# LXXII. PROPERTIES OF CHOLINE ESTERASE IN HUMAN SERUM

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The importance of the physiologically active substance acetylcholine makes it highly desirable that the properties of the enzyme which specifically hydrolyses choline esters be established. Summaries of the past work on this enzyme have recently appeared [Ammon, 1935; Gaddum, 1935; Oppenheimer, 1935]. It is remarkable that although choline esterase has been studied in relation to its partial purification, distribution within the organism, variations in different species, inhibition by compounds of pharmacological interest and in connexion with normal and abnormal physiological functions, some of its most fundamental properties have not yet been adequately investigated. In the present study the activity-*p*H relationship has been definitely established, and the affinity of the enzyme for acetylcholine has been measured.

## Method

The activity of choline esterase may be measured by either pharmacological or chemical methods. In the former, the quantity of unhydrolysed choline ester is estimated by its action upon a sensitive tissue, and in the latter the acid liberated by hydrolysis may be determined either by manometric measurement of the $CO_2$ set free from a bicarbonate buffer [Ammon, 1934; Stedman & Stedman, 1935] or by titration with standard alkali [Stedman *et al.* 1932]. The chemical methods are more exact, may be used over a wider range of conditions and are simpler than the pharmacological procedures. The objection to the titration method made by Bernheim & Bernheim [1936] that it "requires relatively large amounts of acetylcholine and is thus less suitable for the study of the action of inhibitors when such substances may be competing with the acetylcholine for the surface of the enzyme" is not a very serious one. If an inhibiting substance is competing with the substrate for a given enzyme surface, the inhibition produced will be a function of the ratio of the concentrations of inhibitor and substrate. Hence even with a larger amount of substrate the same degree of inhibition may be obtained by suitably changing the amount of inhibitor.

In the course of the present investigation it was necessary to measure the enzyme activity at various hydrogen ion concentrations. The manometric method was inadequate since the *p*H range over which it may be used is greatly limited. The titration method used by Stedman *et al.* [1932] based on the continuous addition of standard alkali to a buffer-free mixture of enzyme and substrate in order to neutralize the acid as fast as it is formed, might have been employed with various indicators for the different *p*H regions. However, there is the possibility that indicators may influence the enzyme as Bamann & Schmeller [1931] have demonstrated for liver esterase. Furthermore, the continuous matching of the colour of the reaction mixture with a standard of known *p*H is a none too exact procedure. Another possibility for the titration method would be to maintain the reaction mixtures at the different *p*H values by a series of suitable buffers, but in this case there is the danger that various buffers would affect the

enzyme in different ways as has been shown for liver esterase [Sobotka & Glick, 1934, 2].

The ideal method would involve measurement of the effects of the interaction of enzyme and substrate in a solution of these two constituents only by a process which would introduce no foreign substances into the mixture or alter the conditions of the reaction. As an approach to this ideal the following procedure was finally adopted.

All measurements were conducted in a constant temperature room maintained at 25° after sufficient time had elapsed for the solutions and vessels to acquire constant temperature.

For the study of the *p*H-activity relationship 0·2 ml. of serum from normal individuals from 20 to 30 years of age was added to 20 ml. of a freshly prepared 0·2% acetylcholine chloride[1] solution. A drop or two of 0·1 *N* NaOH or HCl were added to bring the *p*H to the desired value as measured by a glass electrode (where the adjustment required was slight, 0·01 *N* acid or alkali was used). The glass electrode was left in the reaction vessel during the experiment so that the *p*H might be maintained at a practically constant value by dropping in 0·02 or 0·01 *N* NaOH (depending on the speed of the hydrolysis) from a burette fixed over the vessel. This burette delivered 52 drops per ml., and the number of drops required in each 5 min. period for a total of 30 min. was recorded. Stirring was accomplished by a few up-and-down movements of a glass rod bent into a circle around the glass electrode. With the Lindemann electrometer used, the *p*H could be kept within ±0·1 unit in most cases. Control experiments conducted in

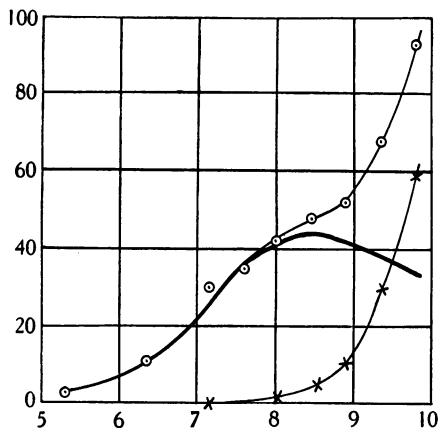


Fig. 1.

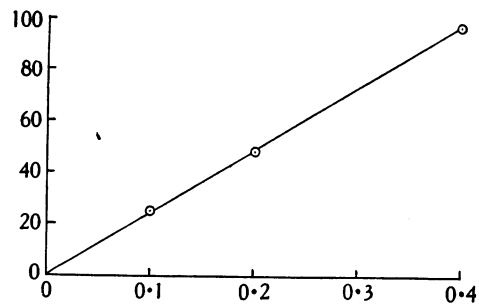


Fig. 2.

Fig. 1. Activity-*p*H curve for choline esterase. Upper curve, total hydrolysis. Lower curve, non-enzymic hydrolysis. Middle curve, enzymic hydrolysis. Abscissa: *p*H. Ordinate: enzyme activity (no. drops 0·02 *N* NaOH for 30 min.).

Fig. 2. Activity-amount of enzyme curve. Abscissa: ml. serum. Ordinate: enzyme activity (no. drops 0·02 *N* NaOH for 30 min.).

the same manner, but without serum, were performed to determine the hydrolysis effected by the hydroxyl ions alone. The activity observed was practically directly proportional to time throughout the 30 min. period in every case. The characteristic curve obtained in this fashion is shown in Fig. 1.

[1] The pure Kahlbaum product was used after it had been kept for several days *in vacuo* over $H_2SO_4$ in a desiccator.

From Fig. 2 it may be seen that in the range of the concentration of serum used in the above experiments there is a linear relation between the activity and the concentration of enzyme. These measurements were carried out at *p*H 8·4 in the manner already described.

The activity-*p*S relationships were investigated by measuring the activity of 0·2 ml. serum upon 20 ml. substrate solution at *p*H 8·4. The substrate concentrations ranged from 0·166 to 0·00111 *M*.

The points in Fig. 3 were obtained from the data in Table I in the manner described in an earlier study [Sobotka & Glick, 1934, 1]. The curve represents the theoretical relations for the dissociation constant of 0·0011. The points are derived from the experimental data, and the extent of the agreement between the theoretical and observed results may be seen from the figure.

### *The activity-*p*H relation*

An attempt at the estimation of the activity-*p*H relation for the enzyme in human serum was made by Plattner *et al.* [1928] using a pharmacological method. However, their work did not establish a definite *p*H optimum. Bernheim & Bernheim [1936], also using a pharmacological procedure, recently reported a *p*H optimum of 8·4 for the serum and brain enzymes of some lower animals. Though they worked with rabbit, rat, guinea-pig, cat and dog, it is not clear whether their activity-*p*H studies were conducted on all, or only on some, of these animals. The peak of their curve is rather sharp, whereas in the curve presented here, a rounded, less sudden optimum may be seen (Fig. 1). The maximum activity was however found at the same *p*H.

### *The activity-*p*S relation*

The difference in activity observed for different substrate concentrations has been explained best by the theory of Michaelis & Menten [1913] that enzyme and substrate combine to form an intermediate compound which in turn yields the original enzyme and the products of the reaction. The equilibrium, or Michaelis, constant, for the reaction leading to the formation of the intermediate compound is a measure of the affinity between the active enzyme group and the substrate (the affinity is inversely proportional to the Michaelis constant). In the present case it may be seen that the Michaelis constant ($K_s$) for the action of the enzyme upon acetylcholine is 0·0011.

If the affinity of human serum choline esterase for acetylcholine is compared with the affinity of liver esterase from various species for methyl butyrate as found by Bamann & Schmeller [1929] it may be seen that the affinity of the former is about 30 times that of the latter in the case of horse liver ($K_s = > 0{\cdot}03$), about 20 times for the ox organ ($K_s = > 0{\cdot}02$), about 8 times for human liver ($K_s = 0{\cdot}008$–$0{\cdot}009$), about 2·5 times for dog ($K_s = 0{\cdot}0028$) and about equal for sheep and rabbit ($K_s = 0{\cdot}0013$ and 0·0010 respectively). Compared with the affinity between hog liver esterase and methyl butyrate ($K_s = 0{\cdot}0020$) [Sobotka & Glick, 1934, 1] the choline esterase-acetylcholine affinity was about twice as great.

One of the characteristics of liver esterase is the inhibition observed in the presence of higher concentrations of substrate [Bamann & Schmeller, 1931; Murray, 1930]. That choline esterase differs in this respect was shown by Stedman & Stedman [1935] who observed small increases in activity as the acetylcholine chloride concentration was increased from 2 to over 16%. From the data in Table I and the curve of Fig. 3 it may be seen that at a substrate concentration

Table I. *The hydrolytic activity of human serum upon acetylcholine at various concentrations*

| *M* concentration acetylcholine... | 0·166 | 0·0554 | 0·0222 | 0·0111 | 0·00554 | 0·00277 | 0·00111 |
|---|---|---|---|---|---|---|---|
| No. drops of 0·02 *N* NaOH after (min.) | Total hydrolysis | | | | | | |
| 5 | 15 | 10 | 8 | 7 | 6 | 5 | 4 |
| 10 | 30 | 20 | 16 | 14 | 12 | 10 | 8 |
| 15 | 45 | 30 | 24 | 21 | 18 | 15 | 12 |
| 20 | 60 | 39 | 32 | 28 | 24 | 21 | 15 |
| 25 | 75 | 49 | 40 | 35 | 30 | 26 | 18 |
| 30 | 89 | 59 | 48 | 42 | 36 | 31 | 22 |
| | Hydrolysis without enzyme | | | | | | |
| 5 | 8 | 4 | 2 | 1 | 1 | 1 | 1 |
| 10 | 17 | 7 | 3 | 2 | 1 | 1 | 1 |
| 15 | 25 | 10 | 5 | 3 | 1 | 1 | 1 |
| 20 | 33 | 13 | 6 | 4 | 2 | 1 | 1 |
| 25 | 41 | 16 | 8 | 4 | 2 | 2 | 1 |
| 30 | 49 | 19 | 9 | 5 | 3 | 2 | 1 |
| | Enzyme hydrolysis after 30 min. | | | | | | |
| | 40 | 40 | 39 | 37 | 33 | 29 | 21 |
| Relative initial velocity (a factor, 2·45 × no. drops representing enzyme hydrolysis after 30 min. | 98·0 | 98·0 | 94·6 | 90·6 | 80·8 | 71·0 | 51·4 |
| | $K_s = 0{\cdot}0011$ | | | | | | |

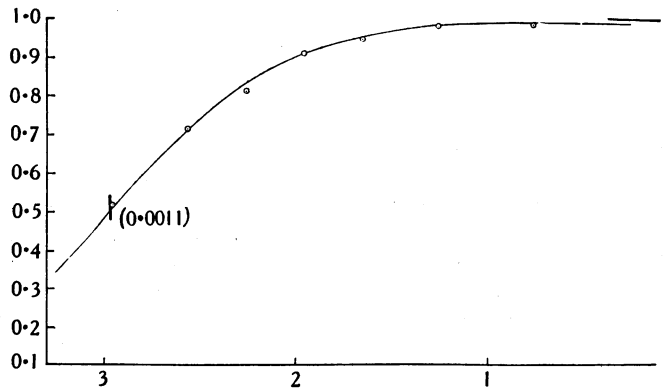


Fig. 3. Activity-*p*S curve. Abscissa: *p*S. Ordinate: relative initial velocity.

of 0·0554 *M* (corresponding to a 1 % solution) the maximum velocity of reaction has been practically attained. The absence of a drop in the curve at higher substrate concentrations confirms the finding of Stedman & Stedman that excess-substrate inhibition does not occur with this enzyme.

## Summary

A modification of the continuous titration method used by Stedman *et al.* was made in order that choline esterase activity might be measured in a solution containing only the enzyme and substrate.

The activity-*p*H relation for the enzyme in human serum was established. A maximum was observed at *p*H 8·4–8·5.

The affinity of this enzyme for acetylcholine was measured, and the dissociation constant was found to be 0·0011.

The absence of excess-substrate inhibition of choline esterase was confirmed.

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