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CHANGES IN TOTAL HISTAMINE CONTENT OF WHITE BLOOD CELLS (WBC) IN ALLERGY PATIENTS

By

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In 1964 Lichstenstein & Osler (6) determined the histamine content in washed WBC, particularly from patients with hay fever. According to these authors the histamine content from any single donor remained fairly constant. The histamine content, however, varied greatly from one individual to another. In their study the cellular histamine content of most donors ranged from about 0.16-0.30 micrograms per 1.5×10^7 cells with variations from 0.1-0.8 micrograms ($= \mu\text{g} = 10^{-6} \text{ gr}$).

In 1972 Assem, Schild & Vickers investigated 5 mite allergic patients and 6 non allergic subjects. The mean histamine content of 10^8 cells from mite allergic patients was 1.78 μg histamine base per 10^8 cells. The corresponding value for non allergic persons was 1.30 μg .

Assem, Turner-Warwick, Cole & Shaw (2) have performed in vitro challenge tests of leucocytes from patients with intrinsic asthma. They showed increased histamine release on challenge with anti-IgE in 60 per cent and anti IgG in 30 per cent of patients. These authors presented results (2) suggesting that the leukocytes of patients with intrinsic asthma are similar to those of extrinsic asthmatic patients with respect to the amounts of IgE and IgG fixed to the leukocytes. Leukocyte challenge tests performed on cells from normal subjects without a history of asthma, hay fever or eczema

showed that less than 25 per cent of histamine was released on challenge with anti-IgE and less than 29 per cent on challenge with anti-IgG. The studies were performed mainly on one occasion after taking away the medical treatment for 2 days.

As far as I know, the literature has not up to now contained any reports of repeated studies of the blood histamine content of asthma patients. Since the clinical troubles of such patients can undergo considerable changes it was felt to be of interest to correlate in time the varying degrees of asthma with the histamine content of the leukocytes. It soon became evident, however, that there is no correlation between the number of leukocytes and the histamine content. The amount of histamine contained in washed leukocytes in 1 ml blood was therefore determined instead of the histamine content in a certain number of washed leukocytes.

METHODS

Patients studied:

Patients with both extrinsic and intrinsic asthma have been studied. For this study only patients have been selected who were treated in the outpatients' department or admitted into medical department II, S:t Görans' hospital on so many occasions that an evaluation of the variations in blood histamine content could easily be performed.

Twenty-two healthy persons were also studied for control.

Lung function

Peak flow, vital capacity and sometimes airway resistance were determined in close connection with the taking of blood samples for measurement of the histamine content.

Cell counts

Leukocytes (WBC), total eosinophils and platelets of both capillary blood and cell suspension were counted. Differential counts of capillary blood were performed. WBC counts (WBCc) of the cell suspensions were made before centrifugation, since after this procedure it is difficult to get reproducible values.

Histamine assay

The method used was described by Lichtenstein & Osler in 1964 (6), though with minor modifications later introduced mainly by Lichtenstein (personal information).

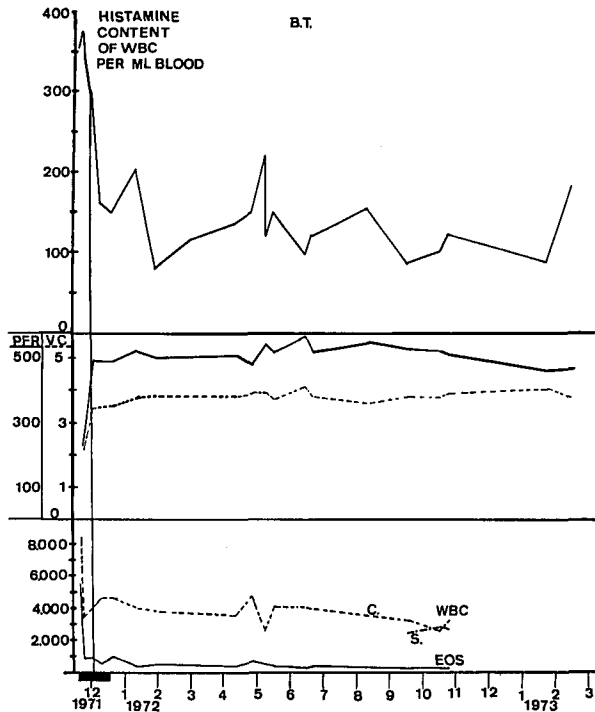


Fig. 1.

Patient 1. B.T. born 26 11 05. Male. Hay fever since childhood. Asthma since 1954, more pronounced during the last few years. Intracutaneous tests positive to house dust, wheat, animal danders, birch and timothy pollen. Nasal and conjunctival provocation tests positive to timothy and wheat. *Ordinate*: Histamine content in nanograms (10^{-9} grams) in leukocytes from 1 ml of blood. PFR l/min. VC l/min. WBC per mm^3 (C in capillary blood, S in suspension of cells before centrifugation). Eos total number per mm^3 , in capillary blood. *Abscissa*: Time. Period in hospital is marked as a thick part of the abscissa. The vertical line in the beginning of Dec. 1971 shows some corresponding points on the different curves where it is seen that the number of WBC and total histamine content are not proportional. *Therapy*: Is on an elimination diet. No ACTH, no steroids.

Blood was taken through a plastic tube directly into a plastic centrifuge tube containing (for 40 ml of blood) 10 ml 6 per cent dextran, 4 ml 0.1 M EDTA and 200 mg dextrose. For different amounts of blood correspondingly different amounts of dextran, EDTA and dextrose were used.

After sedimentation for $1\frac{1}{2}$ hours the red blood cells are found in the lower

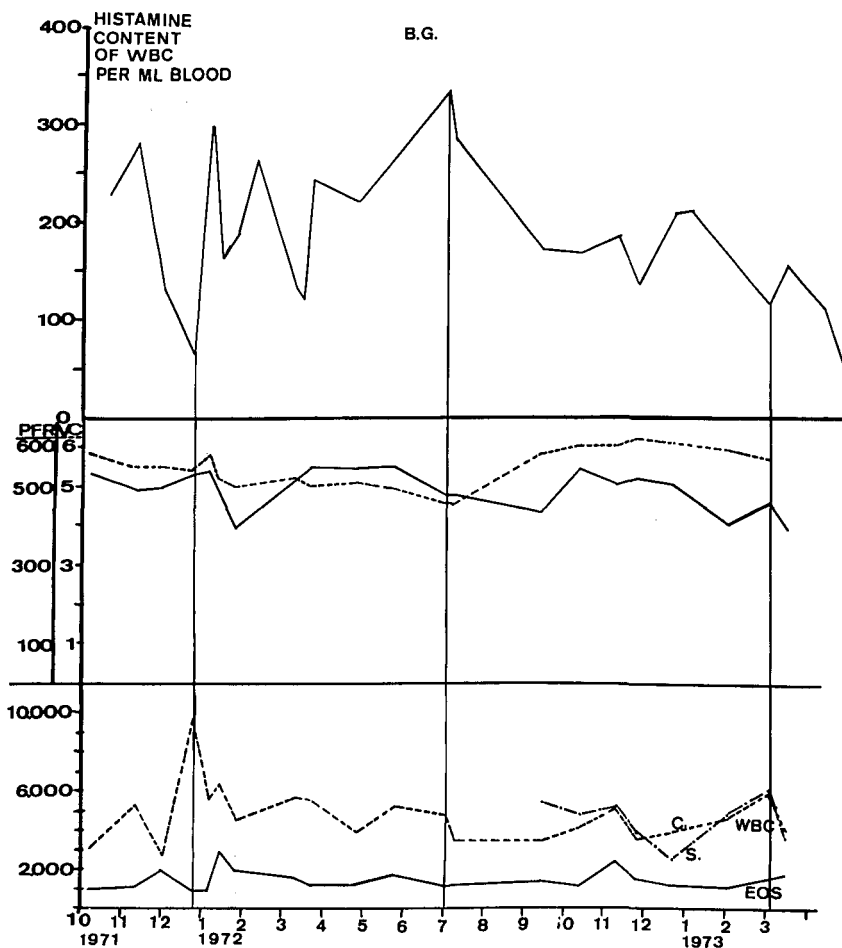


Fig. 2.

Patient 2. B.G. born 35 12 05. Male. Asthma periodically since childhood. Severe troubles in summer. Intracutaneous test positive to pollens, animal danders, and house dust. Intracutaneous titrations: cat + 10^{-3} (w/v), birch + 10^{-5} , timothy 10^{-2} . Nasal and conjunctival provocation tests: + to timothy, birch. See also text to Fig. 1. *Therapy:* Chromoglycate. No ACTH or steroids.

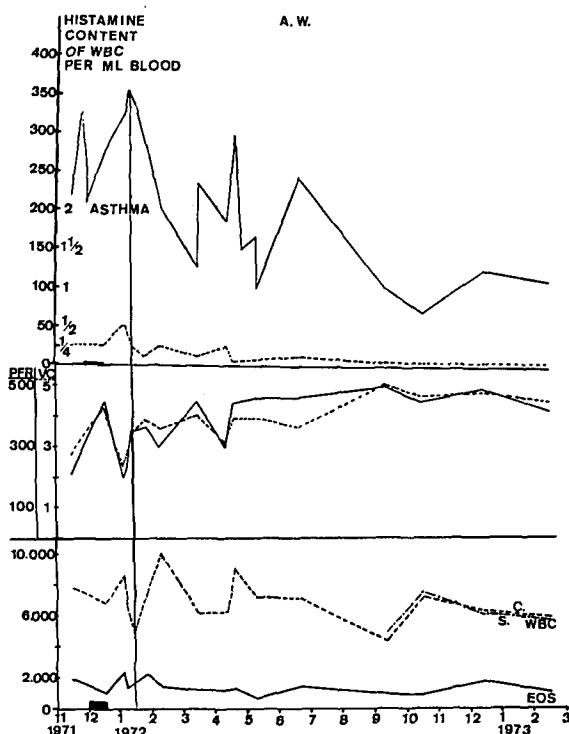


Fig. 3.

Patient 3. A.W. born 11 to 18. Male. Asthma periodically since 1963, more last year. Intracutaneous test negative. Is on an elimination diet consisting of meat, fish, potatoes, vegetables, fruit, rye. See also text to Fig. 1. *Therapy*: Is constantly on a low dose of steroids.

half of the tube. The supernatant containing the WBC is centrifuged (900/min) in plastic centrifuge tubes and the cells washed in tris buffer containing albumen (tris A) and centrifuged after each washing. After the last centrifugation the cells are resuspended in tris A solution containing Ca and Mg (tris ACM), 30 ml for 40 ml of blood.

The composition of tris A buffer is as follows:

Tris 3.029 gr, NaCl 7.013 gr, KCl 0.373 gr, Albumin Kabi 0.3 gr, glass distilled water ad 1000 ml.

Tris ACM is obtained by adding the following to 1000 ml of Tris A: $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.088 gr, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 0.203 gr.

1 ml of the cell suspension is pipetted down in plastic tubes and 0.2 ml tris

ACM is added. The fluorescence readings for these tubes with tris ACM are the blank values which include the fluorescence contributed by the reagents and the spontaneous release of histamine from the cells. If the effect of allergens on histamine release is to be studied 0.2 ml of allergen is added instead of 0.2 ml tris ACM. These tubes are then placed in a water bath 37° C for 1 hour, and are shaken twice. The complete tubes are not placed in water bath and contain 1 ml of cell suspension and 0.2 ml of 8 per cent perchloric acid (HClO_4). After centrifugation (1500 R/min) 15 min, 0.9 ml of the supernatant is extracted in glass tubes (with glass stoppers) containing 1.25 ml butanol, 300 mg NaCl (in substance) and 0.1 ml NaOH (3 N for test tubes and 5 N for complete tubes). After being shaken for 10 min in a shaking machine all tubes are centrifuged for 10 min (2500 R/min). 1 ml of the butanol phase is sucked off and mixed in glass tubes with 1.2 ml 0.12 N HCl and 3.8 ml heptane. The tubes are shaken for 10 min and centrifuged for 10 min (2500 R/min). 1 ml of the water phase is transferred to cuvettes and placed in an icebath for 15 min, 0.2 ml 1 N NaOH and

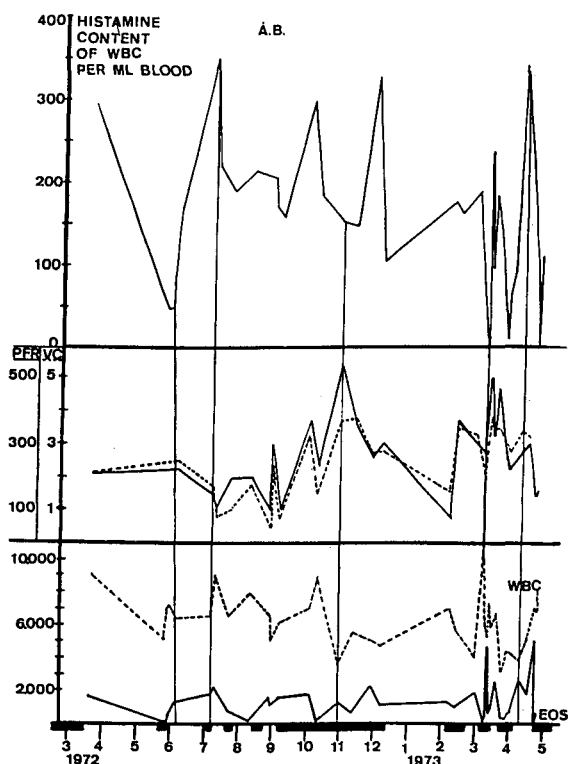


Fig. 4a

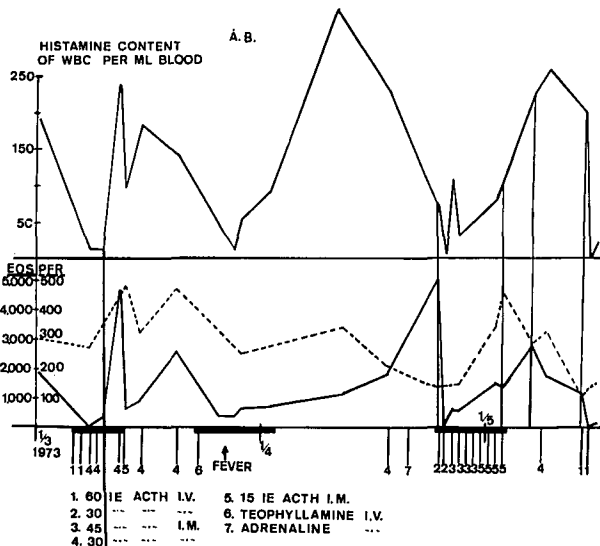


Fig. 4 b

Patient 4. Å.B. born 390220. Male. Has worked as a jockey. Asthma since 1971. Owing to severe asthma he has had to be admitted repeatedly. In Fig. 4 b, which is a detail of Fig. 4 a, the therapy is given. Intracutaneous skin test negative. Nasal and conjunctival provocation test negative (1:10) to horse dander. See also text to Fig. 1. *Therapy:* ACTH injections. No steroids. Is on an elimination diet, from which milk is excluded.

0.1 ml 0.05 per cent OPT is added, the solution is cooled (4° Celcius for 40 min), and 0.1 ml 2 M H_2PO_4 is then added. After mixing, the tubes are kept at room temperature for 15 min and the fluorescence is read on a spectrophotofluorometer.

10-40 ml of blood was taken for preparing the cell suspension; the final volume of the suspension was $\frac{3}{4}$ of the amount of blood taken; the number of cells in the suspension should therefore be $\frac{4}{3}$ of the number of cells in the blood. After incubation with antigen and centrifugation $\frac{3}{4}$ of the total volume in each tube was used. Therefore the histamine determination was performed on the amount liberated from washed leukocytes of 1 ml of blood. However, the number of WBC in the cell suspension was found to be about the same as in the capillary blood. About $\frac{1}{4}$ of the cells have therefore been lost during the washing procedure.

Determination of standard histamine solutions of known strength:

Constant amounts (90 and 45 nanograms) of histamine dihydrochloride (dissolved in 0.2 N HCl) were extracted using the method already described for the other test tubes.

Calculations

The histamine content (complete minus blank values) of the washed WBC of 1 ml blood was calculated from values for extracted histamine of known strength. All values for histamine are given in nanograms (10^{-9} gr) of histamine dihydrochloride. Both complete and blank values were always performed in duplicate.

Error of the determinations

The standard deviation (SD) in the histamine content determinations given in the figures is approximately 10. The calculations are based on double determinations within the same blood samples.

SD is estimated according to the usual formula:

$$\frac{K}{\sqrt{2}} \sqrt{\sigma_c^2 + \sigma_b^2}$$

where $K = 21.33$

$$\sigma_c^2 = \frac{\sum c_i^2}{2n}; \quad \sigma_b^2 = \frac{\sum b_i^2}{2n}$$

where c_1 corresponds to the difference between the first and the second determination of complete values and b_1 to the difference between blank values for the blood sample (i).

The summation is extended over 418 samples for 200 persons ($n = 418$). These represent the total determinations Jan-Aug 1973.

It may be noted that if the difference between two values in the figures exceeds four times the SD (approximately 40) it can hardly ($P \leq 0.005$) be explained as a random variation due to the irregular error in the histamine content determination.

The following chemicals were used:

EDTA (Merck, Darmstadt), Titriplex 3, pa.

Dextrose, D-glucose Anhydrous (Mallinckrodt Chemical Works, St. Louis).

Tris Buffer (Commercial Solvent Corp. Lab. Kemi, Stockholm, Sweden),

Trishydroxymethylaminometan.

Albumen (Kabi, Stockholm) Human lyophilized, grade A.

NaCl (Merck), pa.

NaOH 1 N (SIGMA Chemical Company, P.O. Box 14508, St. Louis Mo. 63178 USA), In 16 gr. vials, No 505-8.

3-5 N (EKA, Kebo, Stockholm) pa, 98.1 % NaOH.

HCl (Merck), Suprapur 30 %.

Heptane (Merck), n-Heptane C_7H_{16} für die Spectroskopie.

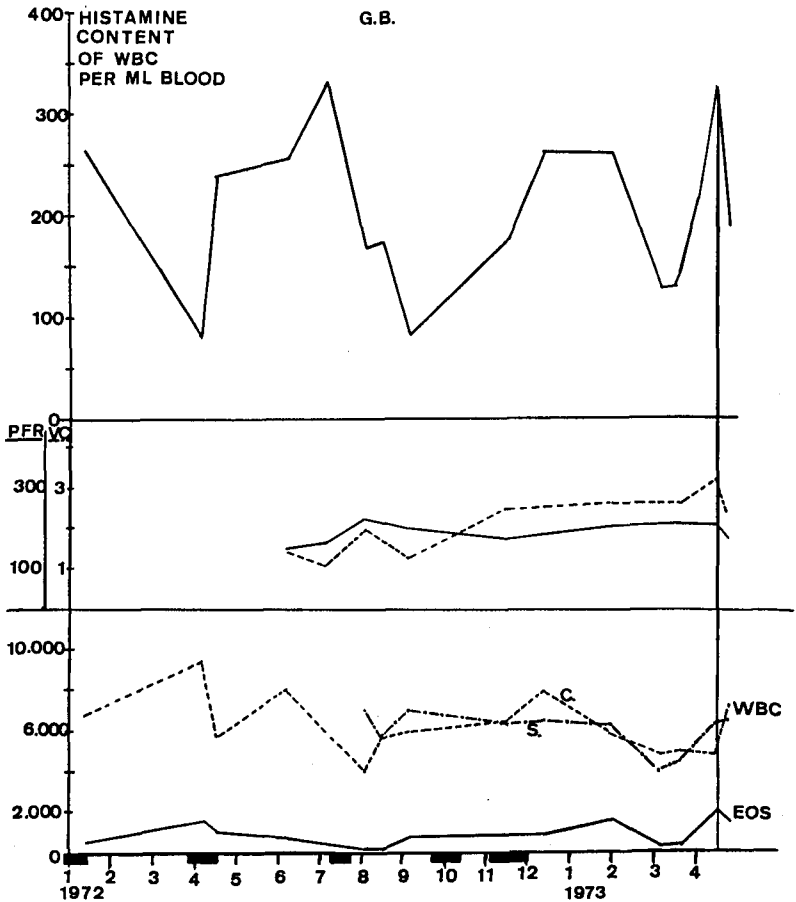


Fig. 5.

Patient 5. GB. born 21 03 11. Female. Asthma since 1949, more in recent years. No provoking allergens are known. Intracutaneous test negative. See also text to Fig. 1. *Therapy:* ACTH is given periodically. Is on a medium-large dose of steroids.

Butanol (Merck), Butanol-(1), für die Spectroskopie.

DPT (Orangeburg, Division of Becton, Dickinson and Co. Schwarz/Mann, New York), o-Phthaldialdehyde, recrystallized in ligroine.

Macrodex, (Pharmacia, Sweden), dextran 70 (6 %) with NaCl (0.9 %).

Perchloric acid, HCl O₄, (Merck), pa, 70 %.

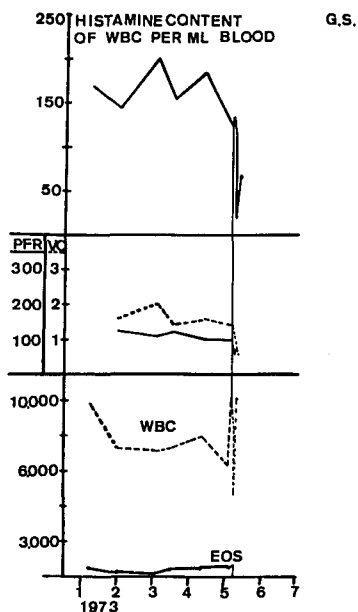


Fig. 6 a

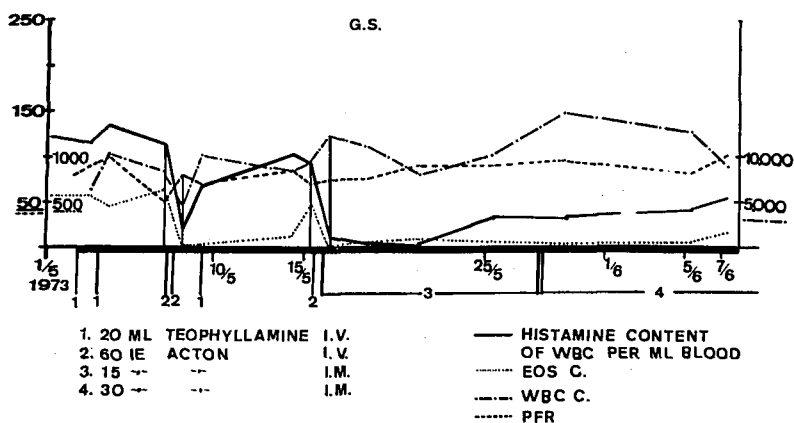


Fig. 6 b

Patient 6. G.S. born 14.09.03. Male. Asthma since childhood. Worse since 1971. Hospitalized several times. Sometimes bronchitis. Intracutaneous test positive to dust, horse and cow dander, tree pollens, moulds. Skin titration test ++ 10^{-3} to birch and hazel, + 10^{-3} to oak and house dust. Inhalation test: Negative with house dust 1:10. See also text to Fig. 1. **Therapy:** ACTH periodically. During the periods marked 3 and 4 ACTH was given 15 IU 3 times daily and 30 IU once daily respectively. Antibiotics were given periodically.

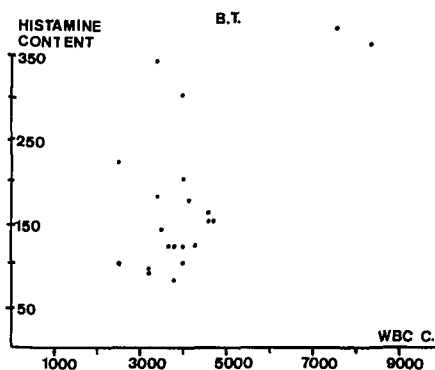


Fig. 7.

Patient 1. B.T. Ordinate: Histamine content in nanogram (10^{-9}) in cells from 1 ml of blood. Abscissa: WBC in capillary blood.

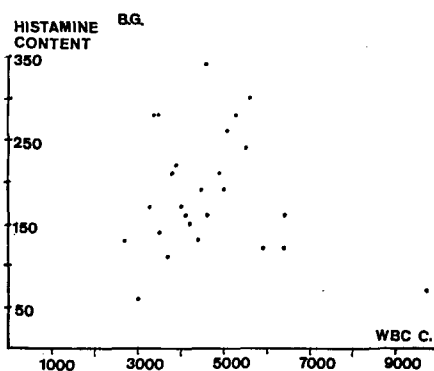


Fig. 8.

Patient 2. B.G. Ordinate and abscissa see Fig. 7.

Ortophosphoric acid, H_3PO_4 , (Merck), pa, 85 %.

Ligroine (Eastman Rochester, N.Y. 14650 USA), does not reduce $KMnO_4$.

RESULTS

There is no correlation between the number of white blood cells in capillary blood and the histamine content in leukocytes of 1 ml of blood.

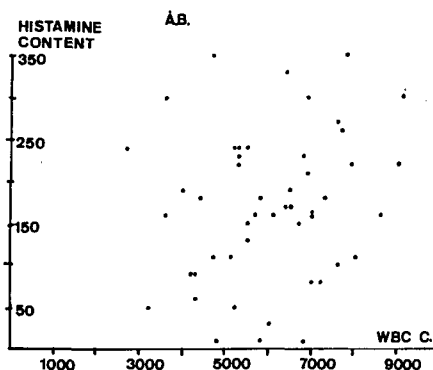


Fig. 9.

Patient 4. A.B. Ordinate and abscissa see Fig. 7.

In three of the patients BT, BG and AB the histamine content in 1 ml of blood is plotted against the number of WBC in the capillary blood. No correlation exists (Fig. 7-9). Therefore the amount of the histamine in the cells of 1 ml of blood is used in this paper instead of correlating the histamine content to a certain number of white blood cells. The number of WBC in the suspension is about the same as in capillary blood. Since the cells in the suspension are concentrated to a factor of $4/3$, $1/4$ of the cells have therefore been lost during the washing procedure. Healthy persons (13 male and 9 female) between 23-64 years old had a histamine content of between 15-94 nanograms in washed WBC from 1 ml of blood. The mean value was 54.1 nanograms. SD 20.3.

In asthmatic patients with varying degrees of asthma troubles the histamine content varied considerably with variations between 0 and 400 nanograms (in washed WBC from 1 ml of blood). Asthmatic patients therefore sometimes had values considerably higher and lower than healthy persons, but only the higher values are statistically significant.

The total histamine content was studied in a large number of patients. The selected patients who are presented below were studied with repeated determinations for several months up to more than a year.

Patient 1. B.T.

Sensitive to several allergens i.a. wheat. He was sensitive to wheat both by skin titration, conjunctival provocation test and also when subjected to WBC provocation test. Admitted into hospital for asthma in November 1971. He was put on an elimination diet without wheat. His PF and VC were lowered, his eosinophils were elevated to about 50 per cent of the total number of WBC. When he left the hospital the histamine content of the cells in 1 ml of blood had gone down to half the value, i.e. from 300 to 150 nanograms per ml of blood. The number of total WBC was the same on both occasions. The eosinophils were constantly at a low level in the later period in hospital (Fig. 1). There is no correlation between the number of white blood cells and the total amount of histamine in 1 ml blood (Fig. 7).

Patient 2. B.G.

This patient is sensitive to certain antigens, especially to timothy, but other factors are also of importance for his allergy. In winter he has nose trouble but only very slightly when on Chromoglycate inhalations. The histamine content in 1 ml of blood changes greatly without very much change in the lung function. A peak of WBC in capillary blood can be connected with a low histamine content. Such findings are the reason why the total histamine content is given as the amount in the cells of 1 ml of blood instead of in a certain number of cells (see also Fig. 8). The most important shock organ of this patient is his nose and in winter time the allergen, if any, is not known (Fig. 2).

Patient 3. A.W.

This patient had low values of PF and VC and a high histamine content when admitted to hospital. On an elimination diet his respiratory function improved and the histamine content decreased and became more stable. It is easily seen that the histamine content is not correlated to the number of white blood cells (Fig. 3).

When on an elimination diet and not in contact with the provoking foods his condition is much better and his blood histamine values are more constant and lower (Fig. 3).

Patient 4. A.B. (Fig. 4).

The patient had repeated periods of severe asthma necessitating hospitalization. No antigen of importance for his asthma has been found. The blood values of total histamine content showed considerable changes. In March 1973 he repeatedly had a very low histamine content, sometimes together with a high number of white blood cells in the capillary blood.

Figure 4 b is a detail of Figure 4 a. The eosinophil count can be high together with a low histamine content and a low PFR (end of April). A high histamine content can occur together with a better lung function (middle of April).

Fig. 4 b further shows that in the last week of March 1973 this patient had a low histamine content during a period of severe asthma associated with fewer (39°). On this occasion the patient had not received ACTH during the preceding 9 days. A low histamine content associated with a low lung function and a low eosinophil count can therefore exist without correlation to ACTH-treatment. This patient is not on steroids.

Patient 5 .G.B.

This patient has had varying amounts of histamine in her white blood cells. The peak values do not correlate with the number of leucocytes. No allergen is known to provoke her asthma. It was necessary to treat her in hospital 5 times in 1972. Both low and high values in total histamine content could be observed in connection with severe asthma that necessitated hospitalization (Fig. 5). The histamine content varied greatly although the number of WBC in capillary blood was unchanged.

Patient 6. G.S.

This patient has had asthma for many years and is sensitive to several antigens, among them birch. At the beginning of May 1973 he was treated in hospital for several days with Teophyllamine i.v. During this time his PFR decreased and the eosinophils were unchanged. The total histamine content remained constant at four determinations. ACTH was given i.v. This led to a marked drop in both total histamine content and eosinophils till the next day. The peak flow rate increased a little. This event was repeated one week later, when ACTH was again given (Fig. 6 a and b).

DISCUSSION

It is evident from the data presented that the histamine content can be either high or low in connection with a period of severe asthma. These findings may most easily be explained by assuming that the histamine content in 1 ml of blood is influenced by both the amount of histamine liberated and the amount of histamine produced in the cells. A low histamine content may depend upon both an increased liberation (with severe symptoms) or a decreased production with no or little liberation (with no or little symptoms). Factors influencing both histamine liberation and production may be of essential importance for the clinical state of the patient.

ACTH can lower the histamine content. This is shown in

Fig. 6. In this patient the histamine content in the cells in 1 ml of blood is lowered at about the same rate as the decrease in the eosinophils. In this patient the PFR increased in the same time period.

The histamine content can also be lowered by severe asthma even if the patient has not had ACTH on the preceding days (patient 4, Fig. 4 b).

The process of histamine formation (histamine forming capacity, HFC) has been investigated by Kahlson, Rosengren & Thunberg and Assem, Schild & Vickers. The last mentioned authors found that the antigen provokes not only a liberation of histamine from the cells but also an increase in histamine production. This is evident from the sum of the histamine content in the cells + that of the supernatant. According to Assem, Shild & Vickers this sum is increased after antigen contact.

ACTH therapy can diminish the histamine content in the cells. This may be due to either an increased histamine liberation from the cells or to a lowered production of histamine in the cells, or to both these processes. In Fig. 6 the histamine content goes down but the PFR increases. It is therefore not likely that more histamine has been liberated. Rather, the combination of a low histamine content and less asthma 24 hours after ACTH treatment could be explained by a lowered HFC. Direct measurements are necessary to prove this hypothesis.

According to several authors the basophils are the most or only important cells for blood histamine content (3, 4).

Differential counts of capillary blood have been investigated in order to get some idea of changes of the basophils. With this insensitive method no changes have been found when the histamine content changes. A more sensitive method of counting basophils has just been started. Patients with asthma can have higher values of histamine in the blood than normal persons. They also show great as well as rapid variations. The changes in histamine content are statistically significant. In contrast to the hay fever patients studied by Lichtenstein &

Osler (6) the histamine content varies greatly in an asthma patient when examined on different occasions when either patient when examined on different occasions, when either grees of intensity.

The histamine content in 1 ml of blood is of the same order of magnitude as that found by Lichtenstein & Osler and Assem, Schild & Vickers. If the number of WBC in 1 ml of blood is assumed to be 5×10^6 , the values found by Lichtenstein & Osler (for 1.5×10^7 cells) should (see page 1) be 3 times as high, and those found by Assem, Schild & Vickers (for 10^8 cells) 20 times as high as those given in this paper.

SUMMARY

Histamine determinations were performed on asthmatic patients over a long period of time.

The following conclusions are drawn:

1. There is no correlation between the amount of histamine in the leukocytes of 1 ml of blood and the number of WBC. In this study the histamine content in the WBC of 1 ml of blood is therefore used instead of the histamine content in a certain number of WBC.

2. The histamine content may vary considerably from one day to another. In connection with severe asthma the histamine content can be high or low. Conversely the histamine content can either be high or low with no or little asthma. In a single patient the histamine content can vary between 0-400 nanograms in the WBC of 1 ml of blood.

These findings may most easily be explained by assuming that the histamine content in 1 ml of blood is determined by both the amount of histamine liberation and the amount of histamine production in the cells.

Factors influencing both histamine liberation and production may be of essential importance for the clinical state of the patient.

3. The histamine content in the WBC of 1 ml of blood in

healthy subjects varies between 15 and 100 nanograms per ml. This contrasts sharply with the wider variations (between 0-400 nanograms per ml) found in asthmatic patients.

4. ACTH therapy can cause a decrease in the histamine content together with an improved lung function.

5. No difference have been found between extrinsic and intrinsic asthma as far as changes in histamine content are concerned.

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