Physiological Reviews

Distribution of Histamine in Human Blood

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INTRODUCTION

In this review we have attempted to present an up-to-date account of work in the field of “histaminology” related to human blood that is concerned both with methods for the determination of the amine and with studies of its distribution between the formed elements and plasma. Our own interests have influenced the choice of topics and we have included sections on blood histamine in asthma and in the newborn infant. Another section deals with the still very controversial topic of histaminopexy. We gladly acknowledge the help afforded to us by earlier reviews, especially those by Code (28), Lindell and Westling (76), and Parrot et al. (103, 104).

Since the early 1930’s workers have been determining the histamine levels in blood and studying the distribution of histamine between the various cells and plasma. Despite this, Lindell and Westling wrote in 1966 (76) that the confusion concerning blood histamine is greater than in any other area of histamine research.

In blood, the largest amount of histamine is found in the cells. As early as 1937, Code (27) considered that strong evidence existed for the assumption that
the majority of histamine in human blood is localized in the leukocytes. Later it was concluded that most of the histamine in the blood of healthy humans is associated with the basophil and eosinophil leukocytes [Graham et al. (52, 53), Code and Mitchell (29)]. At the present time the basophils, like their tissue equivalents, the mast cells, are believed to be important storage sites for histamine; however, no clear role has yet been cast for the eosinophils. The localization of histamine principally in the basophilic leukocytes allows the number of basophils in the blood to be used as an index of the amount of histamine present.

Whole-blood histamine levels give little or no positive evidence for the involvement of histamine in the pathogenesis of various diseases. In myelogenous leukemia, for example, although the histamine levels in whole blood are increased to upward of 500 times normal values [Shimkin et al. (130)], none of the biological effects of histamine are seen. Patients with this disease appear to have normal plasma histamine levels [Thiersch (132)] and a normal urinary excretion of free histamine [Lindell and Westling (76)]. This phenomenon is also seen on a smaller scale in some allergic disorders.

The range of values for histamine in whole blood differs widely between species. Within a species the range is more restricted and individual members of the species normally tend to have a fairly constant range of values [Code (28)].

Comparing the results obtained by different investigators is difficult, for some have not recorded the form of histamine on which they have based their values. Histamine concentration can be expressed in terms of histamine as the "free base," as histamine hydrochloride, or as the acid phosphate. Since 60% of histaminic hydrochloride, but only 36% of the acid phosphate, is equivalent to the free base, the reported values may differ widely depending on the reference used; if this is not stated, the values obtained are ambiguous and comparisons cannot be made. Because the free base is the pharmacologically active portion of the molecule it is therefore the most satisfactory reference [Code (28)]. Throughout this review histamine values are reported as the free base unless stated otherwise.

The concentration of histamine in rabbit blood is the highest in any species so far reported—in the region of 1-5 μg/ml [Rose and Weil (123), Code (27)]. At the other end of the scale are dogs and cats; the values for dogs range from 0 to 40 ng/ml [Barsoum and Gaddum (9)]. Values for man lie between these extremes, and there is far less histamine in human than in rabbit blood. Many values for normal blood histamine in man have been reported; they have a wide scatter of approximately 10-140 ng/ml [Lindell and Westling (76)]. A noticeable difference exists between mean values obtained by different methods. However, variations between mean values obtained by the same method (e.g., biological method) can be as large as those between results obtained by radically different methods (e.g., biological and chemical). The significance of the variation in blood histamine between individuals is unknown, and there seems to be no significant difference between the values for the sexes.

Some values for normal blood histamine are:

1) Normal adults (in ng/ml)—27-50 [Barsoum and Gaddum (10)]; 20-75 [Rose and Browne (121)]; 38-80 [Serafini (129)]; 10-110 [Rorsman and Rosengren (119)]; 26-68 [Porter (106)].
2) Normal children (in ng/ml)—20–80 [Mitchell and Cass (90)]; 22–100 [Maas et al. (78)]; 45 (± 3.4) [Porter and Mitchell (107)].

In 1940 Rose and Browne (121) reported that the histamine content of human blood tends to remain stable when repeatedly studied in one individual. It has been claimed that this stability is not seen in certain pathological conditions, e.g., allergy [Rose (120), Maas et al. (78)] or schizophrenia [Gooszen and Donker (50)]. In 1952 Code indicated that when a substance so pharmacologically active as histamine is found in biological material an important factor to consider is whether or not it is free to produce its physiological and pharmacological effects or whether it is bound to other substances or cells. This gives a reason for the present interest in plasma histamine levels.

The reported values for histamine in blood plasma have become lower as advances have been made in sampling and assay techniques. A very important factor in the techniques used to measure plasma histamine is the treatment of the blood. In order to obtain plasma in a relatively pure form, blood should not be subjected to trauma of any kind as this can cause histamine release from the granulocytes. When adequate precautions are taken, plasma histamine levels are found to be lower than the lowest limits of the most sensitive assay techniques.

Some values obtained for normal plasma histamine levels are: less than 5 ng/ml [Code (27)]; 0–5.5 ng/ml [Noah and Brand (96)]; less than 1.0 ng/ml [Adam et al. (3), Porter and Mitchell (107)].

Little information is available at present concerning plasma histamine levels, estimated by accurate methods, in pathological conditions in man. There seem to be no reliable reports of increased plasma histamine levels when the necessary precautions have been taken for withdrawal of blood and separation of plasma [Lindell and Westling (76)].

METHODOLOGY

A variety of methods exist for the measurement of histamine in blood. Basically they can be divided into two groups—chemical methods and biological methods. The chemical methods measure the colored or fluorescent products of the chemical reaction of histamine with suitable reagents. The major type of biological assay of histamine relies on the contractile effect of the amine on the isolated ileum of the guinea pig. Over the years advances in both types of technique have been made and some very sophisticated methods are now available.

The two chemical methods currently in use for blood histamine studies are the spectrophotometric method of McIntire et al. (82), which involves the use of dinitrofluorobenzene (DNFB), and the spectrophotofluorimetric o-phthalaldehyde (OPT) method of Shore et al. (131). The DNFB method relies on the coupling of histamine to DNFB. This takes place after extraction with trichloroacetic acid and adsorption on Decalso ion-exchange material. The yellow histamine-DNFB complex is then selectively extracted into methyl-n-hexyl ketone and the optical density measured at 360 nm.

The OPT method involves the condensation reaction of histamine with o-
phthalaldehyde in alkaline solution to produce a sensitive acid-stabilized fluorophore (excitation 360 nm; fluorescence 450 nm). The histamine is extracted from the deproteinized biological material into butanol, washed with dilute alkali, and reextracted into acid for the condensation step. A version of the OPT method designed for blood plasma was published by Noah and Brand in 1961 (95) and they later developed a simplified method (96). These workers reported a normal plasma histamine value of 0–5.5 ng/ml, which is lower than values obtained using the DNFB method but not so low as those found by certain bioassay procedures [Adam et al. (3)].

Although the introduction by Schultz (118) of the isolated guinea pig ileum preparation gave pharmacologists a sensitive biological preparation for the measurement of histamine, the first practical biological method for the determination of the histamine content of blood was developed by Barsoum and Gaddum in 1935 (9). Code (26) later produced a somewhat improved and simplified technique that has been used for most human studies. Code's method starts with the precipitation of proteins with trichloroacetic acid (TCA). The TCA filtrate is then boiled with concentrated hydrochloric acid and evaporated to dryness. The residue is next taken up in ethanol and this solution is taken to dryness. At least three ethanol washes are carried out, and the final dried residue is taken up in a solution suitable for biological estimation of histamine on the isolated guinea pig ileum preparation.

The level of histamine in human blood plasma is very difficult to assay accurately, mainly because of the difficulties involved in the separation of plasma and the low histamine concentrations involved. Code (27) found plasma histamine levels in normal humans to be less than 5 ng/ml, whereas Adam et al. (3), using a more sensitive assay technique and special precautions during the withdrawal of blood and separation of plasma, reported plasma histamine levels in normal humans to be less than 1.0 ng/ml. This value was obtained after the blood specimens were withdrawn into polythene syringes and then transferred to chilled centrifuge tubes, also of polythene and containing anticoagulant, in which a two-stage centrifugation procedure was carried out. The anticoagulant used was heparin, each batch being screened for "histamine-like" activity before use. These workers used an extremely sensitive preparation, the superfused guinea pig ileum. Instead of placing the ileum in a bath of nutrient Tyrode solution, it is suspended in air and the solution is dripped over it at a constant rate [Gaddum (49), Adam et al. (2)]. This preparation can detect concentrations of histamine lower than 0.25 ng/ml.

In the method of Adam et al. (2) a purification procedure is carried out before the test material is applied to the preparation. The steps in the method are as follows: first the plasma proteins are precipitated with TCA and this is followed by the preparation of an aliquot of the supernatant for adsorption on buffered cationic resin columns; elution of histamine and other bases is then achieved by displacement with hydrochloric acid; and finally the eluate is converted into a solution suitable for assay on the superfused guinea pig ileum preparation.
Relative Specificities of Methods

The perfect assay technique would be completely accurate, highly sensitive, reproducible, and specific for the compound or compounds it has been developed to measure. This ideal, however, is seldom wholly achieved and techniques are frequently modified to improve one or more of these conditions.

Although various compounds can give false histamine values with the DNFB method, all the potentially interfering compounds known to occur in natural products have apparently been screened [McIntire (81)]. Only histidinol and agmatine give substantially "false" histamine values [McIntire et al. (82), Lowry et al. (77)]. The DNFB method has been used for the determination of histamine in blood and plasma. One criticism leveled at this method is that it does not distinguish between histamine and methylhistamine. However, Swedish workers have separated the DNFB complexes of histamine and methylhistamine by thin-layer chromatography and measured the color of each separately [White (138)].

The newer OPT method has been made more specific, with special procedures for the separation of interfering ammonia, histidine, and some peptides from histamine before the OPT condensation [Weissbach et al. (137), Kremzner and Wilson (72)]. The main advantages of the OPT method over the DNFB method as regards specificity is that methylation of histamine prevents formation of the fluorophore [McIntire (81)]. It has been found, however, that plastic ware cannot be used in conjunction with this method due to the formation of interfering fluorophores with OPT.

In Code's (26) modification of the method of Barsoum and Gaddum (9) the specificity of the assay can be partially checked by the use of mepyramine maleate, which blocks the effect of histamine on the ileum and thus shows whether any spasmogens other than histamine are present in the test solution. However, some critics of these techniques claim that during the initial treatment of the blood, the TCA-HCl digestion stage, histamine can be formed by the decarboxylation of histidine [SchmitterlSw (117)]. Nevertheless, the HCl digestion is thought to be a necessary stage, as it effectively destroys such pharmacologically active substances as acetylcholine, 5-hydroxytryptamine, and certain plain muscle-stimulating polypeptides, e.g. bradykinin [Barsoum and Gaddum (9), Code (28)]. Hughes et al. (60) could produce histamine only from histidine concentrations 100-500 times stronger than those found in blood and plasma, and even then they found that the histamine formation was minimal if the HCl digestion was not too vigorous.

In 1961 Adam (1) modified the earlier method (2, 3) for the estimation of histamine in plasma by the inclusion of an HCl digestion stage after elution from resin columns. This is a gentle digestion procedure carried out in a paraffin bath heated to 100 C; it has enabled the method to be applied to brain tissue, which contains high levels of pharmacologically active bases other than histamine (1).

In our own studies we have used Adam's modified method for the estimation of histamine in whole blood (which also contains 5-hydroxytryptamine, etc.) and
plasma from patients with a variety of diseases. Such samples may contain interfering substances that are destroyed by HCl.

Adam (1) found that HCl digestion destroyed the activity of 5-hydroxytryptamine (100 ng), norepinephrine (100 ng), and substance P (200 units). Also, when he added 10 µg of histidine to a TCA extract and to an eluate, no evidence of histamine formation was found; we have confirmed this observation (1, 106). The methods of Adam et al. (1, 3) and Irvine et al. (64) are more specific than the earlier method of Code (26), as acetylhistamine is effectively separated from histamine during the column chromatography procedure. However, when methylhistamine was added to TCA extracts it was found that it was eluted from the columns along with histamine [Hyc (63)].

**BASOPHIL LEUKOCYTES**

The basophil leukocyte was first described by Paul Ehrlich in 1891 (41). At that time he noted morphological similarities between the basophils and tissue mast cells, both of which contain metachromatically staining granules. Ehrlich also recognized the different origins of these cells and postulated that the basophil leukocyte is derived from the marrow and is morphogenetically analogous to the neutrophil and eosinophil leukocytes. Despite much early criticism [Graham (51)] this view is now widely held.

It was not until some 70 years after the discovery of the basophils that their high histamine content was noted [Graham et al. (52, 53), Code and Mitchell (29)]. In 1952 Behrens and Taubert (13) produced evidence suggesting that the basophils are rich in heparin-like material. This further emphasizes the close relationship between the basophils and their connective tissue counterparts, the mast cells. In 1952 Graham and her colleagues (53) worked on the basis that the unique feature of the blood cell distribution in chronic myeloid leukemia, namely marked basophilia [Ehrlich and Lazarus (42)], had apparently been overlooked as a possible explanation of the equally unique histaminemia.

After publication of their initial findings [Graham et al. (53)], together with the evidence that the mast cells are depots of tissue histamine [Riley and West (113, 115)], Ehrich (40) analyzed earlier data of Valentine and Lawrence (134) to demonstrate a correlation between the basophils of the blood and its histamine content. It was later concluded that the basophil, despite its scarcity—approximately 40–50 per mm³ in human blood [Moore and James (93)]—accounts for at least half the histamine in normal human blood [Graham et al. (52), Code and Mitchell (29)]. Graham and coworkers separated the various leukocytes in specific-gravity graded columns that comprised concentrated albumin mixed with leukocyte-containing plasma. After centrifugation the leukocytes were found to be distributed according to their specific gravities. Histamine in these cell layers was measured using a chemical technique [McIntire et al. (82)] that involves 2,4-dinitrofluorobenzene. From their analyses Graham et al. (52) concluded that the basophils contain histamine in a concentration 20,000 times that of the platelets and “a
million times that of the plasma." We believe, however, that the scarcity of basophilic leukocytes in the blood makes the use of a chamber-counting method [Moore and James (93)] preferable to the centrifugation technique. Thus Code and Mitchell (29) expressed all values in terms of the absolute basophil count: that is, the number of cells per cubic millimeter of blood (i.e., cells mm$^{-3}$).

In 1959 Boscila (20) concluded that human basophils are probably the circulating members of the mast system, representing a mobile source of polysaccharide and histamine, and that they appear in some way to compensate for the tissue mast cells and supplement their functions by being immediately available whenever a condition arises requiring histamine and/or mucopolysaccharide release in the tissue. It has also been suggested that human basophil leukocytes produce their own histamine; in this respect the cells also closely resemble the tissue mast cells [Lindell et al. (75), Rorsman (118)].

Graham et al. (52) made an estimation of the histamine content of a single basophil leukocyte using multiple regression from histamine measured on a series of blood samples from normal adults and patients with myelocytic leukemia. The value obtained was 1 pg of histamine per cell, which is one-twentieth that of a tissue mast cell. Sampson and Archer (125) described a method of obtaining basophil-enriched preparations of white cells from both normal donors and patients with myeloid leukemia. They reported the mean histamine content of the normal basophil to be equivalent to 2.4 ± 0.3 ng histamine acid phosphate per 10$^6$ basophils (1.5 ± 0.2 µg histamine base/10$^6$ basophils)—i.e., 1.5 ± 0.2 ng pg/cell. Patients with chronic myeloid leukemia were found to have a lower mean value (1.8 ± 0.7 ng µg histamine acid phosphate per 10$^6$ basophils (1.1 ± 0.4 ng µg histamine base/10$^6$ basophils)—i.e., 1.1 ± 0.4 ng pg/cell. The mean differences between the two groups of donors were found to be significant (0.01 > P > 0.001). Furthermore, Sampson and Archer (125) showed that the histamine is present in the granular fraction of basophils and is released from the cells in conditions associated with granule lysis.

The blood histamine situation is less clear in species other than man. Minard (87) found that most of the histamine in rabbit blood is localized in the platelets. It is believed that the eosinophil may play a major part in the transport of histamine in the blood of some other species [Code and Mitchell (29)].

**Eosinophil Leukocyte in Relation to Basophil Leukocyte**

The basophil leukocyte was originally thought to be an immature eosinophil [Blumenthal (18)]. Later, however, the morphogenetic independence of the two cell types was shown [Maximow (80)]. Despite this morphogenetic independence, Code et al. (30) suggested an interrelation between the two types of cell based on the parallelism between them in certain conditions. Some workers have claimed that an absolute numerical correlation exists between circulating basophils and eosinophils [Osda (97), Hamerston et al. (57)]. Others, however, have found little or no correlation [Uhrbrand (133), Mitchell (89)]. In 1955 Mitchell (88)
reported that both eosinophils and basophils are more numerous in the newborn infant than in older humans.

The eosinophil was thought to be the chief source of histamine in the body [Code (28)] until evidence came to light that the tissue mast cell and the basophil leukocyte are rich in histamine [Riley and West (116), Graham et al. (52)]. Code's earlier statement was modified in that, "while the histamine content of the basophils is found to be relatively stable, the amount carried by each eosinophil is less and much more variable, and indeed sometimes the human eosinophil may contain no histamine whatever" (29). Moreover, the isolation from eosinophils of granules with antihistaminic properties [Vercauteren (135)] has led to the suggestion that the eosinophil may be concerned with the detoxication and disposal of histamine rather than its production or storage [Riley (114)]. A possible corollary to this was the observation by Archer (6) that after injection of histamine into the skin of horses there is a migration of eosinophil leukocytes to the edematous area. The mechanism of eosinophilia and eosinopenia appears to be that eosinophils are attracted by the release of histamine [Archer (7, 8)]. But Blatt (15) noted that, although histamine may play some role in producing an initial nonspecific increase in eosinophils, it cannot maintain an increase in the number of these cells.

**Basophil Leukocyte-Counting Techniques**

In 1953 Moore and James (93) published their "simple direct method for absolute basophil leukocyte count." They claimed that this method superseded the former indirect methods in which at least 1000 leukocytes must be counted in order to ensure a reasonable estimate of the number of basophils present. Indirect counting methods have a large margin of error due to the uneven distribution of leukocytes in the stained blood film, whereas in the direct method, in which the cells are counted using a graduated counting chamber, the liability to error is very much less.

The method of Moore and James utilizes the metachromatic staining principle described by Ehrlich [Michels (84)], in which the material contained in the cells stains a different color from that of the dye. The granules in the basophil cytoplasm contain a mucopolysaccharide that stains in this way, and Moore and James found that for basophils toluidine blue was the most satisfactory dye. This stains the basophil granules a bright pink-violet and the nuclei of all leukocytes, including the basophil, a blue-violet color, the intensity differing with the type of cell. The eosinophils are easily distinguished by their larger size, the greenish-yellow appearance of their cytoplasm and granules, and the rounded bilobed nuclei. Although the basis of this technique is that only the basophils take on a metachromatic stain, an eosinophil count and a total leukocyte count can be performed simultaneously. The diluting fluid consists of toluidine blue together with a quantity of ethanol and saponin to lyse the erythrocytes.

Moore and James performed basophil counts on 69 normal adults and reported that in the male group (20–38 years) the mean absolute count was 46.7 ±
20.1 \text{sd} \text{basophils mm}^{-3} \text{ and in the female group (18-34 years) was 40.6 } \pm 19.9 \text{sd mm}^{-3}, \text{ therefore finding no significant difference between the means for the sexes. Porter and Mitchell (107) obtained similar results in a group of 35 children (1.5-11 years).}

Mitchell (89) carried out absolute counts of basophil leukocytes in children using a slightly modified version of the Moore and James (93) technique. He found that the method was improved if the pH of the diluting fluid is controlled at 7.75 with sodium hydroxide. For work with the newborn infant, the diluting fluid need contain less ethanol than the amount specified by Moore and James for complete hemolysis to take place (88). Another modification of the Moore and James technique was published by Boseila (21). In this method the toluidine blue stain is first dissolved in Sporen's phosphate buffer at pH 7.7. This has the same effect as the pH adjustment with NaOH (88, 89). Boseila (21) also found that the addition of ammonium-potassium oxalate keeps the blood in a fluid state.

Cooper and Cruickshank (31) criticized the earlier methods of Moore and James (93) and Boseila (21). They claimed that in using these techniques the staining of the basophils was often diffuse due to the water solubility of the polysaccharides (in the basophil granules), making identification difficult and the counting time consuming. It was also claimed that clotting and aggregation of platelets made counting inaccurate and in addition that aggregation was often associated with degranulation of the basophils in that area, leading to counts lower than the true values.

The method of Cooper and Cruickshank (31) is based on the use of cetylpyridinium chloride to lyse the erythrocytes and render the mucopolysaccharides of the basophil granules insoluble. Aluminium sulfate is used to mordant toluidine blue to the granules. The use of ethylenediaminetetraacetic acid (EDTA) as an anticoagulant is said to eliminate any clotting of platelets. The basophils are seen as purple-red metachromatically stained cells. Other leukocytes, platelets, and erythrocytes are unstained. In 10 subjects Cooper and Cruickshank reported a mean absolute basophil count of $40 \pm 7.8 \text{sd mm}^{-3}$. These authors stated that the reduction of the total error was due essentially to their improved staining technique.

One large drawback to the use of the Cooper and Cruickshank (31) method is that parallel basophil, eosinophil, and total leukocyte counts cannot be carried out as with the method of Moore and James (93).

\section*{Asthma and Allergy}

Asthma is characterized by recurrent attacks of breathlessness and wheezing, frequently nocturnal, that may vary greatly both in severity and frequency. The attacks are often very sudden and can be extremely severe. Although many seem to be precipitated by specific allergic reactions this is by no means always the case, and psychological disturbance and other mechanisms are believed to be involved.

The symptoms depend on an increase in airway resistance, which can be due to bronchoconstriction, mucosal edema, infiltration of the bronchial mucosa by...
eosinophils, or the accumulation of quantities of secretions in narrow airways. In adapting to the increased resistance to expiration, the lungs become overinflated and thus provide increased clastic recoil, but this increases also the work of inspiration. Variations in airway resistance between different units of the lung cause maldistribution of ventilation in the lung, giving rise to arterial hypoxemia. Alveolar ventilation may become insufficient, possibly as a result of exhaustion, and this causes the arterial \( P_{CO_2} \) to rise and respiratory acidosis to occur.

**Blood Histamine and Granulocytes in Asthma and Allergy**

Much work has been published concerning blood histamine levels in asthma. However, the values quoted are quite varied and often conflicting, and some of the reports are ambiguous.

Howarth and McDonald (58) and DeGara (36) found high blood histamine levels in asthmatic patients with acute symptoms, and a number of workers [Parrot (98), Randolph and Rackeman (111), Jiminez-Diaz et al. (65)] have reported high blood histamine levels in asthmatic patients during attacks but normal values during symptom-free periods. Rose (120) found a consistent increase of the blood histamine in various forms of asthma with a tendency toward constant higher levels in young asthmatics under 16 years. He noted great fluctuations in blood histamine levels but could not correlate these fluctuations with the onset of asthmatic attacks. Konoshita (70), however, concluded that blood histamine levels increase in bronchial asthma, especially during attacks. On the contrary, Riesser (112) found no increase of blood histamine in asthma and Eggels and Nelemans (39) reported no difference between blood histamine levels of allergic and nonallergic patients.

In 1948 Serafini (129) reported a study of blood histamine levels during asthmatic attacks over a period of some hours from the onset of the asthmatic symptoms, blood samples being withdrawn from the patients at fixed time intervals. Using this procedure he found an initial rise in blood histamine at the onset of the attack, quickly followed by a drop to below normal values. Rose and his colleagues (122) found no increase in blood histamine during the provoking of an “asthma-like” state, but found the blood histamine values generally to be higher in asthmatic subjects than in normals. Maas et al. (78) found that allergic children tended to show definitely raised or definitely lowered histamine levels. Gudowski and Dieckhoff (54) showed a close correlation between the number of circulating basophils and blood histamine concentration in bronchial asthma and during other allergic manifestations in children.

Porter and Mitchell (107) found a much more complex relationship between granulocytes and whole-blood histamine levels in asthmatic children. Although overall they could demonstrate a statistically significant correlation \( P < 0.001 \) between whole-blood histamine levels and basophil counts and between whole-blood histamine levels and log eosinophil counts, no statistically significant correlations were found between the whole-blood histamine levels and basophil...
counts in any of the groups of asthmatic children studied. Their groups included:
1) quiescent asthma (children prone to asthma, not showing signs of symptoms at
time of examination); 2) asthma symptoms group; and 3) a group of children on
long-term steroid therapy.

Lindell and Westling (76) commented that what is certain about allergic
subjects is that their histamine metabolism and their reaction to histamine are
different from the norm. It is also known that asthmatics tend to have “pathologi-
cal” blood histamine levels, i.e., outside of the range 20–100 ng/ml. Lindell and
Westling (76) concluded that, although blood histamine levels are often observed
to rise during attacks, it cannot therefore be assumed that this histamine is released
directly during an attack and that this rise in blood histamine only suggests a
connection between histamine and asthma.

Few measurements of histamine in the blood plasma of asthmatic people have
been published. Beall (11), using the spectrophotofluorimetric method of Noah and
Brand (95), found no significant difference between normal plasma histamine
levels and those of asthmatic and allergic subjects. The mean plasma histamine
concentration of a group of normal subjects was determined as 5.6 ng/ml and this
value was found to be unchanged in the asthmatic patients studied. Porter and
Mitchell (107), using the methods of Adam and his colleagues (3), found less than
1 ng/ml in all the asthmatic children they studied in each of their three groups (a
total of 55 children), which is also in agreement with the values for normal adults
and children (3, 106).

Basophil leukocytes in asthma and allergy were first studied by Cannon in
1892 (25), who reported a slight percentage increase in the basophil count in
asthmatic blood. In 1957 Noah and Brand (94) counted basophils in three asthmatic
patients and found normal values. Criticisms leveled at their work concern the
small number of subjects studied and the method of counting used (the count being
performed on 1000 leukocytes). Rorsman (117) used a slightly modified version
of the method of Moore and James (93) on blood from 20 asthmatic subjects and
was not able to observe a significant increase in the blood basophil count. Rorsman
drew attention to the fact that a large proportion of the patients under study were
being treated with epinephrine or corticosteroids; corticosteroids are known to
produce a lowering of the blood basophil count [Code et al. (30)]. Rorsman (117)
postulated that were an increased basophil count seen in untreated asthmatics,
this could be a possible explanation for the increased histamine content reported
by many workers.

This increased histamine content was also recorded by Porter and Mitchell
(107) in their group of children with asthmatic symptoms. They found a mean of
81 ng/ml (SEM 9.4) and this was significantly higher than the mean whole-blood
histamine level for the quiescent asthma group 57 ng/ml, SEM 5.6) and the long-
term steroid therapy group (51 ng/ml, SEM 5.1). No significant difference was
found between the mean whole-blood histamine level for their control group of
children and the latter two asthmatic groups.

In a study of six asthmatic children Blau and Plenert (16) stated that any rise
in the number of circulating blood basophils was accompanied by a parallel rise
in the number of eosinophils. Unfortunately no values were quoted. We have found that the basophil-eosinophil relationships are more complex in asthmatic than in nonasthmatic subjects and that this relationship appears to vary with the severity of the symptoms (107).

In urticaria, a purely allergic manifestation, basophilic leukopenia is common, together with a lowering of the whole-blood histamine levels [Rorsman and Rosengren (119)]. In a study on 106 patients with various forms of urticaria Rorsman (118) found that the mean number of circulating basophils was significantly lower than that of his control group. As a rule this basopenia disappeared soon after the urticarial symptoms ceased.

Steroid Therapy

Bronchial asthma may be life-threatening in its most acute form (status asthmaticus) or disabling in its chronic form. Corticosteroids are often helpful in terminating an acute attack and in relieving the symptoms of the chronic form. In conjunction with corticosteroid therapy patients are often advised to use sympathomimetic bronchodilator drugs or aminophylline in order to keep the dose of steroid at the lowest possible level for relief of the condition. This is done to cut to a minimum the unwanted effects of long-term steroid administration such as fluid and electrolyte disturbances, hyperglycemia and glycosuria, susceptibility to infections, peptic ulcers, growth retardation, etc.

It has been shown by several workers that administration of corticosteroids produces effects on the granulocytes and blood histamine levels. In 1954 Code and his colleagues (30) reported that cortisone produces basopenia with a simultaneous eosinopenia in healthy humans. They took this as another indication of a relationship between basophils and eosinophils. Code and Mitchell (29) further reported that the basophil and eosinophil counts were decreased by cortisone in dogs and guinea pigs as well as in man. In man and dog whole-blood histamine levels were also diminished but in guinea pigs this change was not constant.

Noah and Brand (94) studied 11 patients mostly suffering from asthma and found marked falls in leukocytes and blood histamine levels in those given therapeutic doses of prednisone. They also reported that the fluctuations in blood histamine levels paralleled more closely the fluctuations in basophil counts than those of any other type of cell. Using a relatively insensitive procedure they found no significant change in plasma histamine values after steroid administration. Porter and Mitchell (107) obtained similar findings with a group of asthmatic children on long-term steroid therapy. Porter et al. (109) studied a group of children on short-term steroid therapy for periods between 5 min and 2.5 hr after administration of a single dose of corticosteroid, and at none of these times did the children demonstrate a rise in the plasma histamine level.

In work on guinea pigs Kovács and Suffiad (71) found that a single massive dose of cortisone (100 mg/kg) brought about a significant increase in the plasma histamine levels. They concluded that this histamine release is mediated by the
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hyperglycemic effect of cortisone, as a similar plasma histamine increase occurred after administration of glucose. Konoshita (70) reported that the high blood histamine levels encountered in status asthmaticus decreased to normal levels in four out of six patients after administration of corticosteroids. In the experiments of Gudowski and Dieckhoff (54) prednisone treatment resulted in degranulation and a drop in the number of circulating basophils with a concomitant decrease in blood histamine.

Although epinephrine and ephedrine are often administered with the corticosteroids [Braunsteiner and Thumb (24)] the administration of epinephrine alone causes no significant change in the basophil count in man.

Melmon and Cline (83) proposed that glucocorticoids may inhibit kinin release by granulocytes and by plasma and tissue kallikreins, and that this interference with kinin production may be a mechanism of the antiinflammatory activity of the glucocorticoids.

Cromoglycate Therapy

In 1967 Cox (34) described the actions of a new drug, disodium cromoglycate (Intal), a chromone derivative. It specifically inhibits the liberation of the mediators of the anaphylaxis initiated by the interaction of antigen with reagin-type antibodies. The compound is neither a bronchodilator nor antiinflammatory and its action is distinct from that of the corticosteroids. Cox (34) added that disodium cromoglycate has few general pharmacological actions, is rapidly excreted from the body, and appears to have a very low order of toxicity. He concluded that the ability of the compound to inhibit the anaphylactic process initiated by the reaginic antibody-antigen interaction may permit a more specific treatment of allergic disease, especially of the lung.

Later an annotation in Lancet (5) expressed an interest in the compound, but drew attention to the fact that the preparation used was a mixture of disodium cromoglycate 20 mg, isoprenaline sulfate 0.1 mg, and lactose. Clinical trials have indicated, however, that the combination of cromoglycate and isoprenaline produces significant improvement when compared with isoprenaline and lactose alone in patients with allergic asthma but not in nonallergic cases [Howell and Altounyan (59)]. Though it is obvious that cromoglycate may have measurable effects on the distribution of histamine in the body, no reports of its effects on blood histamine levels and granulocyte counts have appeared in the literature up to this time.

HISTAMINOPEXY

In 1952 Parrot, Urquia, and Laborde (105) described the binding of histamine by serum. They found that when a solution containing histamine dihydrochloride (1 µg/ml) and 5% normal human serum, previously dialyzed for 24 hr, was tested for biological activity on the isolated guinea pig ileum the effect of the histamine was reduced by approximately 30%. This phenomenon was termed “histamino-
pexy" or histamine binding. When the serum of allergic subjects was tested in the same way, histamine binding was not observed (74). Histaminopexy was thought to be the binding of histamine and not its enzymic destruction, since it was possible to recover the activity by addition of a binding competitor or even by simple dilution (104).

Various techniques have been devised for the estimation of serum histaminopexy: one of the most convenient is the pharmacological method mentioned above; this method was used in our own studies. With the use of a manually operated organ bath, the method described by Laborde et al. (74) subsequently has been employed by many workers, including Mahfouz et al. (79), Wodniansky (140), and Guirgis (55, 56). The technique has also been modified for use with an automatic apparatus such as that described by Boura et al. (23) in 1954 [Flavian et al. (46)]. Kirtchev and Frankland (69) attempted to reproduce Parrot's findings using the automatic apparatus; they did not proceed very far, however, due to their inability to reproduce the basic findings. Guirgis (55) indicated that this may have been due to the high concentration of protein in the 2-ml bath used in their technique.

Parrot and his colleagues (104) claimed that previous dialysis of the serum is important, as the specimen should be free from substances that could interfere with a biological test. It was on this basis that Parrot and his colleagues (104) explained the findings of Craps and Inderbitzin (35), which did not agree with their own (74). Wodniansky (140) produced statistical evidence of the reproducibility of Parrot's findings, using over 600 subjects.

A serological method for the estimation of serum histaminopexy has also been developed [Mikol et al. (86)]; it utilizes histamine bound to particles of latex. Normal human serum was found to agglutinate these coated particles, whereas allergic serum did not. These results have since been confirmed by other workers [Huriez et al. (61, 62), Mikol and Renoux (85)]. The method has been modified and improved by Ruitton et al. (124). In an Australian study using a serumagglutination technique Freeman (47) reported that in 315 cases allergic sera gave 82% negative results and in 88 control sera 66% gave positive agglutination.

It has been shown that the serum of normal subjects binds histamine (100), 5-hydroxytryptamine (99), and acetylcholine (45). In all their large-scale investigations Parrot and his colleagues have studied histamine binding using the pharmacological technique. A group of 79 subjects without allergic symptoms and without familial allergy all showed histaminopexy. Later, when a group of 225 subjects who were not so carefully selected was studied, low values were found in 7% of the subjects (102). Parrot and colleagues (104) claimed that histaminopexy values equal to or greater than 15% could be regarded as normal.

Normal serum histaminopexy has been attributed to the presence of a gamma globulin—plasmapexin I [Laborde et al. (73)]. This material represents only 0.01% of all the globulins present in serum, but these workers argue that it is adequate to produce the serum histaminopexic action. This fraction is also believed to bind 5-hydroxytryptamine and acetylcholine (45, 99). Beall (12), using an equilibration dialysis technique, studied histamine binding by a number of serum protein fractions including plasmapexin I and heat-agglutinated gamma globulins with negative results and concluded that the binding of histamine by serum proteins
is not the cause of histaminopexy. Guirgis (55) demonstrated that the gamma globulins separated from human serum and the gamma globulin obtained commercially were free from histaminopexic activity. He did find, however, the full histaminopexic power (30%) in plasmapexin I. When Guirgis (55) carried out cellulose acetate electrophoresis of plasmapexin I, it was found to contain beta, alpha 1, and alpha 2 globulins as major constituents and gamma globulin in a very much smaller concentration. He concluded that further purification of the binding factor was required before a conclusion as to its identity could be reached.

Freeman (48), in studies using immunoelectrophoresis, reported that the identity of one of the fractions showing histaminopexic activity (that precipitated with 0.8 M Na₂SO₄) was IgG. The active fraction was also precipitated in vitro by specific anti-IgG serum, and after removal of the precipitate the histaminopexy using the latex test was negative. However, she found in addition that some sera also precipitated their active fraction with 0.96 M Na₂SO₄ and that these fractions contained traces of alpha globulins.

It has been shown that the allergic subject's serum does not bind histamine, 5-hydroxytryptamine, or acetylcholine (45, 99). Parrot and colleagues (104) wrote that lack of histaminopexy had sometimes been noted in the course of systematic investigations, even when the subject showed no clinical signs of allergy; however, in some of these subjects asthmatic attacks did occur subsequently. From this they (104) concluded that the loss of histaminopexy is likely to precede the clinical symptoms. Parrot and Laborde (102) examined a group of 74 patients with asthma and hayfever. In 97% of the subjects histaminopexy was absent. In the same study, all of a group of 14 patients with urticaria and angioneurotic edema had zero histaminopexy.

Porter and Smith (108) carried out histaminopexy determinations in a group of control children, a group of children with asthmatic symptoms, and a group of quiescent asthmatic children. They found a mean histaminopexy of 18% in the control group, 17% in the quiescent asthmatic group, and 12% in the group of children with asthmatic symptoms. When the Student t test was applied to the means the difference between the group of control children and that of children with asthmatic symptoms was significant at the 5% level. However, no significant difference was found between the means for the control children and the children with quiescent asthma. The authors suggested that one of the reasons why their results were not as decisive as those of other workers was because asthma may not always be a purely allergic phenomenon. If attacks are consistently precipitated only by an antigen-antibody reaction the condition is clearly allergic, but when symptoms can be provoked by physical or emotional stimuli the etiology may be more complex.

Laborde et al. (73) claimed that there is no plasmapexin I in allergic serum. They did find, however, an antipexin that is an alpha or beta globulin and inhibits the binding of histamine by plasmapexin I. Together with this they found plasmapexin II, which is a gamma globulin able to bind histamine, but exerting no action in the serum. These findings, however, have not yet been verified by other investigators.

Little work has yet been published on the effect of steroid therapy for asthma.
on histaminopexy. However, Parrot and Laborde (101) carried out a systematic study with several doses of cortisone injected into rats (range 0.5–10 mg/100 g). They found that the histaminopexic power of the serum was lowered after 4 hr and fell to zero 12–18 hr after administration of the drug. Parrot and his colleagues (103) suggested that the cancellation of the histaminopexy could be due to a steroid-induced general diminution of the gamma globulins. Porter and Smith (108) studied the percentage histaminopexy in a group of asthmatic children on long-term steroid treatment (prednisolone 10–20 mg/day) and found that the difference between the mean histamine binding power for this group (10%) and that for a group of control children (18%) was significant at the 2% level.

BLOOD HISTAMINE AND GRANULOCYTES IN NEWBORN INFANTS

Several workers measuring the levels of histamine in the blood of women before and after delivery have found that pregnant women have normal or slightly lowered blood histamine levels when studied some weeks before delivery. Effkenmann and Werle (38) reported whole-blood histamine levels of 30–45 ng/ml in the 7th and 8th months of pregnancy. However, at delivery these values seem to be decreased; Bjurö and his colleagues (14) reported whole-blood histamine levels of 6–18 ng/ml at delivery. This lowered histamine level is probably due to the presence of the enzyme diamine oxidase (histaminase). The levels of this enzyme in the blood are very much increased in pregnant women [Åhlmark (4)].

Mitchell and Cass (90) found slightly elevated levels in blood from the umbilical vein at birth (1 case in 10 was above 100 ng/ml). Bjurö et al. (14) quoted values for blood from both the umbilical vein and artery. Those for the umbilical vein were 33–125 ng/ml and for the artery 40–135 ng/ml, which is a difference of approximately 15% between the arterial and venous levels. Whole-blood histamine levels in newborn infants thus appear to be 2–5 times higher than those of the mothers at delivery (14).

Dieckhoff and Cobet (37) also carried out a study of the blood histamine levels in the newborn. In a group of 11 newborn infants at term the whole-blood histamine levels were in the ranges 15–67 ng/ml arterial and 5–37 ng/ml venous. Again a tendency to higher levels was seen in the arterial blood. In a group of 9 premature newborn infants the histamine levels in the umbilical cord blood were 10–33 ng/ml arterial and 10–26 ng/ml venous. Here no significant differences were observed between the arterial and venous ranges. In normal term and preterm infants we have found whole-blood histamine levels similar to those in healthy adults and no significant difference between the mean values in umbilical arterial and venous blood [Mitchell and Porter (91)]. In 10 distressed newborn infants the mean values for histamine in both arterial and venous umbilical cord blood were slightly higher than normal, but the difference was not significant (110).

Wicksell (139) reported no difference between the plasma histamine levels of mothers and their newborn infants. His experiments, however, were carried out before the present accurate methods for the measurement of plasma histamine levels had been developed [Adam et al. (3), Noah and Brand (95, 96)].
Kahlson, Rosengren, and White (66) reported that human fetal plasma in early pregnancy contains greater amounts of histamine than normal; together with other evidence, this led them to the hypothesis that the fetus has a high histamine-forming capacity (66, 67). Dieckhoff and Cobet (37) made a study of plasma histamine concentrations in the newborn. Using blood from the umbilical vein they reported a range of plasma histamine levels of 5–15 ng/ml, whereas values in the umbilical artery were 5–30 ng/ml. These workers obtained similar figures with blood from premature infants. Kahlson and his colleagues (66) suggested that the somewhat lower histamine content of umbilical venous plasma is due to diffusion between fetal and maternal blood in the placenta.

Cooper et al. (33) found small amounts of histamine in the plasma of healthy term newborn infants. Their specimens were obtained by heel pricks and the histamine estimated spectrophotofluorimetrically. Recently, however, we studied normal and distressed infants born before and at term, using the methods of Adam and his colleagues, and found histamine levels in umbilical arterial and venous blood plasma to be consistently less than the lower limit of the assay method (91, 110), viz. less than 1 or 2 ng/ml (depending on volume of blood obtainable). These findings are in contrast to those of Dieckhoff and Cobet (37) and Kahlson et al. (66). Lindell and Westling (76) suggested that if any histamine appears in the plasma all that can be concluded safely is that the histamine-containing cells in the condition studied are more fragile than normal. We feel this provides an acceptable explanation of the very low levels found in our studies using scrupulously careful collection and separation techniques.

The earliest method of counting basophil cells in the newborn was by a differential method, the counting being carried out on smears. In 1895 Edler and Hutchison (43) found no basophils in the cord blood of 12 infants. Kato (68) reported in 1935 that basophils are comparatively numerous in the newborn period but gave no values. Wegelius (136) in 1948 found average counts of 50 basophils mm⁻³ at birth and a fall to 45 mm⁻³ after 2 hr. In 1955 Mitchell (88), using a more accurate technique, basically that of Moore and James (93), confirmed that circulating basophils in the peripheral blood are numerous in the period immediately after birth, but found an increase during the first 24 hr with a rapid decrease thereafter. This general pattern contrasted with the eosinophil counts, which were found to fluctuate widely. Later work in 1965 by Dieckhoff and Cobet (37) showed similar ranges of basophils in both arterial and venous umbilical blood from both term and premature infants, viz. approximately 5–50 mm⁻³. In the term group the upper range was somewhat higher in the arterial specimens, i.e., 58 mm⁻³ compared with 45 mm⁻³. Eosinophil counts were also made in the cord blood by Dieckhoff and Cobet (37); they found no significantly different values between any of their groups, the range being approximately 5–55 mm⁻³ in all four groups.

However, Mitchell and Porter (91) found a wider range of eosinophil counts—approximately 50–1000 mm⁻³. The distribution was found to be skew and this was normalized by taking logarithmic transforms; this distribution is in agreement with the findings of Cooper et al. (33).

In a study of 40 premature infants Blau and PLENERT (17) counted circulating
basophils and eosinophils. They found that the absolute eosinophil and basophil counts increased with the age of the infant and that this rise was more marked in premature infants with low birth weight. They also reported a statistically significant positive correlation between the numbers of these two cell types.

CONCLUSION

Although much work has been done in the field of blood histamine there are still large areas of disagreement and inconclusiveness, as is the case with the other autacoids [Mitchell and Porter (91, 92)]. It appears that as sampling techniques have improved so the reported plasma histamine values have become lower. The improved techniques now available allow more specific estimations to be made and indicate that normally the level of histamine in human blood plasma is very low indeed. A pathological increase in plasma histamine has not been verified in any of the conditions studied.

Histaminopexy still appears to be a controversial phenomenon, in need of further clarification as to its occurrence in different subjects and its localization to certain specific protein fractions.


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