THE BASOPHILE LEUKOCYTE IN URTICARIAL HYPERSENSITIVITY TO PHYSICAL AGENTS*

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Studies of peripheral blood by Ehrlich (1, 2) in 1890 led to the first description of the basophile leukocyte which he originally called a "mast leukocyte". Since that time, there has been considerable interest and speculation as to the role of the basophile leukocyte in health and disease as well as its relationship to the tissue mast cell.

Metachromatic staining, (3, 4, 5) which is common to both the basophile leukocyte and the tissue mast cell at first led to the assumption that these were the same cell. However, as early as 1906, Maximov (6) published evidence suggesting that they were not the same. Maximov postulated that the basophile is a leukocyte with an independent derivation and development from the tissue mast cell. Reviews by Doan and Reinhart (7) and Naegeli (8) have served to point out morphologic differences in the size and shape of the cells, nuclei and granules of these two types of cells. The more recent literature, however, has shown biochemical similarities which indicate that physiologically these cells in certain respects behave alike. Both types of cells contain heparin (9, 10) and histamine (11, 12) in man.

It was felt by Valentine, (13) Graham (14) and others (15) that the basophile leukocyte was the major carrier of histamine in the blood of man. While all the evidence supports the concept that the tissue mast cell and the basophile leukocyte are different cells, Bunting (16) called attention to an interesting numerical relationship between these cells in the different species of animals. Thus, those species which have a relatively large number of basophile leukocytes in the circulating blood (*e.g.*, rabbits and birds) have a relatively small number of mast cells in the tissues. Conversely, species with few basophile leukocytes in the circulating blood (*e.g.*, man) have large numbers of tissue mast cells.

Because the basophile leukocyte contains such a large amount of histamine and because of the report (17, 18) decreased level of blood histamine in patients with urticaria, Rorsman (19) was first led to investigate the role of the basophile leukocyte in the circulating blood in patients with urticaria. Rorsman, and subsequently also Bersani (20) and Meneghini *et al* (21) demonstrated that in patients with acute urticaria there is a decrease in the number of circulating basophiles and that this can be correlated with a decrease in blood histamine (22).

In studying the basophile leukocyte count in urticaria, engendered by different etiologic agents, it was noted that several patients with urticaria due to cold did not show a decreased basophile count. It was, therefore, decided to investigate the basophile leukocyte counts in a larger series of cases with urticarial hypersensitivity to physical agents.

MATERIALS

The number of basophile leukocytes per mm^3 blood was determined in 151 individuals, forming three categories: a control group, a "non-physical" urticaria group, and a "physical" urticaria group.

I. The control group was composed of 96 normal individuals†, all of whom were in good general health and had no history of urticaria or of other dermatologic disorders.

II. The non-physical urticaria group consisted of 36 patients with urticaria of varying severity. The urticaria was thought to be due to penicillin in eleven and to acetylsalicylic acid in fourteen patients. Foods were the cause or the cause was unknown in eleven patients. The basophile counts were performed during a phase when the patients had urticarial lesions. However, basophile counts were done only in patients who had received no medications (antihistamines, ACTH, or adrenocorticosteroids) for at least 48 hours.

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† This control group is the same as that previously presented in the paper by Dr. Rorsman, "Basophil Leukocytes and Urticaria", Acta Derm. Venereol., **37:** 121, 1957.

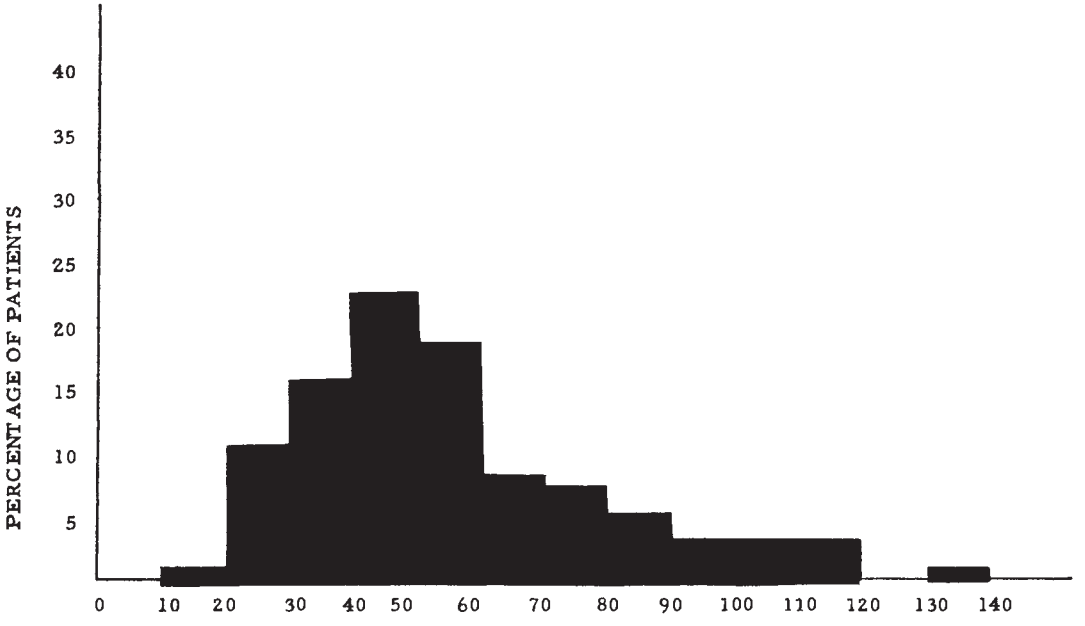

FIG. 1 Distribution of Basophil Leukocyte counts in 96 normal individuals

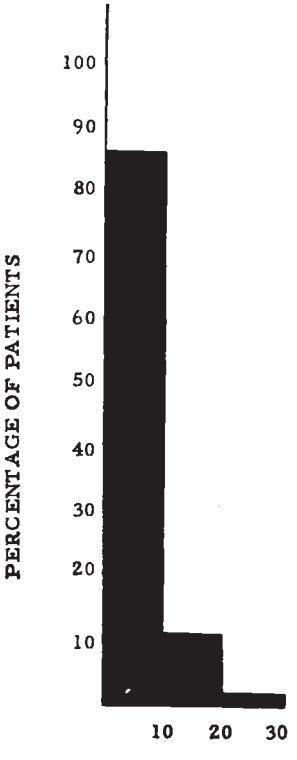

FIG. 2 Distribution of Basophil Leukocyte counts in 36 patients with non physical urticaria

III. The physical urticaria group consisted of 19 patients: 14 with cold urticaria, 2 with solar urticaria, 2 with cholinergic urticaria, and one with the very rare localized form of heat urticaria. Samples of blood were obtained before, during and after the urticaria was elicited by the appropriate stimuli.

METHODS

The number of basophile leukocytes per mm^3 of blood was determined by the direct and indirect methods as follows:

I. *The direct method:* This is a modification (Rorsman (19)) of the method described by Moore and James (23). Twenty-five mm^3 of venous blood was diluted with 215 mm^3 of a solution (Schellenberg, Wright and James(24)) containing:

Na_2HPO_4, M/15 ..... 9.3 ml
KH_2PO_4, M/15 ..... 0.7 ml
NaCl, 0.85% ..... 5.0 ml
Toluidine Blue, .01% ..... 5.0 ml
Absolute Alcohol ..... 5.0 ml

Five minutes after mixing, 10 mm^3 of a freshly prepared and filtered solution of 10% Saponin (Riedel-de-Haen) dissolved in 20% alcohol was added to produce hemolysis. The final dilution of blood thus was 1/10. A Jessen counting chamber with a depth of 0.4 mm and a volume of 10 mm^3 was filled and the cells in the entire chamber were counted.

Using this method, the basophile leukocytes have a red, metachromatic color, which is localized to the granules in the cytoplasm. Occasionally, the stain spreads throughout the cytoplasm rather than the granules. The nucleus is at times obscured by the staining. The cytoplasm of other leukocytes stains pale blue while eosinophil granules stain a greenish-grey.

II. *The indirect method:* Smears were made from venous blood and then the number of basophile leukocytes per 2000 white blood cells was determined, and related to the total white blood cell count. The smears were stained with 1% Toluidine blue (25) in methyl alcohol for five minutes and washed with distilled water.

A very close correlation was found in the results of the direct and indirect methods when the same sample of blood was used for both methods. This is in agreement with previous findings (19, 22). For the sake of simplicity and clarity, only the directly obtained values are graphically represented.

RESULTS

The average number of basophile leukocytes in capillary blood for the control group was 45/mm^3. Figure I shows the distribution of basophile leukocytes in this group.

The average number of basophile leukocytes in capillary blood from the "non-physical" urticaria group was 4.5/mm^3. Figure II indicates the distribution of basophile leukocytes in this group. Table A gives the individual basophile counts and causes of the acute "non-physical" urticarias.

TABLE A

Basophile counts of patients with acute urticaria

Patient	Cause of Urticaria	Basophil Count [Direct] [per cmm blood]
1.	Penicillin	8
2.	Penicillin	18
3.	Penicillin	11
4.	Penicillin	2
5.	Penicillin	0
6.	Penicillin	0
7.	Penicillin	1
8.	Penicillin	1
9.	Penicillin	4
10.	Penicillin	3
11.	Penicillin	0
12.	Acetylsalicylic acid	0
13.	Acetylsalicylic acid	0
14.	Acetylsalicylic acid	22
15.	Acetylsalicylic acid	0
16.	Acetylsalicylic acid	1
17.	Acetylsalicylic acid	6
18.	Acetylsalicylic acid	8
19.	Acetylsalicylic acid	0
20.	Acetylsalicylic acid	1
21.	Acetylsalicylic acid	3
22.	Acetylsalicylic acid	6
23.	Acetylsalicylic acid	1
24.	Acetylsalicylic acid	0
25.	Acetylsalicylic acid	2
26.	Miscellaneous foods & unknown	14
27.	Miscellaneous foods & unknown	10
28.	Miscellaneous foods & unknown	0
29.	Miscellaneous foods & unknown	2
30.	Miscellaneous foods & unknown	0
13.	Miscellaneous foods & unknown	19
32.	Miscellaneous foods & unknown	0
33.	Miscellaneous foods & unknown	2
34.	Miscellaneous foods & unknown	6
35.	Miscellaneous foods & unknown	9
36.	Miscellaneous foods & unknown	1

The average number of basophile leukocytes

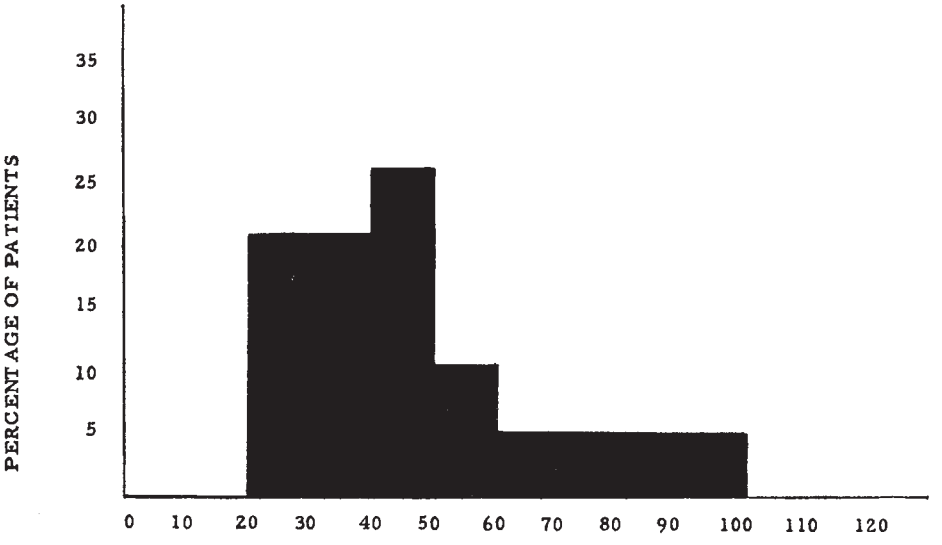

FIG. 3 Distribution of Basophil Leukocyte counts in 19 patients with physical urticaria.

TABLE B

Basophile counts of patients with "physical" urticaria

Patient	Type of Urticaria	Basophil Count [Direct] [per cmm blood]
1.	Cold urticaria	25
2.	Cold urticaria	18
3.	Cold urticaria	69
4.	Cold urticaria	14
5.	Cold urticaria	37
6.	Cold urticaria	21
7.	Cold urticaria	81
8.	Cold urticaria	38
9.	Cold urticaria	47
10.	Cold urticaria	27
11.	Cold urticaria	36
12.	Cold urticaria	11
13.	Cold urticaria	36
14.	Cold urticaria	47
15.	Cholinergic urticaria	18
16.	Cholinergic urticaria	30
17.	Solar urticaria	59
18.	Solar urticaria	38
19.	Heat urticaria	72

in capillary blood for the "physical" urticaria group was 38.1/mm³. As previously stated, tests were done before, during and after the deliberate production of urticaria. No significant differences were noted and the values given are those obtained from the patients at the time of their urticarial response. Figure III shows the distribution of basophile leukocytes in the "physical" urticaria group. The individual counts and causes of the "physical" urticarias are given in Table B.

DISCUSSION

The average number of basophile leukocytes in individuals not suffering from urticaria in our series was 45 per mm³ blood. This is in about the same range as that reported by Alder (26) who found an average of 35 basophiles per mm³ in normals. Moore and James (23) reported 45.7 cells per mm³ blood and called attention to the individual variation in the number of circulating basophiles. These variations were also evident in Bersani's (20) series. In his study of 62 individuals with no history of urticaria he found a mean basophile leukocyte count of 42.2 basophiles per mm³.

Rorsman (27) found that these variations are not attributable to prior food intake, or moderate exercise. The mean value of basophiles was the same in males and females and was somewhat higher in subjects between the ages of 40 and 59 years.

There are a variety of factors which have been reported to influence the number of circulating basophile leukocytes and of tissue mast cells.

Shelley and Juhlin (28) reported degranulation of basophile leukocytes in association with postprandial lipemia. However, the test meal consisted of large amounts of fats and cannot very well be considered a physiologically normal diet. Pathologic increases in the number of circulating basophile leukocytes have been reported in lymphatic and myelogenous leukemia (7, 8, 26), in polycythemia, secondary anemias, splenic stasis, congenital hemolytic jaundice and thyrotoxicoses. Pathologic decreases have been reported by Naegeli (8) in thyrotoxicoses and by Alder (26) in pernicious anemia. More recently, Boseila (29) found a significantly decreased number of circulating basophile leukocytes in 47 of 76 patients with rheumatoid arthritis of long standing. Ten patients in the early stages of disease were found to have basophile counts that were higher than normal. Experimentally, a decrease in basophile leukocytes can be produced with the administration of ACTH, cortisone and Thyroxin (30, 31, 32, 33).

Rorsman (34) investigated possible mechanisms causing urticarial basopenia and found normal basophile counts in the bone marrow of these urticaria patients. The basophiles are not degranulated in heparin or histamine solutions or in serum from patients with urticarial basopenia. There is no noteworthy accumulation of basophile leukocytes in wheals. Rorsman concluded that in acute "non-physical" urticaria, urticarial basopenia is caused by degranulation of the basophiles in the circulating blood.

Basophile counts in our group of patients with "physical" urticarias did not show the decrease which is regularly seen in patients with "non-physical" urticaria. Indeed, the distribution of basophile counts is quite similar to that of the control group. It is not within the scope of this paper to decide what percentage of cases of urticaria due to cutaneous hypersensitivity to physical agents is based upon an allergic mechanism. We merely wish to discuss the role of the basophile leukocyte in the circulating blood of patients with this particular variety of urticaria. Our findings indicate that this cell is not involved in, nor affected by, the reaction mechanism in urticaria due to physical agents. This is in contradistinction to the role of this cell in urticaria due to non-physical causes. It is also noteworthy that while acute urticaria due to non-physical causes is associated with a lowered blood histamine level (22) this is not the case in urticaria due to physical stimuli.

The tissue mast cell is known to participate in the urticarial reaction due to both physical and non-physical causes (35). Biochemical and perhaps also functional similarities of the tissue mast cell and basophile leukocyte suggest that, under appropriate conditions *in vitro* degranulation of the basophile leukocytes would be expected when they are in contact with a histamine liberator (*e.g.*, a circulating antibody-antigen system). This is well demonstrated for urticaria due to cold, penicillin, etc. by the basophile reactions described by Shelley (35) and Juhlin. These authors (36) were of the opinion that in cold urticaria the basophile leukocyte as well as the tissue mast cell may release histamine in association with the occurrence of wheals. They support this assumption by their findings that when blood from patients with cold urticaria is chilled *in vitro* the basophile leukocytes in this blood undergo degranulation. However, their data indicate that in the very patients with cold urticaria whose blood they used to demonstrate this reaction, a basopenia was not evident. It should also be noted that the temperature required to produce the degranulation in their test is so low as to preclude its existence in the blood *in vivo*. The possibility might be considered, that the size of the area which is made to urticate may have a bearing on the occurrence or absence of basopenia. However, among those cases reported by us were a few patients where we deliberately exposed at least 30% of the total body surface to the urticariogenic stimulus; yet, no basopenia was noted. It seems justified to assume that wheeling this large surface area involves as much as, or more than, the body surface involved in most cases of acute urticaria due to non-physical causes.

What then is the most likely explanation for the differences in basophile leukocyte counts in "physical" and "non-physical" acute urticarias? It is that the interaction between the stimulus (allergic or non-allergic) and the target (circulating or cell bound antibody or other target) in urticaria due to physical agents takes place

exclusively in the skin. On the other hand, in urticaria due to non-physical causes (drugs, foods, etc.), there is likely to be an interaction between the causal agent and the antibody or other target in the skin as well as in the circulating blood. However, it must be remembered that in most cases of urticaria due to drugs and foods, circulating antibodies cannot be demonstrated in the blood. There is a possibility that such antibodies exist and that it is only the presently available technics which are inadequate for their demonstration. Unless such antibodies are present in the circulating blood of patients with urticaria due to foods and drugs, it is difficult to understand why there should be a basopenia in such cases.

SUMMARY

The number of basophile leukocytes in the circulating blood was determined in 151 individuals. Ninety-six normal individuals had an average count of 45 cells per mm^3 blood. Thirty-six patients with acute "non-physical" urticaria showed a basopenia with an average count of 4.5 cells per mm^3 blood. Unlike the "non-physical" urticaria group, the 19 patients with "physical" urticaria showed no basopenia. The average cell count was 38.1 per mm^3 blood. The significance of this finding is discussed. Apparently in contrast to "non-physical" urticarias, in "physical" urticaria the circulating basophile does not participate in the reaction.

REFERENCES

1. Ehrlich, P.: Metholologische Beitrage zur Physiologie und pathologie der verschiedenen Formen der Leukocyten. Z. Klin. Med., 1879–80.
2. Ehrlich, P.: Farbenanallytische Untersuchungen zur Histologie und Klinik des Blutes. Berlin, Hirschwald 1891.
3. Ehrlich, P.: Beitrage zur Kenntnis der Anilinfarbungen und ihrer Verwendung in der Mikroscopischen Technik. Arch. f. Mikro. Anat., **13:** 263, 1877.
4. Ehrlich, P.: Beitrage zur Theorie und Praxis der Histologischen Farbung. Inaugural-Dissertation Universität Leipzig, 1878.
5. Ehrlich, P.: Beitrage zur Kenntnis der granulierten Bindegewebszellen und der eosinophilen Leukocyten. Arch. Anat. Physiol. (Physiol. Abt), **3:** 166, 1879.
6. Maximov, A.: Ueber die Zellformen des lockeren Bindesgewebes. Arch. f. Mikro. Anat., **67:** 680, 1906.
7. Doan, C. A. and Reinhard, H. L.: The basophil granulocyte, basophilocytosis and myeloid leukaemia, basophil and "mixed granule" types; an experimental, clinical and pathological study, with a report of a new syndrome. Amer. J. Clin. Path., **11:** 1, 1961.
8. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, 5th ed. Berlin, Springer, 1931.
9. Behrens M. and Taubert, M.: Der Nachweiss von Heparin in den Basophilen Leukocyten, Klin. Wschr., **30:** 76, 1952.
10. Martin, H. von and Rosa, L.: Zur Frages des Heparin Gehaltes der Blutmastzellen des Menschen. Acta Haemat., **10:** 26, 1953.
11. Riley, J. F. and West, G. B.: The presence of histamine in tissue mast cells. J. Physiol., **120:** 528, 1953.
12. Graham, H. T., Wheelwright, F., Parish, H. H. Jr., Marks, A. R. and Lowry, O. H.: Distribution of histamine among blood elements. Fed. Proc., **11:** 350, 1952.
13. Valentine, W. N., Lawrence, J. S., Pearce, M. L. and Beck, W. S.: The relationship of the basophil to blood histamine in man. Blood, **10:** 154, 1955.
14. Graham, H. T., Lowry, O. H., Wheelwright, F., Lens, M. S. and Parish, H. H. Jr.: Distribution of histamine among leukocytes and platelets. Blood, **10:** 467, 1955.
15. Wintrobe, M. M.: Clinical Hematology, 4th ed. Philadelphia, Lea & Febiger, 1956.
16. Bunting, C. H.: The granuler leukocytes. Special Cytology, 685–707. ed. by Dowdry, E. V. II: New York, Hoeber, 1932.
17. Rose, B.: Studies on blood histamine in cases of allergy. I. Blood histamine during wheal formation. J. Allergy, **12:** 327, 1941.
18. Nilzen, Å.: Studies in histamine (H-substance) with special reference to the conditions obtaining in urticaria and related skin changes. Acta Dermat., vener., **27:** suppl. 17, 1947.
19. Rorsman, H.: Basophil leucocytes and urticaria. Acta Dermat., vener., **37:** 121, 1957.
20. Bersani, R. B.: The blood basophil in urticaria and other dermatoses. Thesis, University of Zurich, 1959.
21. Meneghini, C. L. and Levi, L.: Istaminemia e mastociti circolanti in alcune dermatosi allergiche. G. Ital. Derm., **100:** 449, 1959.
22. Rorsman, H. and Rosengren, E.: Basophil leucocytes and blood histamine in urticaria. Acta Derm., vener., **38:** 377, 1958.
23. Moore, J. E. III and James, G. W., III: A simple direct method for absolute basophil leukocyte count. Proc. Soc. Exp. Biol. Med., **82:** 601, 1953.
24. Schellenberg, R. F. and Wright, D. V. and James, G. W. III, Personal communication, 1955.
25. Undritz, E.: In Hamatologische Tafeln Sandoz, p. 23. Basel, Sandoz AG 1950.
26. Alder, A.: Uber Klinisches Verhalten und Diagnostische Bedeutung der Basophilen Leukocyten (Mastzellen). Folia Haemat., **28:** 249, 1923.
27. Rorsman, H.: Normal variation in the count of circulating basophil leucocytes in man. Acta. Allerg., **17:** 49, 1962.
28. Shelley, W. B. and Juhlin, L., Degranulation of the basophil in man induced by alimentary lipemia. Amer. J. Med. Sci., **242:** 211, 1961.
29. Boseila, A. W. A. and Toone, E. C., Jr.:

Basophil leucocytes (Blood Mast Cells) in rheumatoid arthritis. Acta. Rheum. Scand., **7:** 183, 1961.
30. CODE, C. F., MITCHELL, R. G. AND KENNEDY, J. C.: The effect of cortisone on the number of circulating basophils and eosinophils: Is there a relationship between these cells. Proc. Mayo Clin., **29:** 200, 1954.
31. OSADA, Y.: Parallel changes in the numbers of circulating eosinophils and basophils inducted by ACTH administration. Bull. Inst. Pub. Health, **4:** 12, 1954.
32. BOSEILA, A. W. A. AND UHRBRAND, H.: Basophil-eosinophil relationship in human blood. Studies on the effect of corticotrophin (ACTH). Acta Endocr., **28:** 49, 1958.
33. CAMERADA, P., AND LEO, P.: Modificazioni indotte dalla tioxina sul tasso ematico dei leucocite basophile. Ross. Med. Sarda, **6:** 163, 1955.
34. RORSMAN, H.: Basopenia in urticaria, Acta Allerg., **16:** 185, 1961.
35. SHELLEY, W. B. AND JUHLIN, L.: A new test for detecting anaphylactic sensitivity: The basophil reaction. Nature, **191:** 1056, 1961.
36. JUHLIN, L. AND SHELLEY, W. B.: Role of the mast cell and basophil in cold urticaria with associated systemic reactions. J. A. M. A., **177:** 371, 1961.

DISCUSSION

DR. HERBERT MESCON, Boston, Mass.: Did you get much difference in the counts in using the direct and indirect methods?

DR. PETER FLESCH, Philadelphia, Pa.: Did I understand you correctly, that if these people are exposed for some time to urticarial agents, the basophiles presumably would remain unchanged for a long time?

DR. ALLAN L. LORINCZ, Chicago, Ill.: I enjoyed Dr. Harber's presentation very much and would just like to make a suggestion for further testing the interpretation given for the observed phenomena. Namely, it would be of interest to determine whether urticaria elicited by topical exposure to antigen in persons with urticarial contact allergy to materials such as wool or other animal hair is associated with changes in basophile count.

DR. L. C. HARBER, [in closing]: In response to the question regarding the direct versus the indirect methods of basophile counting we were able to approach an accuracy in comparing the two kinds of counts of $P < .05$. However, in the beginning when one starts basophile counts, I believe they will find the direct method is much simpler to use though it is quite time-consuming and may take from two to three hours.

Dr. Flesch's question concerning the recovery phase is an interesting point and I regret Dr. Rorsman is not here to answer it. He did extensive studies on just this phenomenon and found that the vast majority of patients who have urticaria will have a basophile count approaching normal values within three or four days of the appearance of the last urticarial lesion [1]. However, he did mention in his publication on this phenomenon that there were selected cases in which basophile recovery took several weeks. So, I would say as a generalization, the recovery rate is usually quite rapid. Dr. Rorsman also did bone marrow studies in these urticaria patients during the early stages of his investigations in order to ascertain whether or not there was a depression in the bone marrow that might account for the basopenia. His studies indicated that bone marrow was normal in individuals who had urticaria [2].

Dr. Lorincz raises an interesting point concerning the participation of the basophile following the application of a topical urticariogenic agent. Unfortunately, I have had no experience in this matter. The closest analogy that perhaps might be satisfactory can be found in work that Dr. Shelley has published recently [3]. I am under the impression that in skin biopsy specimens of individuals with cold urticaria, he was able to demonstrate a depletion in the mast cells after cold stimulation. In the same paper, I believe, there is also a statement that in those cold urticaria patients who manifested a markedly abnormal sensitivity of their basophiles in *in vitro* chilling at 4° C., the total circulating blood basophile count at the time of testing was normal and no basopenia could be demonstrated.

REFERENCES

1. RORSMAN, H.: Basophil leucocytes in urticaria, asthma and atopic dermatitis. Acta Allerg., **12:** 205, 1958.
2. RORSMAN, H.: Basopenia in urticaria. Acta Allerg., **16:** 185, 1961.
3. SHELLEY, W.: Role of mast cell and basophil in cold urticaria with associated systemic reactions, W. L. Juhlin, JAMA, **177:** 371, 1961.