ADVENTURES WITH THE BASOPHIL*

WALTER B. SHELLEY, M.D., PH.D.

For many, the word adventure conjures travel to distant lands or the exploration of endless outer space. I hope this afternoon to show you that adventure still exists no further away than your fingertips. This is a chronicle of the adventures we have had with a "cinderella" cell in a drop of blood. Our trail began on December 1, 1958 when a routine peaceful afternoon's practice was shattered by the near-tragedy of anaphylactic shock following a penicillin injection. Twenty physicians and two hours later the patient "returned" to the living and I was left haunted by our inability to predict these sensitivity states. With all our knowledge of modern immunochemistry could not the patient (and physician) be spared such episodes?

Dr. Harry Hurley and I reviewed the classical modalities for the demonstration of circulating antibodies known to participate in anaphylaxis. The precipitin reaction with its elegant versions, the wide range of agglutination phenomena, and the complement fixation test all seemed to fail in the clinical scene. The more specialized tools of the Schultz-Dale reaction and passive cutaneous anaphylaxis were again lacking in clinical appeal. Direct skin testing had been shown to be hazardous and at times misleading. After Doctor Hurley's departure in the Spring of 1959, Dr. Ralph Florence and I decided to study the problem intensively and exclusively. It was hoped to take testing out of the skin and into the laboratory. Extensive reading and long discussions for weeks gradually made it clear that a new technic was necessary. The logical approach was to use the mast cell as our cytologic index. Here was a cell containing histamine granules and one known to undergo visible alteration in anaphylaxis in animals. Here was the cell responsible for the positive skin test. But where to find and how to harvest this cell? Skin biopsies from patients proved too unwieldy and too low in mast cell density. Umbilical cord specimens showed but few intact mast cells. Turning to the animals we tried to passively sensitize the mast cells of the ears of the rat, mouse and guinea-pig. They remained strangely quiet in the presence of antigen and serum from patients known to be penicillin sensitive. The mesentery mount was our next battle site. After a year of effort and experiments with 1500 animals of many species, our sole positive contribution was the discovery of a new fixative and a method of capturing the fragile mast cells of the guinea-pig, but still no laboratory test for penicillin hypersensitivity (1, 2).

Dr. Florence returned to Canada and I turned our focus on a cousin cell, the basophil leukocyte (3). It had not seemed proper for dermatologists to begin by exploring the behavior of this blood-borne histamine carrier, and yet its role in anaphylaxis might be even more central than that of the cutaneous mast cell. Hence, when Lennart Juhlin came from Sweden to work on our laboratory in the fall of 1960, it was agreed to step outside the "union" and observe the behavior of the basophil. With virtually only one basophil per million cells in the blood stream, it became imperative to attempt to concentrate this elusive fragile test object. After innumerable adsorption trials failed, we explored liquid fixation of the blood basophil using a version of the fixative which had been so effective in capturing the morphology of the delicate guinea-pig mast cell. Fortunately this fixative was remarkably effective when minute quantities of blood were sprayed into it (4). Although the basophils were smaller than as seen in dilated form in smears on glass, their morphology was precise and clear when filtered out on a cellulose paper and stained with toluidine blue. No erythrocytes remained due to the acetic acid in the fixative and the other white cells were faintly stained.

The first tests on patients with penicillin hyper-

* From the Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

These studies were conducted under the sponsorship of the Commission on Cutaneous Diseases of the Armed Forces Epidemiological Board and were supported by The Surgeon General, Department of the Army.

Presented as the Presidential Address at the Twenty-Third Annual Meeting of The Society for Investigative Dermatology, Inc., Chicago, Illinois, June 25, 1962.

sensitivity uniformly showed *in vitro* degranulation of the basophils upon the addition of penicillin in a concentration of 1/10,000. Subjects who had taken penicillin without incident showed no change in the basophil (5). The basophil apparently was providing us with a miniaturization of anaphylaxis (6). Morphologic change was the key rather than alteration in number. Accordingly we devised a typing classification of the basophil to facilitate reading and perceiving the degree of degranulation. Based on the number, size and intensity of staining of the granules, as well as their location, it proved helpful in standardizing the test.

We soon moved to other areas of immediate type allergic hypersensitivity. One of our early studies was on patients with cold urticaria (7). Again the basophil proved an accurate index of this physical allergy. Next came observations on the urticarias from stinging insects and from drugs other than penicillin. Correlations continued to be excellent. The basophil showed a remarkable sensitivity to the antigen-antibody union. We apparently had trapped the true physiologic test object for study of the immediate type allergic reactions.

The responsiveness of the basophil to allergic reactions made us review its other potential functions (8, 9, 10, 11). It did store histamine (and as is now known, synthesize it as well) (12), but actually our stain technic measured the amount of heparin present in cell. Could not this second powerful pharmaco-dynamic agent, heparin, be playing a role we might observe? It is well known that heparin will regularly and rapidly erase the visible cloud of lipemia which follows a fatty meal. Is it possible, we reasoned, that lipemia itself will mobilize endogenous heparin and so initiate an automatic clearing reaction? Our studies adduced evidence to support an affirmative answer to this question (13). High fat meals were followed within two to three hours by significant degranulation of the basophil. Carbohydrate and protein test feedings were without this effect. It thus appeared likely that the presence of large amounts of fat in the blood stream did mobilize the heparin of the basophil. Significantly some individuals showed no response and there is need to explore these individual differences in reference to disease states such as xanthomatosis and atherosclerosis, and indeed aging itself.

Juhlin returned to Uppsala and William Caro joined the laboratory team last summer. Upon reviewing our past work for points of dissatisfaction, we realized that, although the basophil degranulation response correlated well with the histories given by the patients, we still had no cogent direct evidence. We were reluctant to test the "positives". It became evident that the value of the basophil test would have to be documented by critical experiments on animals. Using rabbits, because of their routinely high basophil counts, a program of sensitization to egg albumin was undertaken. The results of the *in vitro* basophil test were most gratifying. In every instance the sensitized rabbit could be detected using a battery of titred tests (14). There were no consistent false positives. Many new facts became evident. The best diagnostic results were obtained using a strong antigen titre, *viz.* 1/100. Incubation of the blood tests at room temperature for fifteen minutes seemed to be optimal. Indeed the reaction could not be elicited in an ice bath and it proceeded too rapidly at 37°C. Heparin did not interfere but oxalate as an anticoagulant poisoned the system indicating a presumed need for calcium. Finally the basophil degranulation was induced and shown *in vivo* as the result of allergen challenge of these animals. Significantly, Juhlin and Westphal (15) have recently demonstrated this in a man with milk hypersensitivity.

Returning to the patient we have found expanding vistas of research and study. Contact allergens such as poison ivy extract, paraphenylenediamine, have also shown positive reactions in sensitized patients. Apparently the basophil reaction detects circulating antibody in any allergy. In the penicillin area we have shown that the sensitizing portion of the molecule commonly resides in amino-penicillanic acid (16). Furthermore, strong sensitivity to penicillin G usually denotes sensitivity to the newer synthetic penicillin derivates, since all are variants of amino-penicillanic acid. Not only can we now predict the anaphylactic reactors, but also by means of this laboratory test it should be possible to effectively approach the problem of preparing a non-antigenic penicillin. The agility of the chemists in preparing molecular variants has not been previously matched by the allergists' ability to assay these freely.

Possibly our greatest area of interest has been

in atopic dermatitis. Here the basophil has given a new dimension in diagnosis. We have been able to detect regularly the significant allergens using the patient's blood rather than his skin (16). Using a scout tray of common allergens it is possible to assess the suspect atopic. Some early observations on those suffering from the related atopic problems of asthma and hay fever extends the usefulness of the basophil to these common afflictions. Thus, although the localization of disease may be organ directed, the homogeneous basophil population of the blood seems regularly sensitive.

The test permits extension of our concept of allergic disease. One area of particular note in our laboratory has been the detection of food allergens causing diarrhea. This has been extended to patients with ulcerative colitis. Here, Priest, Rebuck and Havey's work on tissue basophil infiltrates is intriguing (11). Our own data with the basophil test indicates that these patients have marked specific food allergen sensitivities. Other areas of promise include detection of anaphylactic sensitivity in patients receiving radiopaque dyes, blood transfusion reactions, migraine, and auto-immune disease.

With the realization of the expanding horizons for the basophil degranulation test, we have devoted our latest efforts to simplification of the procedure so that it might be done in any laboratory boasting a centrifuge and a microscope. This has been accomplished. The results obtained with the filter paper technic can now be duplicated in a micro-method involving centrifugation of a drop of antigen-treated blood in a plastic tube supported by a glass shield (17, 18). The buffy coat is removed, smeared, and stained on a glass slide for immediate rapid reading of twenty consecutive basophils. This second technic—the buffy coat smear—may be done with capillary blood and is rapid and simple.

A third useful technic has been the observation of the degranulation response in the living basophil, stained supra-vitally by neutral red. This pH indicator dye selectively stains the acidic granules of the basophil a brick pink. With this technic it has been possible to follow the course of specific allergic degranulation in a single basophil.

Our final adventure has concerned itself with the problem of the patient with few or no circulating basophils. In the highly allergic individuals contact with the allergens degranulates the cells. Thus while the central role of the basophil in immediate allergy is confirmed, the diagnostic test becomes very difficult in the absence of the test object. It is precisely these patients who need study. Fortunately, donor basophils may be used, either from the rabbit or man. We have designated this the *indirect* test in distinction to the *direct* tests described above where antigen is simply added to blood. In the indirect test the patient's serum, rabbit buffy coat, and antigen are mixed and degranulation observed either by the fixative-filter paper technic, by the buffy coat smear technic, or by supra-vital staining (19). Hence, we have several arrows in our quiver. Each has advantages but for large scale multiple allergen testing the indirect, supra-vital stain technic seems most useful. Serum may be mailed from anywhere to a central laboratory where it may be stored indefinitely. Using this micro-cyto-immune technic one may test as many as 200 separate allergens on 1 ml. of serum. For the determination of possible sensitivity to one or two drugs or a radiopaque dye the direct buffy coat smear technic would appear preferable. Here the capillary blood sample is an attractive feature.

In conclusion we have taken you on a three year trail followed by our group. I hope you have become interested in the basophil and will look for diagnosis if not adventure at your patient's fingertip.

REFERENCES

1. Shelley, W. B. and Florence, R.: Ethylene glycol monomethyl ether, a new fixative for histological work. Nature, **191:** 719–720, 1961.
2. Shelley, W. B. and Florence, R.: A technique for the study of mast cells: the guinea pig mesentery. J. Invest. Derm., **37:** 195–199, 1961.
3. Boseila, A.-W. A.: The Basophil Leucocyte and Its Relationship to the Tissue Mast Cell, 59 pp., Copenhagen, Munksgaard, 1959.
4. Shelley, W. B. and Juhlin, L.: Functional cytology of the human basophil in allergic and physiologic reactions: technique and atlas. Blood, **19:** 208–216, 1962.
5. Shelley, W. B. and Juhlin, L.: A new test for detecting anaphylactic sensitivity: the basophil reaction. Nature, **191:** 1056–1058, 1961.
6. Mongar, J. L. and Schild, H. O.: Cellular mechanisms in anaphylaxis. Physiol. Rev., **42:** 226–270, 1962.
7. Juhlin, L. and Shelley, W. B.: Role of mast cell and basophil in cold urticaria with associated systemic reactions. J.A.M.A., **177:** 371–377, 1961.

8. JUHLIN, L. AND SHELLEY, W. B.: The basophil response: a new area in cytopharmacology. Biochem. Pharmacol., **8:** 13, 1961. Abstract, First International Pharmacological Meeting, Stockholm, August 1961.
9. THONNARD-NEUMANN, E.: The influence of hormones on the basophilic leukocytes. Acta Haemat., **25:** 261, 1961.
10. BRAUNSTEINER, H., POUTUZHEK, O. AND THUMB, N.: Variations Observees Dans le Nombre des Leucocytes Basophiles Chez L'Homme. Rev. d'Hemat., **15:** 241–249, 1960.
11. PRIEST, R. J., REBUCK, J. W. AND HAVEY, G. T.: A new qualitative defect of leukocyte function in ulcerative colitis. Gastroenterology, **38:** 715, 1960.
12. Conference on Mast Cells and Basophils, May 17–19, 1962. Ann. N. Y. Acad. Sci., in press.
13. SHELLEY, W. B. AND JUHLIN, L.: Degranulation of the basophil in man induced by alimentary lipemia. Amer. J. Med. Sci., **242:** 211–222, 1961. Trans. Ass. Amer. Physicians, LXXIV: 118–133, 1961.
14. SHELLEY, W. B. AND CARO, W. A.: Detection of anaphylactic sensitivity by the basophil degranulation response. An experimental study. J.A.M.A., in press, 1962.
15. JUHLIN, L. AND WESTPHAL, O.: Degranulation of basophil leukocytes in a case of milk allergy, In press, 1962.
16. SHELLEY, W. B.: Unpublished observations. 1962.
17. SHELLEY, W. B.: Methods of observing the basophil leucocyte degranulation response. Ann. N. Y. Acad. Sci., in press, 1962.
18. SHELLEY, W. B.: Rapid technique for obtaining leucocytes from small blood samples. in press, 1962.
19. SHELLEY, W. B.: New serologic test for allergy in man. Nature, in press, 1962.