THE DISTRIBUTION OF HISTAMINE IN THE BLOOD OF
HEALTHY AND ASTHMATIC CHILDREN

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SUMMARY

1. Histamine levels in whole blood and blood plasma, together with circulating basophil, eosinophil and total leucocyte counts, were determined in three groups of asthmatic children and in a control group of non-asthmatic children.

2. Blood histamine levels were significantly higher than the control levels during asthmatic symptoms, but fell to near normal values in the quiescent phase of asthma and during long-term steroid treatment.

3. Plasma histamine levels were found to be minimal in all the subjects studied (<1 ng/ml).

4. Significant correlations were found between whole blood histamine levels and basophil counts and between whole blood histamine levels and log eosinophil counts in the control children. The findings in the asthmatic children suggested that the relationships between these phenomena in asthma are more complex than in normal individuals.

It is by now evident that the amount of histamine in the blood of healthy adult humans is within the range 10–100 ng/ml (Rose & Browne, 1940; Rorsman & Rosengren, 1958; Lindell & Westling, 1966) and that most of it is located in the basophil leucocytes (Graham et al., 1955; Code & Mitchell, 1957).

Few reports have appeared concerning blood histamine in children and fewer still have included parallel basophil and eosinophil counts. Mitchell & Cass (1959) found that the levels of blood histamine in a group of healthy children fell within the normal adult range which was in agreement with the work of Maas, Fehmers & Strengers (1956). Mitchell & Cass (1959) also reported that the amount of histamine in the blood is closely related to the number of circulating basophils in childhood and they could find little or no histamine in the plasma.

Much work has been published concerning blood histamine levels in asthma. The values quoted, however, vary considerably and are often conflicting. Some authors have reported

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high levels of blood histamine during acute asthmatic symptoms (Haworth & McDonald, 1937; DeGara, 1951). The work was extended by Jimenez-Diaz, Arjona & Perianes (1955) and Konoshita (1963) who confirmed the increased levels during attacks and also reported normal or only slightly elevated levels in symptom-free periods. Rose (1941) found a consistent increase in the blood histamine in various forms of asthma, with a tendency towards constantly higher levels in young asthmatics under 16 years. Other workers have found no increase of the blood histamine in asthmatic and allergic subjects (Riesser, 1937; Eggels & Nelemans, 1966). In their study of children, Maas et al. (1956) found that allergic subjects tended to show definitely raised or lowered blood histamine levels.

A close correlation between the numbers of circulating basophils and blood histamine levels in bronchial asthma and other allergic manifestations was found by Gudowski & Dieckhoff (1967).

Few measurements of histamine in the blood plasma of asthmatic subjects have been published. Beall (1963), using a chemical method, found no significant difference between normal plasma histamine levels and those of allergic and asthmatic subjects. The mean plasma histamine concentration for a group of normal individuals was 5.6 ng/ml and a similar value was recorded for the asthmatic patients studied.

It has been reported that after corticosteroid administration, the high blood histamine levels encountered in status asthmaticus are decreased to normal (Konoshita, 1963) and Gudowski & Dieckhoff (1967) found a concomitant fall in the numbers of circulating basophil leucocytes.

In work on guinea-pigs, Kovács & Suffiad (1968) showed that a single massive dose of corticosteroid brought about a significant increase in the plasma histamine level. Earlier, in 1957, Noah & Brand studied the effects of therapeutic doses of corticosteroids in humans and found no increase in plasma histamine. These values were obtained, however, using a relatively insensitive method and few patients.

The present investigation was undertaken to determine the levels of histamine in the blood of children with various degrees of asthmatic symptoms and of children undergoing long-term corticosteroid therapy. The whole blood histamine–basophil count and whole blood histamine–eosinophil count relationships were also investigated. The concentrations of histamine in the blood plasma of normal and asthmatic children were determined using the very sensitive techniques of Adam, Hardwick & Spencer (1954, 1957).

METHODS

Specimens of blood were obtained from groups of children of both sexes.

Control group

It was not practicable to obtain specimens from healthy children outside hospital and so thirty-six children, aged between 1½ and 11 years, who were in hospital and had either been investigated for functional enuresis or recovered from minor infections, were selected as controls. None of these children had asthma or had been given antihistamines, corticosteroids or aspirin. This group was divided into two sub-groups according to sex.
Blood histamine in childhood asthma

Asthmatic groups

Atopic asthma is sometimes divided into extrinsic and intrinsic types but these are not clearly distinct in young children, in whom the clinical picture changes with development, and we prefer to define asthma descriptively as 'recurrent dyspnoea of an obstructive type without other demonstrable cause'. All the children in this study had been investigated in hospital and the diagnosis of atopic asthma made, and most of them were attending the allergy clinic at the Children's Hospital. They were sub-divided further as follows:

Quiescent asthmatic group. This was a group of twenty-one children aged from 2 to 13 years who, although prone to acute attacks of asthma, were not showing any clinical signs of the condition at the time of study. Again, none of the children was taking antihistamines, steroids or aspirin.

Asthmatic symptoms group. This contained twenty-three children aged between 3 and 12 years displaying clinical symptoms of asthma. These were roughly classified into two sub-groups, viz. 'chronic symptoms' and 'acute symptoms'. None of these was receiving steroids, antihistamines or aspirin. Some, however, were using catecholamine sprays.

Long-term steroid therapy group. These were children with chronic asthma who were receiving corticosteroids (usually prednisolone) daily. The group comprised eleven children aged between 3 and 14 years.

Withdrawal and partitioning of blood specimens

Blood specimens were obtained from the antecubital veins using 20 ml capacity disposable polythene syringes fitted with wide bore needles.

One ml of the blood was immediately added to a dipotassium ethylenediamine tetra-acetate (EDTA)-coated 5 ml capacity siliconed glass specimen tube and the contents mixed by gentle inversion. The concentration of EDTA in the blood was 5 mg/ml which was sufficient to prevent clotting. This was left at 4°C until the cell count could be performed.

Of the remaining blood in the syringe, 10 ml was added to a chilled siliconed 'Pyrex' glass centrifuge tube containing heparin, 100 I.U. This was also stoppered, gently inverted to mix the contents and stored at 4°C until needed for the histamine estimation—this storage time was usually less than 10 min.

The estimation of histamine in whole blood and plasma

This was carried out using slightly modified versions of the methods of Adam et al. (1954, 1957) and Adam (1961), and entailed an initial two-stage centrifugation at 4°C to separate the plasma from the cells. Plasma and whole blood specimens were then extracted with trichloroacetic acid, centrifuged to remove the proteins and further purified on composite cationic resin columns. The eluates, after heating with 6 M HCl and drying, were reconstituted and assayed.

The bioassay was performed on the superfused guinea-pig ileum preparation, using a semi-automatic apparatus similar to that described by Adam et al. (1954). The dried residues were dissolved in a reconstituting solution, the composition of which was calculated to compensate for the concentration of NaCl in the eluates. Thus, when the dried eluates were dissolved in 5 ml of the reconstituting solution, the test was isotonic with the atropinized Tyrode's solution used to superfuse the preparation.
Frequently, test solutions were re-assayed in the presence of Tyrode's solution containing mepyramine in a concentration of 3 ng/ml.

On several occasions, the potassium content of eluates from whole blood and plasma specimens was estimated. This was thought to be especially important as excess potassium in a test solution could produce anomalous contractions of the preparation and thus give 'high' histamine levels. It was found, however, that on no occasion was the potassium concentration of the test high enough to interfere with the histamine assay.

**Leucocyte counting procedure**

Blood from the 5 ml tubes was diluted 10 times with a diluting fluid containing Toluidine Blue which metachromatically stained the basophil and eosinophil cells. The cells were counted in a Fuchs-Rosenthal haemocytometer using both chambers. The method used was based on Mitchell's modification (1955) of the method of Moore & James (1953).

All whole blood histamine and, where possible, plasma histamine estimations were carried out in duplicate, as were the leucocyte counts.

### RESULTS

**Recovery experiments**

When histamine, 5 and 25 ng, was added to specimens of plasma (2.5 ml), the mean recoveries were respectively 98 (SEM 6.9, n = 6)% and 87 (SEM 4.6, n = 6)%.

The recovery of histamine, 25 ng, from the whole blood (0.5 ml specimens) was 92 (SEM 3.4, n = 11)%.

**Control group**

The results for this group are shown in Table 1. When Student's *t* test was applied to the mean whole blood histamine levels (ng free base/ml) for the two sub-groups (male and female) it was found that there was no significant difference (0.7 > P > 0.6).

In none of the samples of plasma in this group was activity due to histamine detected; the values are recorded as less than the smallest amount which could be measured by the method used, i.e. <1 ng/ml.

<table>
<thead>
<tr>
<th></th>
<th>Whole blood histamine (ng/ml)</th>
<th>Plasma histamine (ng/ml)</th>
<th>Basophils (mm⁻³)</th>
<th>Eosinophils (mm⁻³)</th>
<th>Total leucocytes (mm⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>46 (SEM 4.0, n = 26)</td>
<td>&lt;1 (n = 24)</td>
<td>38</td>
<td>133* (n = 25)</td>
<td>7490 (SEM 530, n = 25)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>42 (SEM 6.7, n = 9)</td>
<td>&lt;1 (n = 10)</td>
<td>37</td>
<td>134* (n = 10)</td>
<td>7000 (SEM 790, n = 10)</td>
</tr>
<tr>
<td></td>
<td>Males and females (pooled)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45 (SEM 3.4, n = 35)</td>
<td>&lt;1 (n = 34)</td>
<td>37</td>
<td>133* (n = 35)</td>
<td>7350 (SEM 440, n = 35)</td>
</tr>
</tbody>
</table>

*Antilogarithm of logarithmic mean.*
Comparing the mean basophil counts for the two subgroups, it was also found using the $t$ test that there was no significant difference for the sexes ($0.9 > P > 0.8$).

The logarithmic mean eosinophil counts were calculated in this study as it was found that the distribution was more normal when these transforms were used. Here there was also no significant difference between the sexes ($P = 0.995$). A similar lack of significance was found between the mean total leucocyte counts ($0.7 > P > 0.6$).

Fig. 1 shows the relationships between whole blood histamine levels and basophil counts and whole blood histamine levels and log eosinophil counts. In both cases, positive significant correlations were found.

![Fig. 1. Relationship between the histamine level and the number of basophils (left: $r=0.67$; $P < 0.001$) and the number of eosinophils (logarithmic transforms) (right: $r=0.62$; $P < 0.001$) in the blood of a group of control children. Circles represent males and squares females.]

**Groups of asthmatic children**

The results for these groups are shown in Table 2.

When the means of the estimations for the group with quiescent asthma were compared with the means of the control group (combined male and female results), no significant differences were found between the whole blood histamine levels ($0.10 > P > 0.05$), the basophil counts ($0.9 > P > 0.8$) or the total leucocyte counts ($0.8 > P > 0.7$). The logarithmic mean eosinophil counts were, however, significantly different ($P < 0.001$), there being a higher log mean count for the quiescent asthma group than for the control children. No linear correlation was found between the whole blood histamine levels and the basophil counts for the quiescent asthma group ($r = 0.27$; $P > 0.1$). A significant linear correlation, however, was shown to exist between the whole blood histamine and log eosinophil counts ($r = 0.48$; $0.05 > P > 0.02$).
TABLE 2. Mean blood histamine levels and leucocyte counts in groups of asthmatic children

<table>
<thead>
<tr>
<th></th>
<th>Whole blood histamine (ng/ml)</th>
<th>Plasma histamine (ng/ml)</th>
<th>Basophils (mm⁻³)</th>
<th>Eosinophils (mm⁻³)</th>
<th>Total leucocytes (mm⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quiescent asthma</td>
<td>57 (SEM 5·6, n = 21)</td>
<td>&lt;1</td>
<td>38</td>
<td>460*</td>
<td>7120</td>
</tr>
<tr>
<td>Acute asthma</td>
<td>106 (SEM 20·3, n = 6)</td>
<td>&lt;1</td>
<td>62</td>
<td>570*</td>
<td>6940</td>
</tr>
<tr>
<td>Chronic asthma</td>
<td>94 (SEM 10·9, n = 15)</td>
<td>&lt;1</td>
<td>88</td>
<td>760*</td>
<td>7610</td>
</tr>
<tr>
<td>Chronic and acute</td>
<td>97 (SEM 9·4, n = 23)</td>
<td>&lt;1</td>
<td>81</td>
<td>700*</td>
<td>7420</td>
</tr>
<tr>
<td>symptoms (pooled results)</td>
<td>51 (SEM 5·1, n = 11)</td>
<td>&lt;1</td>
<td>49</td>
<td>210*</td>
<td>8330</td>
</tr>
<tr>
<td>Long-term steroid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>treatment</td>
<td></td>
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</table>

* Antilogarithm of logarithmic mean.

Considering the asthmatic symptoms group; no significant differences were found between the mean histamine levels (0·7>P>0·6), basophil counts (0·3>P>0·2), total leucocyte counts (0·6>P>0·5) or log eosinophil counts (0·4>P>0·3) for the chronic and acute sub-groups when the t test was applied.

Correlation coefficients were determined for whole blood histamine levels against basophil counts (r = 0·40; P>0·05) and log eosinophil counts (r = 0·03; P>0·1) for the asthmatic-symptoms group and in neither case was a significant linear correlation obtained.

When the mean values for the symptomatic group were compared with those for the control children group, significant differences were found between the means for whole blood histamine (P<0·001), basophil counts (P<0·001) and log eosinophil counts (P<0·001). In each case the mean value was higher for the asthmatic symptoms group. However, no significant difference was found between the mean total leucocyte counts for the two groups (P>0·9). Similar findings were obtained when the t test was applied to the mean values for the quiescent asthma and asthmatic symptoms groups.

In the long-term steroid therapy group, the correlation coefficient was found not to be significant for the whole blood histamine–basophil count relationship (r = 0·05; P>0·1) but the correlation coefficient for the log eosinophils and whole blood histamine levels was just significant (r = 0·61; 0·05>P>0·02).

The mean values for this group were compared with the means for the other groups using the t test. When compared with the control children group, no significant differences were found between the means of the whole blood histamine levels (0·4>P>0·3), total leucocyte counts (0·3>P>0·2) and the logarithmic means of the eosinophil counts (0·1>P>0·05). A significant difference was found, however, between the means of the basophil counts for the two groups (0·025>P>0·02), the mean basophil count for the steroid-treated group being higher than that for the control children.

Comparing the steroid-treated group with the quiescent asthma group, no significant differ-
ences were found between the mean whole blood histamine levels \((0.5 > P > 0.4)\) and basophil counts \((0.1 > P > 0.05)\). Significant differences did occur, however, between the logarithmic mean eosinophil counts \((0.02 > P > 0.01)\) and the mean total leucocyte counts \((0.05 > P > 0.025)\).

The mean values for the whole blood histamine \((0.005 > P > 0.001)\), basophil counts \((0.05 > P > 0.025)\) and mean eosinophil counts \((P > 0.001)\) were all significantly lower for the steroid-treated group than for the asthma-symptoms group. No significant difference was found between the total leucocyte counts for the two groups \((0.3 > P > 0.2)\).

In all the groups of asthmatic children no histamine was detected in the plasma of any of the subjects. This was again reported in Table 2 as \(< 1 \text{ ng/ml}\).

**DISCUSSION**

As found by previous workers (Mitchell & Cass, 1959; Maas et al., 1956), the range of histamine levels in this group of children was in agreement with the results obtained for groups of adult subjects (Rose & Browne, 1940; Rorsman & Rosengren, 1958).

The virtual absence of histamine from the plasma accords with the findings of Adam et al. (1957) who, using larger volumes of plasma, recorded minimal levels of histamine \((< 1.0 \text{ mg/ml})\) in the plasma of healthy adult subjects. These levels are consistently lower than those found by certain chemical techniques (Noah & Brand, 1963).

The basophil counts were in agreement with those found by Moore & James (1953) in a group of adults. Moore & James found no significant sex difference in basophil counts and this observation was confirmed here for the control children. These counts are also in accord with the findings of Mitchell & Cass (1959).

The clinical grouping of the asthmatic children studied, although imprecise, was found to be the most satisfactory basis for comparing the results. The study confirms reports by previous workers of high blood histamine levels in asthmatic subjects while symptomatic, with normal values during symptom-free periods (Jimenez-Diaz et al., 1955; Konoshita, 1963), and the return of high values to normal levels after steroid treatment (Konoshita, 1963).

Comparable results were obtained for the basophil counts. Rorsman (1958) did not find a significant increase in basophils in the blood of twenty asthmatic subjects, but a large proportion of his patients were treated with corticosteroids, and in the present study the mean basophil count for the steroid-treated group, although higher than that for the control group, was only just significantly different.

The eosinophil counts for the groups of control and asthmatic children also differed. All the groups of asthmatic subjects had higher log mean counts than the controls. The log mean count for the steroid-treated group was not significantly different from that for the controls but was significantly lower than that for the asthma symptoms group. This is in agreement with the findings of some other workers. Thus Blatt (1966) stated that in allergic asthma, a blood eosinophilia is present in patients who are not in a state of shock and, when corticosteroids are administered, an eosinopenia is produced. An explanation of the mechanism of eosinophilia and eosinopenia is given by Archer (1960, 1968) who showed that eosinophils are attracted by the release of histamine.

No histamine was detected in the plasma of the children in any of the asthmatic groups studied. This is in general agreement with the work of Beall (1963), to the extent that he found no significant difference between ‘control’ and ‘asthmatic’ levels, and with the rather scanty
data of Noah & Brand (1957), who found no increase in plasma histamine levels in steroid-treated subjects.

Kovács & Suffiad (1968) concluded that the release of histamine into the plasma after massive doses of corticosteroids were administered to guinea-pigs could be due to the hyperglycaemic effect of the steroids. It would therefore be interesting to study histamine levels of plasma specimens from hyperglycaemic patients.

Correlations between basophil counts and whole blood histamine levels were calculated. Whereas a significant linear correlation was found in the control group of children, there were no such correlations in the asthmatic groups. This differs from the reports by Gudowski & Dieckhoff (1967), who found significant correlations during both symptomatic asthma and steroid treatment. On the other hand, just significant correlations were found for log eosinophil counts and whole blood histamine levels in the quiescent and steroid-treated groups, suggesting that relationships between these phenomena in asthma are more complex than in normal subjects.

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REFERENCES


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