RESEARCH PAPERS

A NEW METHOD FOR THE ESTIMATION OF HISTAMINASE ACTIVITY

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The effects of enzyme and substrate concentrations on the rate of inactivation of histamine by histaminase of rat ileum have been investigated. The fact that the time taken for one-half of the histamine to be destroyed is inversely proportional to the enzyme content of the incubation mixture has been used as a basis for a new method for the estimation of histaminase activity in tissue extracts.

WICKSELL in 1949 described a simple biological method for estimating the histaminolytic activity of various tissues, and several workers have used this method (e.g. Carlsten and Wood, 1950; Haeger and Kahlson, 1952; Paratt and West, 1960). However, the rate at which histamine is destroyed by histaminase in rat tissues is not constant, as the relationship is exponential. To express activity in terms of the amount of histamine destroyed in unit time by a unit amount of tissue is therefore not accurate, and consequently activities determined in different laboratories by different workers vary widely.

The present paper describes experiments using ileum histaminase of the rat to obtain a linear relationship between histamine concentration and duration of incubation. During this work, the effect of changes both in enzyme and in substrate concentration on the rate of histamine destruction have also been investigated.

METHODS

Source of enzyme. Male Wistar albino rats, weighing 120–160 g. were used. The small intestine was removed from groups of 4 or 5 rats immediately after killing by a blow on the head. The tissue was washed with normal saline solution, the first 6 in. and the last 2 in. being discarded. It was then weighed.

Extraction of histaminase activity. The bulked tissue was ground in a glass mortar with a little sand. Tyrode solution (5 ml./g.) was added and extraction proceeded for 5 min. The suspension was then centrifuged at 2,800 r.p.m. for 10 min., and the resultant supernatant constituted the enzymic extract. Longer times of grinding, extraction and centrifugation did not increase the yield of enzyme.

Incubation. To 95 ml. of Tyrode solution in a 250 ml. conical flask, containing a known amount of histamine and equilibrated to 37°, was added 5 ml. of the histaminase extract. After shaking the flask to mix

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the contents, a first sample was immediately removed and the remainder incubated at 37° with frequent shaking. Further samples were removed at 10 min. intervals. Each sample was brought to the boil to arrest enzyme activity, cooled and stored at 4° until it was assayed for its histamine content. In experiments in which the effect of changes in enzyme or substrate concentration were investigated, different volumes or quantities of extract and histamine were used and the volume of Tyrode solution varied so that all incubation mixtures were initially 100 ml. in volume. Throughout the first 90 min. of incubation, the mixture remained within the limits pH 7·0 to 7·4.

Estimation of histamine. The samples were assayed directly on the atropinised ileum of the guinea-pig. All estimations were in duplicate and the means have been used to compile the Figures and Table I.

Results

Inactivation of histamine by ileum histaminase. Using an initial histamine concentration of 1·04 μg./ml. in the mixture and an extract from 1·0 g of rat ileum, the rate of inactivation of histamine was slowly reduced throughout the period of incubation. An exponential relationship between histamine concentration and time of incubation was produced (see Fig. 1). Experiments using initial histamine concentrations

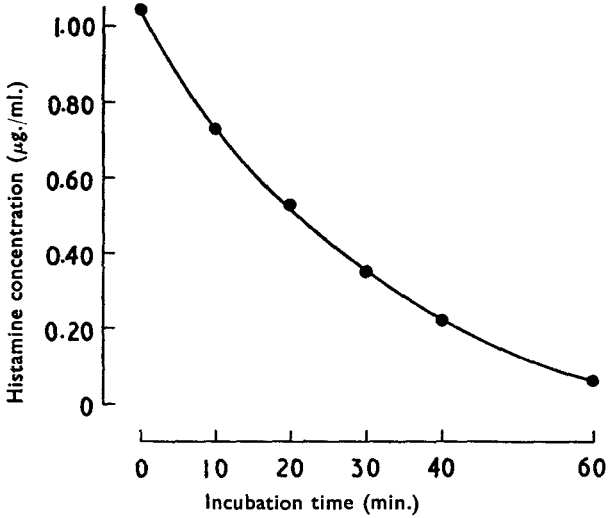

FIG. 1. Rate of inactivation of histamine by histaminase of rat ileum at 37°. Plot of histamine concentration (μg./ml.) against incubation time (min.).

of approximately 0·5, 1·5, 2·0, 2·5 and 5·0 μg./ml., or with histaminase activity from 0·5 to 2·0 g. of rat ileum tissue produced similar exponential relationships.

Huennekens (1953) has stated that observations on the rates of enzyme-catalysed reactions have disclosed two fundamental features: (*a*) at constant initial substrate concentration, the rate is proportional to the

enzyme concentration over a wide range, and (*b*) at constant enzyme concentration the rate increases (or decreases) with the concentration of the substrate in conformance with the curve of a rectangular hyperbola, eventually levelling off asymptotically. Providing the initial substrate concentration remains low, then at constant enzyme concentration the reaction should be a first order reaction with respect to the substrate. If the above destruction of histamine is a first-order (or pseudo first-order) reaction under the conditions of the experiment, then from the simplified equation for a first-order reaction,

$$k = \frac{2{\cdot}303 \log_{10} (x_o/x_t)}{t}$$

then a plot of $\log_{10} (x_o/x_t)$ against t should produce a straight line relationship. (x_o and x_t are the initial histamine concentration and the concentration after incubation for time t, respectively).

Fig. 2 shows the results from Fig. 1 expressed in this manner. A linear relationship exists for most of the reaction, and this fact has been utilised as a basis for the estimation of histaminase activity.

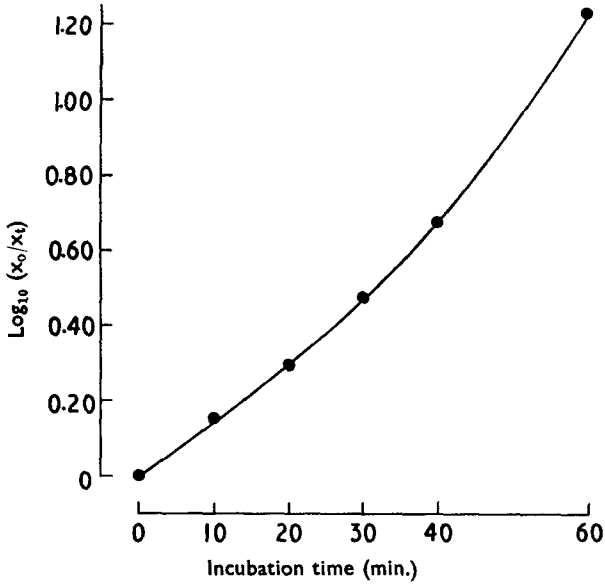

FIG. 2. Rate of inactivation of histamine by histaminase of rat ileum at 37°. Plot of $\log_{10} (x_o/x_t)$ against incubation time (min.).

Effect of changes in enzyme concentration. In a second series of experiments, the effect of varying the enzyme content using a constant initial histamine concentration was investigated. Results from a typical experiment are presented in Fig. 3.

Incubation mixtures containing 1·3 (A), 1·0 (B), 0·8 (C) and 0·5 g. (D) of ileum tissue and an initial histamine concentration of approximately 2·00 μg./ml. were sampled after 0, 20, 40 and 60 min. incubation

at 37°. By plotting $\log_{10}(x_o/x_t)$ against time, linear relationships are obtained in all incubations for at least 40 min., and in those mixtures containing the two lower enzyme concentrations (C and D) the graphs are still linear after 60 min. of incubation. In Fig. 3, a line has been drawn through the 50 per cent destruction level (where $\log_{10}(x_o/x_t) = 0{\cdot}3010$), and the times obtained at which 50 per cent of the histamine was destroyed (the DT50 values) (see Table I). By comparing incubations A, C and D with B (1·0 g. of tissue), time ratios of 1·27, 1·00, 0·82 and 0·53 were obtained, and these agree very closely with enzyme ratios in the four incubations of 1·30, 1·00, 0·80 and 0·50, respectively. Thus the time taken to reach 50 per cent destruction (or any other point on the linear part of the graphs) is inversely proportional to the enzyme concentration.

TABLE I

EFFECT OF TISSUE (ENZYME) CONTENT ON THE DT50 TIMES OF 4 INCUBATION MIXTURES, A, B, C AND D, WITH SIMILAR INITIAL HISTAMINE CONCENTRATIONS OF APPROXIMATELY 2 μG./ML.

Inbucation mixture	A	B	C	D
ml. of extract added	6·50	5·00	4·00	2·50
Amount of ileum tissue (g.) ..	1·30	1·00	0·80	0·50
DT50 values (min.)	22	28	34	53
Ratio of values (B = 1)	1·27*	1·00	0·82	0·53

* $\frac{\text{DT50 B}}{\text{DT50 A}} = \frac{28}{22} = 1{\cdot}27$

The relationship is to be expected theoretically. From the original statement by Huennekens (1953), the rate of reaction is proportional to the enzyme concentration, i.e. $k \propto E$. But in the equation, $k = (2{\cdot}303/t).\log_{10}(x_o/x_t)$, when $\log_{10}(x_o/x_t)$ is made a constant (for example 50 per cent destruction), then $k \propto 1/t$. Therefore E (enzyme concentration) $\propto 1/t$, and this is shown in Fig. 3.

Effect of changes in initial histamine concentration. The extraction of varying quantities of endogenous histamine during preparation of the enzyme extract resulted in a variable initial histamine content of an incubation mixture. The rate of inactivation of histamine in mixtures containing different amounts of initial histamine and constant amounts of enzyme, was therefore investigated.

Three incubation mixtures containing initial histamine concentrations of 2·49, 2·01 and 1·53 μg./ml. and enzyme from 1·0 g. of tissue, were followed for up to 60 min. incubation. Fig. 4 shows the conventional plot of histamine concentration against length of incubation, and three distinct rates of inactivation are seen. However, when the results are plotted by a different method (that is $\log_{10}(x_o/x_t)$ against t), three superimposable graphs are obtained, with a common DT50 time of 26 min. (Fig. 5).

When a wider range of initial histamine concentrations was investigated, the graphs were superimposable and only deviated markedly at incubation times beyond 40 min.

Suggested method of calculating histaminase activity. In the experiments described above, the rate of histamine inactivation, expressed as μg./hr., varied not only with different enzyme concentrations, but also with different histamine concentrations. Thus the rate varied between

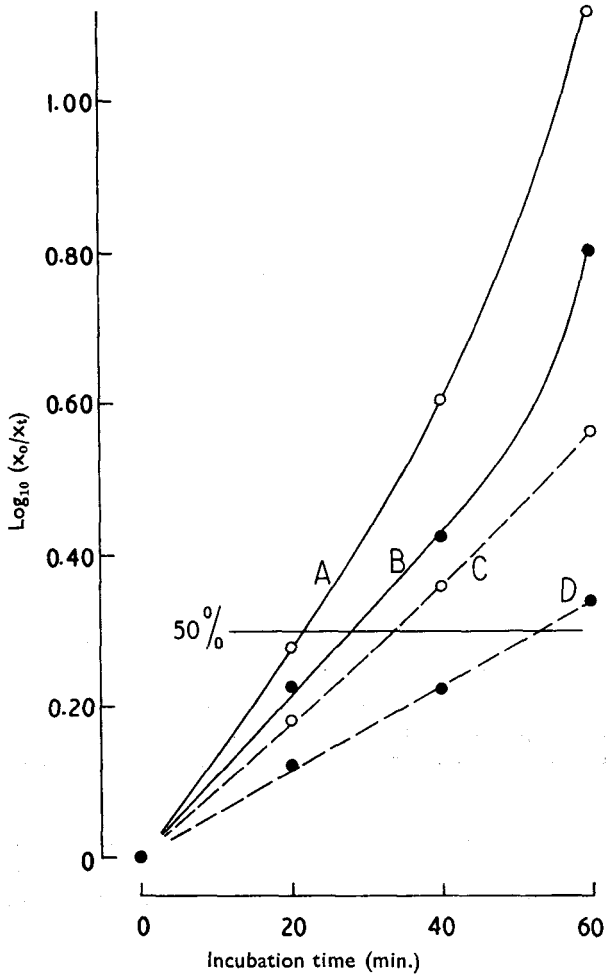

FIG. 3. Effect of changes in enzyme content on the rate of inactivation of histamine. A, B, C and D contain the enzyme activity from 1·30, 1·00, 0·80 and 0·50 g. of ileum tissue respectively. Plot of $\log_{10}(x_0/x_t)$ against incubation time (min.).

mixtures containing the same amount of enzyme, and also in the same mixture when examined at different times. To express enzyme activity as μg. of histamine inactivated in 1 hr. by 1 g. of tissue is therefore impossible under these conditions.

However, by plotting $\log_{10}(x_0/x_t)$ against t, then within a fairly wide range of initial histamine concentrations, the time taken for 50 per cent

of the histamine to be inactivated was a constant for a given amount of tissue.

that is, E (enzyme concentration) $\propto$ 1/DT50

The following method is proposed to determine enzyme activity. Histamine concentrations are determined at 0 min. and various time intervals afterwards and a plot of $\log_{10}(x_0/x_t)$ against t is made. A linear relationship should exist over most of the reaction, at least as far

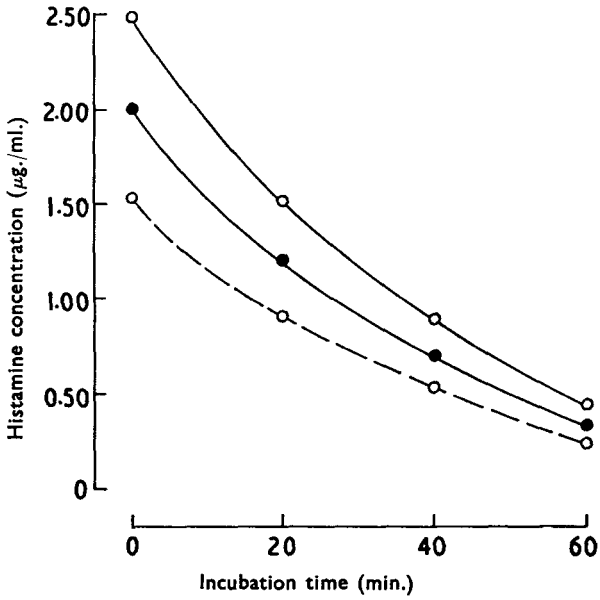

FIG. 4. Effect of changes in initial substrate concentration on the rate of inactivation of histamine. Plot of histamine concentration (μg./ml.) against incubation time (min.).

as 70–80 per cent inactivation. The time taken for 50 per cent (or another fixed percentage within the linear part of the graph) to be destroyed, is read; this is the DT50 value. The ratio of enzyme activity in different tissues is then:

$$\frac{E_1}{E_2} = \frac{DT50\ (2)}{DT50\ (1)}$$

As a percentage of control tissue activity,

$$E\ (\text{experimental}) = \frac{DT50\ (\text{control})}{DT50\ (\text{experimental})} \times 100$$

Using this method, maximum errors of $\pm$ 7 per cent have been obtained in experiments using known amounts of tissue-extract in different incubation mixtures. If an initial histamine concentration within the range

1·50–2·50 μg./ml. is used, then dilution before assay is possible throughout the range of samples obtained, and this excludes difficulties in assay due to interference by tissue proteins.

Enzyme activity in other tissues. Pilot experiments have been made with histaminase activity from rat lung. A similar exponential relationship exists between histamine concentration and length of incubation, and this becomes linear when plotting $\log_{10}(x_0/x_t)$ against t. Comparison by the method described above showed the lung to possess about 1/5th of the activity of the ileum. Mouse ileum and lung show similar exponential curves, and the ratio of activity is approximately the same as in the rat.

Initial experiments with 5-hydroxytryptamine and rat kidney as a source of amine oxidase suggest that a similar method may be used for estimating amine oxidase activity.

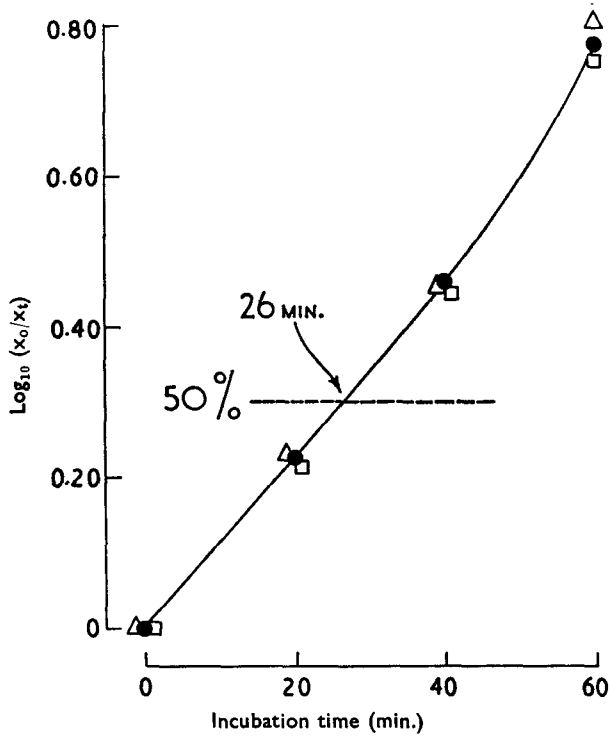

FIG. 5. Effect of changes in initial substrate concentration on the rate of inactivation histamine. Plot of $\log_{10}(x_0/x_t)$ against incubation time (min.) giving 3 superimposable graphs. Approximate initial concentrations: △ = 2·5, ● = 2·0 and □ = 1·5 μg./ml.

DISCUSSION

The results presented in this paper show that the rate, in μg./min. or μg./hr., at which histamine is inactivated by rat ileum histaminase is not constant, but decreases throughout the period of incubation. This is

not due to a progressive change in the pH of the medium, but appears to be the natural development of a pseudo first-order reaction. Examination of previous work describing biological (Wicksell, 1949) and biochemical methods (Kapeller-Adler, 1951) for estimating histaminase activity, reveals that an exponential curve is common to both types of procedure.

It is impossible to assess histaminase activity using an expression which is not constant for a particular incubation mixture, and it follows that such an assessment is a compromise and obtained under strictly controlled conditions. Wicksell (1949) for example stipulated that the rate should be calculated from data obtained when not less than 30 per cent and not more than 60 per cent of the initial histamine had been destroyed.

By converting the exponential relationship between histamine concentration and incubation time to the linear one of $\log_{10}(x_0/x_t)$ against time, a constant—the slope of the new graph—for a particular incubation mixture is obtained, and the time taken to obtain a certain proportion or percentage inactivation is dependent on the enzyme content of that mixture. This is the basis of the present method. Using this method, changes in histaminase activity after thyroxine treatment have been detected in the rat (Spencer and West, 1962a) and the mouse (Spencer and West, 1963).

The method offers the following advantages:

1. The DT50 time depends on a constant and does not change for a given concentration of enzyme.

2. The value is obtained from data from all of the samples assayed for histamine in a given mixture. Thus, one false assay only slightly affects the result, although the initial sample is perhaps the most important in any mixture.

3. Incubations containing different initial amounts of histamine can be compared, providing they fall within the range 75 per cent to 150 per cent of the mean initial histamine concentration. Varying amounts of endogenous histamine will not alter the answer.

4. A maximum experimental error of less than 10 per cent can be expected.

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