Placental and Blood Histaminase in Human Pregnancy

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The histaminase content of human pregnancy serum has been investigated by a number of workers (Ahlmark, 1944; Anrep, Barsoum & Ibrahim, 1947) but the results on the whole have not been consistent and some of the methods have been criticized. Recently Kapeller-Adler (1951) has described a simple volumetric method and has suggested a new unit, the permanganate unit (P.U.). Using this method she has determined serum and placental histaminase in cases of normal and toxæmic pregnancy. The equation that is most commonly used to represent the histamine-histaminase reaction is written as follows:

Laskowski (1942) has cast some doubt on the accuracy of this equation, but the work of Stephenson (1943), though not finally establishing it, supports its principal requirements.

Kapeller-Adler's method is essentially an attempt to estimate the hydrogen peroxide by its action on a dye. Buffered serum is incubated with histamine in the presence of indigocarmine and oxygen and the hydrogen peroxide liberated decolorizes some of the dye. The mixture and a control containing no histamine are then titrated with permanganate until decolorization is complete. The difference between the volumes of permanganate required is proportional to the concentration of the enzyme, the unit being defined as the amount of enzyme which, when incubated with 1 mg. histamine for 24 hr. at 37° and pH 7.2 inactivates sufficient substrate to give a difference of 0.1 ml. 0.002N permanganate. According to the above equation this will correspond to a rate of histamine destruction of 0.463 µg./hr.

Quite apart from the question of the equation representing the reaction, the definition of the permanganate unit assumes that all the hydrogen peroxide evolved and all the permanganate used are concerned with the oxidation of the dye to the exclusion of the many other oxidizable substances present. Kapeller-Adler offers no new evidence that this is so. On the contrary, she finds that the volume of permanganate used was 6% more than that required theoretically on the basis of the volume of oxygen consumed.

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In the present investigation an attempt was made to confirm the findings of Kapeller-Adler regarding histaminase concentrations in blood and placental extracts from normal patients and from a few cases of toxæmia. Certain modifications in the technique were also studied.

MATERIAL AND METHODS

The specimens investigated were obtained from patients at the Rotunda Hospital, Dublin.

Estimations of histaminase were carried out by the method of Kapeller-Adler (1951) and extracts from placenta were prepared in the manner described by Kapeller-Adler (1952). In order to secure good agreement between triplicate estimations it was found necessary to have all apparatus scrupulously clean. Test tubes were cleaned in bichromate cleaning fluid and all rubber bungs were boiled.

Use of colorimetry in place of titration. The maximal extinction values of both the experimental and control mixtures were determined after incubation, using the Spekker photoelectric absorptiometer and Ilford Spectrum Orange (no. 607) filters, peak transmission 6000 Å. It was hoped that the difference between the extinction values could be correlated with the histaminase concentrations as determined by permanganate titration.

Ammonia estimation. Since ammonia is evolved at the same time as hydrogen peroxide in the histamine-histaminase reaction, it should be possible to estimate the concentration of enzyme by determining the amount of ammonia liberated, using the Conway (1940) microdiffusion technique.

RESULTS

Histaminase content of pregnancy serum. Eighty-three cases of normal pregnancy ranging from the 14th to the 40th week were investigated and the results are shown in Fig. 1. It will be seen that (i) different cases at the same period of pregnancy showed wide variations in serum histaminase levels, (ii) the values obtained bore no relation to the period of pregnancy, and (iii) the range of values varied from 0.6 to 10.6 P.U./ml., the majority lying between 2 and 6 P.U./ml.

Four cases of toxæmia were investigated, two at 36 weeks and two at full term. The histaminase levels were 2.0, 8.4, 3.4, and 4.8 P.U./ml. respectively.

Placental histaminase. Fourteen normal placentae were extracted and the histaminase content was determined. The results are given in Table 1, where it will be seen that values vary from 2.5 to 21 P.U./g.

Fig. 1. Histaminolytic activity of normal pregnancy serum at intervals of 2 weeks, from the fourteenth week onwards.

Table 1. *Histaminase content of normal placentae*

Results expressed in permanganate units/g. fresh tissue (P.U./g.).

Placenta (no.)	After extraction for 1 hr. at room temp. (P.U./g.)	After extraction overnight in refrigerator (P.U./g.)
1	5	—
2	10	—
3	21	—
4	15	—
5	13	—
6	12	—
7	5	6.5
8	5.5	9
9	10	20
10	12	16
11	7	13
12	8.5	11
13	2.5	10
14	5	6.5

of fresh tissue. In a single case of severe toxæmia, the placenta showed a histaminase content of 8 P.U./g.

In eight normal cases, after the placental tissue had been extracted for a period of 1 hr., as required by the Kapeller-Adler method, the extraction was allowed to proceed overnight in the refrigerator. It will be seen from the third column of Table 1 that the histaminase content of the extract at the end of this period greatly exceeded that of the 1 hr. extract and that the relative increase varied considerably from one specimen to another.

Use of colorimetry instead of titration. Satisfactory results could not be obtained by substituting colorimetry for permanganate titration. The solution

after incubation was always cloudy, and when it was filtered some of the dye was always adsorbed on the filter paper. The extent of this adsorption was variable, so that triplicate estimations rarely agreed. Furthermore, in those cases where there was agreement between triplicates, the difference in colour between the test solutions and controls was extremely small, and the probable experimental error therefore great. The modification compared unfavourably with permanganate titration from both points of view.

Ammonia estimation. The results of attempts to estimate histaminase in serum and in extracts from fresh placenta or from acetone dried placenta by determining the amount of ammonia liberated were also unsatisfactory. Triplicate estimations differed widely and the control samples frequently showed a greater evolution of ammonia than the samples containing histamine. It seems likely that these inconsistencies may have been due to the liberation of ammonia from nitrogenous constituents other than histamine by micro-organisms, as it is extremely difficult to maintain sterile conditions.

DISCUSSION

The results obtained differ from those of Kapeller-Adler, serum histaminase levels being very much lower and the scatter very much wider than reported by her. The levels showed no relationship to the period of pregnancy, whereas those of Kapeller-Adler showed a fairly steady rise from the 14th to the 24th week, followed by a fall through the remainder of the pregnancy and through labour to

Table 2. *Range of histaminolytic activity of normal pregnancy serum at intervals of 2 weeks from the 14th week onwards, as found by various investigators*

Results expressed in $\mu\text{g.}$ histamine inactivated during 1 hr. incubation ($\mu\text{g./hr.}$) by 1 ml. serum.

Week of pregnancy	Ahlmark (1944) ($\mu\text{g./hr.}$)	Kapeller-Adler (1951) ($\mu\text{g./hr.}$)	Present authors ($\mu\text{g./hr.}$)
14	1.0-2.1	0.9-3.2	0.5-1.4
16	1.1-3.0	1.6-3.2	0.7-4.5
18	1.3-4.0	2.3-5.1	2.3
20	2.0-4.0	4.6-5.6	0.6-3.9
22	2.2-6.0	5.5-8.7	1.1-2.4
24	2.0-5.2	9.0-10.8	1.8-3.4
26	2.1-5.6	9.7	0.3-2.4
28	2.1-5.0	6.7-9.0	1.2-3.8
30	3.1-4.1	6.7-6.9	1.3-4.9
32	2.0-4.3	5.1-9.3	2.6-4.2
34	1.5-6.0	5.5-6.0	0.9-2.8
36	2.0-4.0	6.0-6.4	1.1-3.1
38	2.1-4.1	4.8-5.3	1.4-2.8
40	2.0-5.6	4.1-7.1	1.6-3.1

the puerperium. Ahlmark (1944) recorded levels of histaminolytic power (determined biologically) which do not differ greatly from those reported here. His figures, those of Kapeller-Adler, and ours, all expressed in $\mu\text{g.}$ histamine inactivated/hour by 1 ml. serum are shown in Table 2.

There is a similar discrepancy between Kapeller-Adler's results and ours for normal placentae at full term. Whereas we found a range of 2.5 to 21 P.U./g. tissue, with an average of 9.4 P.U./g., Kapeller-Adler reported an average of about 10 times this figure, 99 P.U./g.

SUMMARY

1. When certain precautions were taken, the Kapeller-Adler technique gave consistent results in

the estimation of histaminase in pregnancy serum and in placental extracts. Attempts to develop a colorimetric modification or to use the ammonia evolved as a basis for the estimation were not successful.

2. In eighty-three cases of normal pregnancy, ranging from 14 to 40 weeks, serum histaminase varied from 0.6 to 10.6 permanganate units (P.U.)/ml., the majority lying between 2 and 6 P.U. The values bore no relation to the period of pregnancy and different cases at the same period showed wide variations.

3. In fourteen cases of normal full-term pregnancy, placental histaminase varied from 2.5 to 21 P.U./g. fresh tissue, the majority lying between 5 and 10 P.U. Extraction in the refrigerator overnight increased the yield of histaminase.

4. In four cases of toxæmia, serum histaminase values were within normal limits and the histaminase content of placenta in one toxæmia case was also normal.

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The Effect of Ergosterol and Ergocalciferol on the Anaerobic Glycolysis of Rat-liver Slices

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The exact biochemical role of vitamin D has not been established (Nicolaysen & Eeg-Larsen, 1953), but the vitamin is known to affect carbohydrate metabolism. In avitaminosis D, the rate of phosphorylation of carbohydrates is diminished and administration of vitamin D to rats leads to increased glycogen deposition in liver and muscle, as well as to an increased carbohydrate/lactic acid ratio in blood, liver and muscle (Pincussen, 1941). Zetter-

ström (1951) recently investigated the effect of adding phosphorylated vitamin D to a suspension of kidney mitochondria containing glutamate as oxidizable substrate, when an increased rate of oxygen uptake was found.

Simonsen & van Wagtenonk (1947) found that ergosterol increased the lowered anaerobic glycolysis rate of liver homogenates from guinea pigs suffering from the 'stiffness syndrome'. It has been