlier mass of literature on this subject, while the prefix "pseudo-" was selected to emphasize the intrinsic property of non-specificity and to avoid the hitherto indiscriminate application of the term cholinesterase, suggestive of specificity towards choline esters.

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[10] G. A. Alles and R. C. Hawes, SCIENCE, 100: 75, 1944.
[11] H. Rudney. To be published shortly.
[12] B. Mendel, J. M. Gunter and E. Mortimer. To be published shortly.
[13] A. Hottinger and H. Bloch, *Helv. Chim. Acta*, 26: 142, 1943.

THE ANTIBACTERIAL ACTION OF PENICILLIN AGAINST GRAM NEGATIVE ORGANISMS[1]

SINCE the discovery of penicillin and the subsequent demonstration of its antibacterial activity *in vivo*, considerable interest has centered on the group of bacterial agents susceptible to its action. Fleming reported in 1929[2] that penicillin possessed a marked bacteriostatic effect against many of the Gram positive organisms, including staphylococci, streptococci and the diphtheria bacillus. This observation was confirmed by Chain *et al.*[3] in 1940 and the list of susceptible organisms extended by Hobby *et al.*[4] and by other workers. With the exception of the meningococcus and gonococcus, however, no activity could be demonstrated against Gram negative organisms.

In 1941 Kocholaty[5] demonstrated that *Penicillium notatum*, from which penicillin is formed, produced a second substance, notatin (also known as penatin, penicillin B or *E. coli* factor) which possessed an antibacterial action against Gram negative as well as Gram positive organisms. It was subsequently shown, however, that notatin is an enzyme effective only *in vitro* in the presence of glucose.

In 1944 Helmholz and Sung[6] demonstrated a weak bactericidal effect of penicillin in urine on *Streptococcus fecalis* and on *Proteus ammoniae* but not on *E. coli* or Aerobacter aerogenes.

In the present communication preliminary data are presented to demonstrate that penicillin produced by *Penicillium notatum* or *Penicillium chrysogenum* possesses an antibacterial action *in vitro* against other Gram negative organisms and is effective in the absence of glucose.

[1] From the Biological Laboratory of Charles Pfizer and Co., Brooklyn, N. Y.
[2] A. Fleming, *Brit. Jour. Exp. Path.*, 10: 226, 1929.
[3] E. Chain *et al.*, *Lancet*, 2: 226, 1940.
[4] G. L. Hobby, K. Meyer and E. Chaffee, *Proc. Soc. Exp. Biol. and Med.*, 50: 227, 1942.
[5] W. Kocholaty, *Jour. Bact.*, 46: 313, 1943; *Arch. Biochem.*, 2: 73, 1943.
[6] H. F. Helmholz and Chieh Sung, *Proc.* of the Staff Meetings of The Mayo Clinic 19(14): 370, 1944.