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# A New Volumetric Method for the Determination of Histaminase Activity in Biological Fluids

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Different methods for the estimation of histaminase activity in biological fluids and many different units have been proposed by various workers. The methods described were either chemical (Zeller, 1940; Zeller, Stern & Wenk, 1941; Koloszynski, 1946), manometric (Zeller, Birkhäuser, Mislin & Wenk, 1939; Zeller & Birkhäuser, 1940; Laskowski, 1942; Stephenson, 1943; Swedin, 1943*a*, *b*, 1944) or biological ones (Marcou, Chiriceanu, Cosma, Gingold & Parhon, 1938; Ungar & Parrot, 1939; Albus, 1939; Werle & Effkemann, 1940; Ahlmark, 1944; Anrep, Barsoum & Ibrahim, 1947*a*, *b*; Wicksell, 1949). These methods were applied either to purified histaminase preparations, made from hog kidneys, or to the serum, plasma or placentae of pregnant women. Since the conditions under which these tests were carried out varied widely, the results obtained by various workers can hardly be compared.

In the present investigation a simple volumetric method for the determination of histaminase activity in biological media has been developed and a new histaminase unit proposed. Moreover, an attempt has been made to correlate the results obtained for histaminase activity with chemical, manometric and biological methods by applying these methods simultaneously to a purified preparation of histaminase made from hog kidneys. Finally, the discrepancy in the behaviour of histaminase towards histamine on the one hand and towards cadaverine on the other hand, described and discussed in previous work (Kapeller-Adler, 1944, 1949), was confirmed by means of this new volumetric method. The substance of this paper was communicated at the First International Congress of Biochemistry at Cambridge in August 1949.

## METHODS

### *New volumetric method for the estimation of histaminase in biological media*

This method is a modification of Zeller's qualitative indigo test (Zeller *et al.* 1941); it is based on the theory that during the action of histaminase 1 mol. of $H_2O_2$ is formed for each mol. of histamine oxidized (Kiese, 1940; Zeller, 1938*a*, 1942; Stephenson, 1943; Swedin, 1943*a*, *b*, 1944; Kapeller-Adler, 1949).

A purified preparation of histaminase made from hog kidneys (Kapeller-Adler, 1949) was used. The activity of this preparation was expressed in Laskowski units (L.U.), one unit corresponding to the amount of enzyme which at 37°, pH 7·2, in air, with 1 mg. of histamine dihydrochloride as substrate, utilizes 1 $\mu$l. $O_2$/min. (Laskowski, 1942).

*Reagents required:* (1) M/15-phosphate buffer (Sörensen) pH 7·2. (2) Histamine solution in M/15-phosphate buffer, containing 10 mg. of histamine dihydrochloride/ml. (3) Indigo disulphonate solution. Indigo Carmine (200 mg.) is dissolved in 300 ml. water and the solution stored in a brown bottle. (4) 0·002 N-$KMnO_4$ solution. (5) $CHCl_3$.

*Procedure.* To increasing amounts of enzyme solution, placed in Pyrex test tubes (0·1–1 ml. of a solution of the histaminase preparation in phosphate buffer, corresponding to 0·0025–0·025 L.U.), 1 ml. of the indigo solution and 0·1 ml. of the histamine solution are added and the volume made up with phosphate buffer to 4 ml. $CHCl_3$ (1 drop) is added as a

preservative. $O_2$ is passed through the fluid for 1 min., the test tube is closed with a rubber stopper, the solution well mixed by shaking and incubated for 24 hr. at 37°. A control for each individual test without the addition of histamine is always carried out simultaneously with the test. After 24 hr. the controls and the tests containing an excess of the indigo compound are titrated with 0·002 N-$KMnO_4$ until the end point of the titration is reached, when the blue colour has just disappeared and a pink colour just appears and persists for a few seconds. The amount of $KMnO_4$ utilized in the assay is subtracted from the amount taken up by the control. The difference indicates the amount of $H_2O_2$ formed by the action of histaminase on histamine and thus the enzyme activity.

### *Estimation of histaminase activity of serum by the volumetric method*

Add to 1 ml. of non-haemolysed serum 2·5 ml. of phosphate buffer pH 7·2, 0·1 ml. of the histamine solution, 0·5 ml. of the indigo solution and 1 drop of $CHCl_3$. Bubble $O_2$ through for 1 min. and proceed as above. A control serum without the addition of the histamine solution is worked up simultaneously with the test serum. If possible, controls and test sera should be done in triplicate.

### *Estimation of histaminase activity in serum by a biological test*

The biological test of Anrep *et al.* (1947*a*, *b*) has been slightly modified. Place 1 ml. of serum in a small conical flask in an incubator (37°) for 5 min. Add 0·3 ml. of an aqueous solution of histamine acid phosphate containing 3·6 μg. of histamine base, and incubate for 30 min. at 37°. After that time dilute rapidly with 4·7 ml. of Ringer solution and heat the fluid to 80°. After cooling, the solution is assayed on the isolated guinea pig ileum against the control solution.

*Control test.* To 1 ml. of serum 4·7 ml. of Ringer solution are added and the fluid is heated to 80°. After cooling 0·3 ml. of histamine acid phosphate solution, containing 3·6 μg. of histamine base is added, the mixture incubated for 30 min. at 37° and then assayed on the guinea pig ileum.

Anrep's modified biological test was also used for the estimation of histaminase activity in histaminase preparations. More histamine (6·0 μg. instead of 3·6 μg. of base) was added to enzyme solutions containing more than 0·0125 L.U., and to more active sera.

## RESULTS

*Simultaneous application of the new volumetric method and Anrep's biological test to histaminase preparations.* Fig. 1, *a* and *b*, show that when both tests were simultaneously applied to increasing amounts of a purified histaminase preparation the volume of permanganate taken up and the amount of histamine inactivated was directly proportional to the amount of enzyme present. The volume of permanganate utilized was 6% more than might have been expected theoretically from the oxygen consumption.

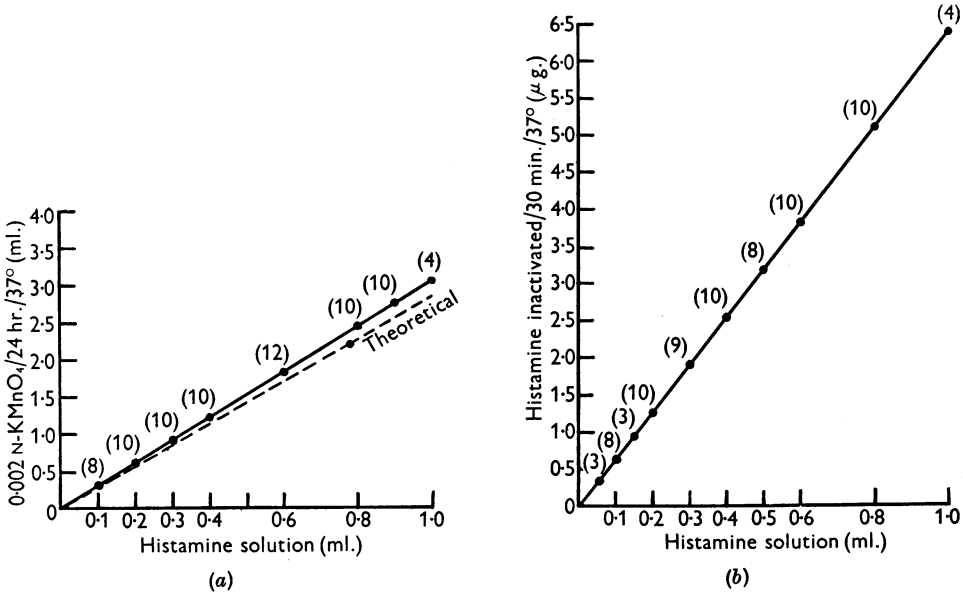


Fig. 1. Effect of increasing amounts of histaminase from hog kidneys on histamine. (*a*) Volumetric test. (*b*) Biological test. The number in brackets is the number of separate estimations on which the average result shown was calculated.

*New histaminase unit.* The results of the new volumetric test are expressed in terms of a new histaminase unit. This unit, conveniently called a permanganate unit (P.U.), represents the amount of enzyme which, after an incubation of 24 hr. at 37° and pH 7·2, in an atmosphere of oxygen with 1 mg. of histamine hydrochloride as substrate and with an

aqueous solution of indigo disulphonate, takes up 0·1 ml. 0·002N-potassium permanganate.

If this quantitative indigo test is carried out in air only about 35% of the histaminase activity in an atmosphere of oxygen is obtained. Table 1 shows

Table 1. *Activity of one permanganate unit* (P.U.)

| | Histamine inactivated (μg./hr.) | $O_2$ taken up (μl./hr.) |
|---|---|---|
| Theoretical | 0·463 | 0·053 |
| Observed | 0·417 | 0·050 |
| % of theory | 90·00 | 94·00 |

Table 2. *Comparison of permanganate units with units depending on oxygen consumption*

| | P.U. |
|---|---|
| Laskowski's unit | 1200 |
| Zeller's unit | 224 |
| Stephenson's unit | 120 |
| Torantil unit | 50 |

the rate of histamine destruction and the rate of oxygen consumption by one permanganate unit compared with values calculated on the theory that 1 mol. of histamine is equivalent to 1 mol. of hydrogen peroxide and 0·5 mol. of oxygen. 1 P.U. thus corresponds to the destruction of 0·46 μg. of histamine and to an uptake of 0·05 μl. oxygen/hr. The results obtained were 10% less for the histamine inactivation and 6% less for the oxygen uptake that might have been expected theoretically.

In Table 2 a comparison is made between the permanganate unit and units calculated on the basis of oxygen consumption.

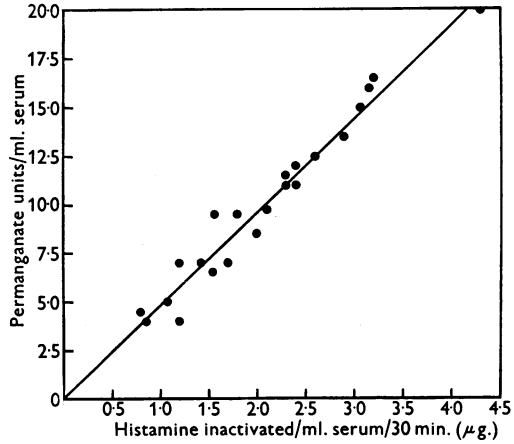


Fig. 2. Relationship between the volumetric test and the biological test when both tests were applied simultaneously to pregnancy sera.

*Estimation of histaminase activity in sera.* When the new volumetric test and Anrep's biological test were applied to non-pregnancy sera no histaminase activity was detected.

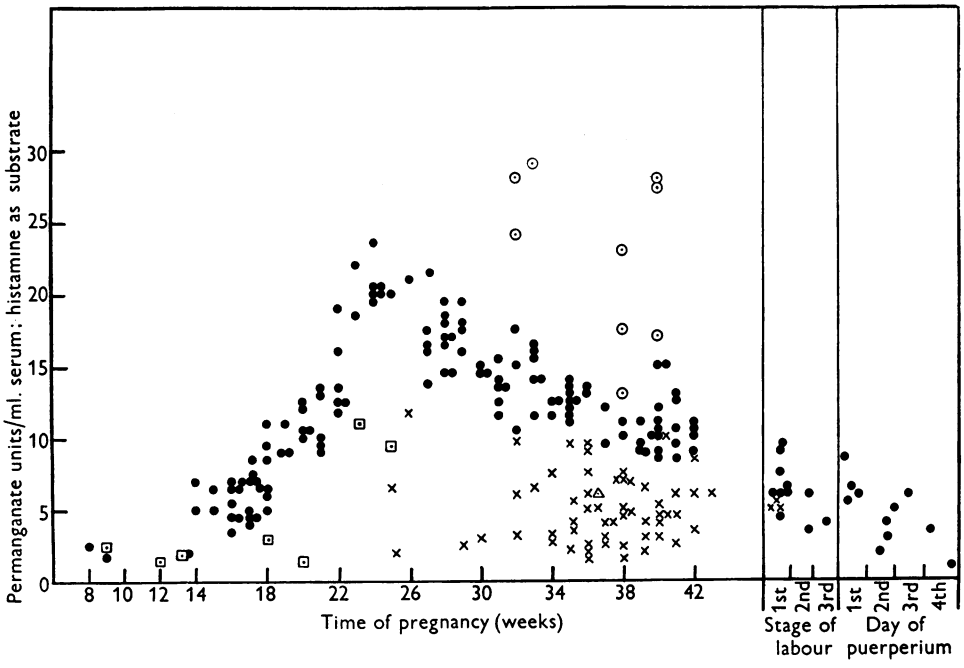


Fig. 3. Histaminase content of 1 ml. of serum of women at various stages of normal pregnancy, ●; twin pregnancy, ⊙; triplet pregnancy, △; toxaemic pregnancy, ×; and threatened abortion, ⊡. Results obtained during labour and in the puerperium are also shown.

The results found when both methods were simultaneously used for the investigation of sera from

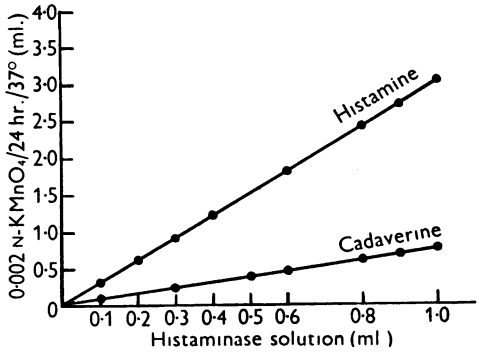


Fig. 4. Effect of histaminase on histamine and cadaverine determined by the volumetric method.

twenty-two women in various stages and conditions of pregnancy are shown in Fig. 2, the values obtained by the biological test being plotted against the values by the volumetric method. This Figure shows that a reasonably good agreement between the two methods was obtained. The central line represents the relationship between the two tests calculated from results obtained when both methods were simultaneously applied to purified enzyme preparations.

In view of the good agreement between the chemical and the biological test it was decided to use only the volumetric method for all subsequent estimations of histaminase activity. In Fig. 3 results are recorded which were obtained when this method was applied to the sera of about 230 pregnant women, women in labour and in the puerperium. The abscissa indicates weeks of pregnancy, stage of labour and days of the puerperium, and the ordinate the P.U./ml. of serum. According to the results obtained histaminase activity becomes apparent in the serum at the end of the 2nd month of pregnancy, reaches its maximum between about the 22nd and 26th week of gestation, and somewhat decreases in the 7th month of pregnancy to remain

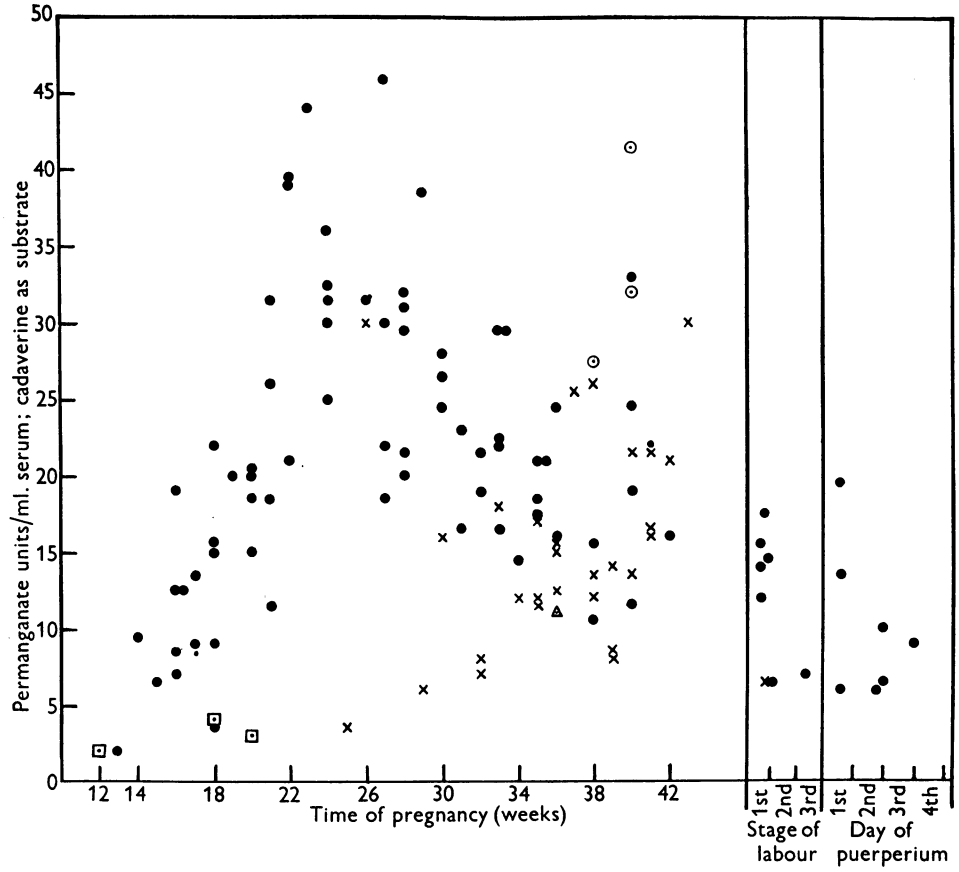


Fig. 5. Effect of serum histaminase on cadaverine in normal pregnancy, ●; twin pregnancy, ⊙; triplet pregnancy, △ toxaemic pregnancy ×; and threatened abortion, ⊡.

more or less stationary until delivery. During labour, and still more rapidly in the puerperium, histaminase activity tends to decrease. Abnormal results were found in three types of cases. Significantly low results were obtained in cases of pre-eclamptic toxaemia, the severe cases showing a very small histaminase activity. Significantly high results were found in most of the cases of twin pregnancy. An unexpected low result was encountered in a case of triplet pregnancy. In several cases of threatened abortion the estimated histaminase activity was exceedingly low.

Figure that histaminase preparations from hog kidneys are four times more active on histamine than on cadaverine.

The results shown in Fig. 5 were obtained when the volumetric test with cadaverine as substrate was applied to some of the sera of pregnant women whose histaminase action on histamine was presented in Fig. 3.

On comparing Figs. 3–5 one finds a discrepancy in the behaviour of serum histaminase and of histaminase preparations from hog kidneys towards cadaverine. Whereas the latter preparations are four

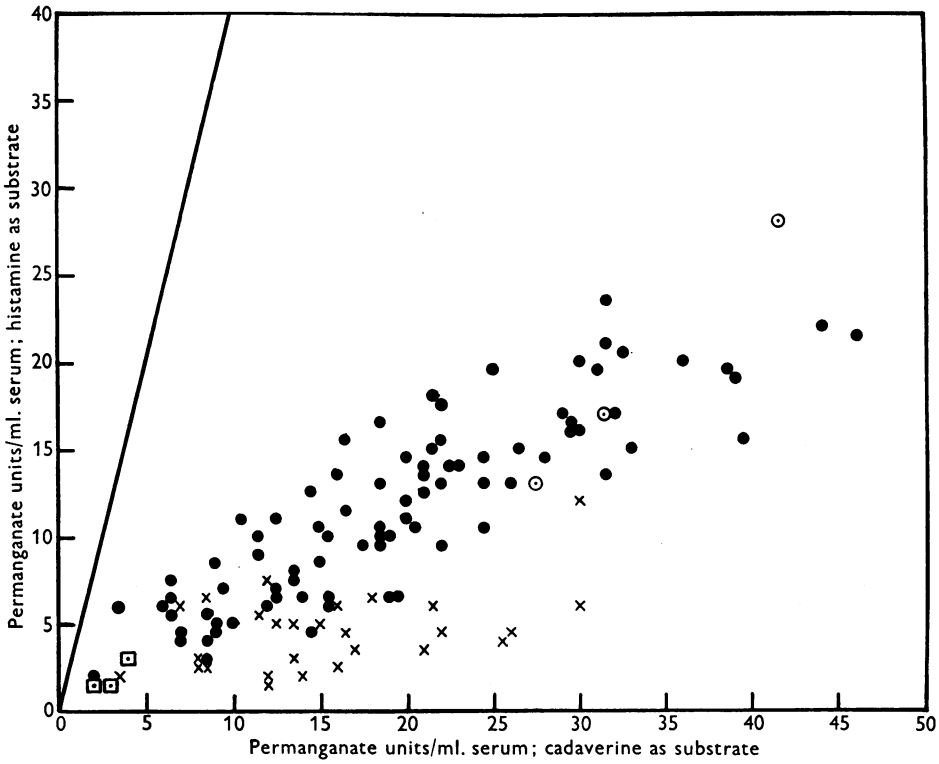


Fig. 6. Relationship between the effect of serum histaminase on histamine and that on cadaverine. Normal pregnancy, ●; twin pregnancy, ⊙; toxaemic pregnancy, ×; and threatened abortion, ⊡. The line indicates the relationship in histaminase preparations from hog kidneys.

*Effect of histaminase on cadaverine.* In view of contradictory observations concerning the action of histaminase on cadaverine (Zeller, 1938*a*, *b*; Zeller, Birkhäuser, Mislin & Wenk, 1939; Zeller, Schär & Stähli, 1939; Zeller, Stern & Wenk, 1940; Kapeller-Adler, 1944, 1949) the volumetric method was also used to study this reaction. Fig. 4 shows results obtained with various purified preparations of histaminase from hog kidneys with known activity acting on 1 mg. of histamine-dihydrochloride and 1 mg. of cadaverine dihydrochloride in parallel assays. It follows from this

times more active on histamine than on cadaverine (Fig. 4), serum histaminase is more active on cadaverine than it is on histamine. More striking results were obtained in cases of pre-eclamptic toxaemia, confirming previous observations (Kapeller-Adler, 1944). Whereas a significantly decreased enzymic activity was encountered in the sera when histamine was used as substrate, normal results were obtained in most of the toxaemic cases with cadaverine as substrate.

In Fig. 6 the values for P.U./ml. serum with cadaverine as substrate and those with histamine as

substrate are plotted against each other. If the behaviour of histaminase in pregnancy sera towards cadaverine had been identical with that of histaminase preparations from hog kidneys the results would be expected to fall on or near the line drawn on the left side of the diagram.

## DISCUSSION

For the quantitative estimation of histaminase activity in biological fluids only biological methods could be successfully used up to now. Chemical methods, mainly published by Zeller (1940) and Zeller and his co-workers (1941), did not prove satisfactory in the investigation of sera. Koloszynski (1946) tried to transform Zeller's qualitative indigo test into a colorimetric one by extracting the excess indigo dye with a mixture of acetone and saturated ammonium sulphate and measuring the dye in a colorimeter. In an attempt to repeat this work in this laboratory it was found, however, that the indigo dye did not readily dissolve in the mixture mentioned above, and very unreliable results were obtained.

The manometric method for the estimation of histaminase activity in pregnancy serum (Zeller & Birkhäuser, 1940) was not any more satisfactory. The oxygen uptake in the controls without the substrate was always very high, sometimes even higher than in the tests containing histamine as substrate. The results shown in Table 1 of the present paper furnish the explanation for the shortcomings of Zeller's manometric method. The amounts of histaminase present in pregnancy sera are far too small to show an oxygen consumption which could be accurately measured in Warburg manometers (0·25–1·25 $\mu$l. oxygen/hr.).

The best known biological method for the determination of histaminase activity in biological media is that published by Ahlmark (1944). This method, being very sensitive, allows the estimation of very small amounts of histaminase in plasma. It has, however, the disadvantage of being very tedious and requiring large amounts of material (13–17 ml. of plasma from each patient). Besides, one serious criticism has to be made when this method is used for the determination of histaminase activity in not very active plasmas. Ahlmark incubates these plasmas with histamine at 37° for up to 22 hr. without a preservative. Under these circumstances the possibility cannot be excluded that the loss of histamine might be due not to the activity of histaminase but to bacterial influence. Moreover, Anrep *et al.* (1947*a*) point out that the histaminolytic effect of non-pregnancy plasma, observed by Ahlmark, is not abolished by raising the temperature to 80° and cannot, therefore, be attributed to histaminase activity. The biological method of Anrep and his co-workers (1947*a*, *b*) in the modification mentioned above is simple and gives satisfactory results. Its main disadvantage is that it depends on animal material. Wicksell (1949) tried to simplify Ahlmark's method, but his method still remains rather complicated and does not offer any advantages over the modified Anrep method.

As to the results obtained in the present work it may be said that in normal pregnancy cases they agree quantitatively with those obtained by Ahlmark (1944). Whereas in cases of severe pre-eclamptic toxaemia a significant decrease of serum histaminase was shown with the new quantitative indigo method, Ahlmark found in cases of pregnancy toxaemia, independent of the severity of cases, normal, diminished and increased histaminase activity in the plasma. Anrep investigated twelve cases of severe pre-eclamptic toxaemia in the 35th to 41st week of gestation. Of these cases eight showed a normal histaminase activity and four a reduced one. He did not, however, find any increased histaminase activity. In cases of threatened abortion all methods indicate a significant decrease in histaminase activity. No extensive data are available in the literature referring to the increased histaminase activity in the serum of women with twin pregnancy, observed in this work. Swanberg (1948) mentions one case of a mother of twins in labour whose blood histaminase was a little higher than that of other women in labour.

In agreement with Anrep no other condition has so far been encountered in which histaminase could be detected in human blood. Nor has histaminolysis been found to occur in the blood of pregnant or non-pregnant animals. These findings emphasize the importance of the presence of histaminase in the blood during human pregnancy. It may perhaps be a prophylactic measure against a potential histamine intoxication. More work will have to be done on cases with pre-eclamptic toxaemia to find out the significance of the decreased values for serum histaminase in women with severe pre-eclamptic toxaemia.

Whereas Best, who discovered histaminase in 1929, considered this enzyme to be specific for histamine, Zeller, 1938*a*, *b* and Zeller and his co-workers in a number of papers (1939, 1940) advanced evidence that histaminase also acts on diamines such as cadaverine, putrescine and agmatine. They therefore replaced the term of histaminase by that of diamine oxidase. Though most of the subsequent workers accepted this suggestion of Zeller's school, some doubts were cast on the identity of histaminase and diamine oxidase when Zeller's qualitative indigo test was applied to the investigation of sera from women with pre-eclamptic toxaemia, and of placental extracts (Kapeller-Adler, 1944). The question was, therefore, reinvestigated (Kapeller-Adler, 1949).

From the results obtained it appeared probable that histaminase is an enzyme with various modes of behaviour towards histamine, for which it shows the greatest affinity, and towards diamines such as cadaverine, putrescine and agmatine. In the present work it has been demonstrated that histaminase preparations made from hog kidneys and serum histaminase of pregnant women differed in the relation between their actions on histamine and cadaverine. This discrepancy in the behaviour of histaminase, depending on the origin of the enzyme, suggests that the effect of histaminase on cadaverine, unlike that on histamine, is a non-specific one. In this connexion it should be mentioned that Blaschko (1949), in a recent communication, has intimated that diamine oxidase was active only on diamines up to a chain length of eight carbon atoms. Diamines with seven or more carbon atoms were, however, oxidized by amine oxidase. This enzyme did not act on diamines with six or less carbon atoms.

On account of all these observations it appears to be reasonable to drop the name of diamine oxidase and to re-establish that of histaminase for the enzyme which specifically acts on histamine.


## SUMMARY

1. A new simple volumetric method for the estimation of histaminase activity in biological media is described and a new histaminase unit, called permanganate unit (P.U.), is proposed. The effect of this new unit is compared with that of other known histaminase units. 1 P.U. inactivates 0·46 $\mu$g. of histamine/hr. with the uptake of 0·05 $\mu$l. oxygen.

2. This new method was applied to the investigation of histaminase activity in non-pregnancy sera and in sera during normal and abnormal pregnancy. The results obtained are discussed.

3. The discrepancy in the effects of histaminase on histamine and cadaverine is well shown when the new volumetric method is applied to the investigation of histaminase preparations from hog kidneys and serum histaminase.


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