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Short Communications

Calibration of an Electrometric Method for the Determination of Cholinesterase Activity

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The intention of this investigation was to test the accuracy of Michel's [1] method for the determination of cholinesterase activity by means of continuous registration of the pH change. Michel determines the pH change in a buffered solution caused by the acetic acid from the hydrolysis of acetylcholine. He is of the opinion that the rate of the pH change is a satisfactory measure of the enzyme activity, which is true if the interest is limited to the comparison of different enzyme activities determined in the same buffer solution. However, it seemed more reasonable to use the change in the acetic acid concentration per unit time. This measure makes it possible to compare results obtained from different buffer solutions. Furthermore, we will also describe a method that makes it possible to convert measured pH changes into changes of the acetic acid concentration.

Apparatus. For the determination of pH we used a glass electrode in combination with a saturated calomel electrode. This cell was connected to a potentiometer of the type PHM 22d, Radiometer, Copenhagen. To make it possible to record the continuous change in pH a "Brown recorder" was connected to the potentiometer.

The determination of the change in the acetic acid concentration as a function of pH demanded the use of an "Agla micrometer syringe" driven by a synchronous motor and giving 10 μl per minute. Temperature was kept at 25° C in the solutions by an oil thermostat.

Experiments. The buffer used was prepared according to Michel and had the following composition. 4.1236 g (0.02 moles) sodium barbital, 0.5446 g (0.004 moles) KH_2PO_4 and 44.730 g (0.60 moles) KCl were dissolved in 900 ml of water. 10 ml of 0.1000 *M* HCl were added and the solution made up to 1 000 ml with water. The water used in all solutions was redistilled in pyrex glass ware.

The enzyme preparation used was a hemolysate of human erythrocytes prepared according to Augustinsson [2]. Heparinised blood was centrifuged and the corpules washed twice with 0.9 % NaCl and made up to blood volume with water. 1 ml of this solution was diluted to 20 ml with water. Acetylcholine chloride was used as a substrate of which a 0.11 *M* solution was made immediately before addition.

3 ml of the erythrocyte hemolysate and 3 ml of buffer were mixed. They were then placed in the oil bath for 15 minutes to obtain temperature equilibrium. After that 0.6 ml acetylcholine solution was added and the pH registration started. In experiments with tetraethyl pyrophosphate (TEPP) the inhibitor was added half an hour before the acetylcholine.

The apparatus was calibrated in the following manner. The pH scale was set against three standard buffer solutions (pH 7.98, 7.40 and 7.00). By means of the

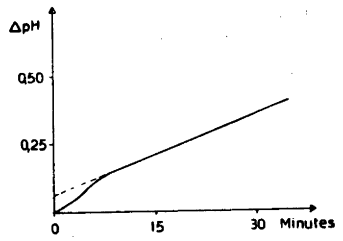

Fig. 1. The variation of pH as recorded by our apparatus in an experiment with uninhibited cholinesterase.

micrometer syringe acetic acid was infused in a solution where the substrate was substituted by water of the same volume. The acetic acid was 0.1379 *M*. The rate of the addition of the acetic acid was of the same magnitude as by the enzymatic decomposition.

Results. The method was tested in an experiment, where the enzymatic activity was determined as a function of various TEPP concentrations. The activities were then determined from the slopes of the lines obtained by the "Brown recorder". The velocity of the recorder paper (0.332 cm/minute) and the aforementioned calibration diagram can be used for the calculation of the "activities" expressed in μ moles of acetic acid per ml per minute.

A deviation from the straight line obtained by the recorder was observed during the first five minutes (see Fig. 1), and this deviation became more permanent, if the

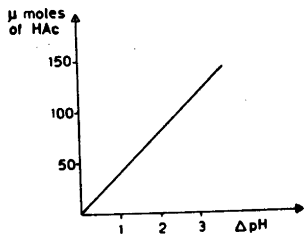

Fig. 2. The calibration curve gives μ moles of acetic acid as a function of pH.

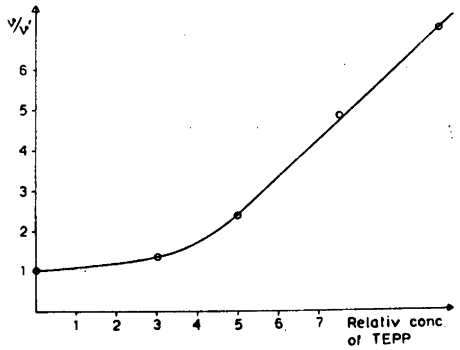

Fig. 3. Inhibition of erythrocyte cholinesterase as a function of the TEPP concentration.
v = reaction rate with uninhibited enzyme.
v' = » » » inhibited enzyme.
1 rel. unit for TEPP conc. = $2.86 \cdot 10^{-9}$ M.

protein content was increased. In experiments with enzyme preparations of low activity, where the concentration of the hemolysate must be increased in order to obtain a measurable slope of the line this deviation may cause erroneous determinations. Under any circumstances it is not advisable to use the points taken during the first five minutes, if point measurements are made according to Michel [1] or Davies and Rutland [3]. A buffer solution which is known not to contain inhibiting substances [4–5] but contains activators as calcium and magnesium ions and which is more sensitive to changes in the hydrogen ion concentration will possibly make this method more useful for enzyme solutions of low activity.

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