

From the Allergy Department of Rothschild Foundation, Paris, France.

Head: P. Gervais, M.D.

CELLULAR EVOLUTION IN THE SKIN- WINDOW TEST WITH A SPECIFIC ANTIGEN

By

THEANO DIKEAKOU & PIERRE GERVAIS

Histological studies have shown that a relationship exists between the type of hypersensitivity and the inflammatory cells (16, 42). The polynuclear cells are characteristic of the immediate type of hypersensitivity (3) while mononuclear cells of the delayed type (6, 17, 22, 31, 32, 39, 45).

Recent and more advanced studies have established more precisely the relationship of these cells and have determined their specific role during the various stages of the immune mechanism.

We know now, that during the primary reaction lymphocytes as well as eosinophils and macrophages participate in the rapid transport of the antigen (Ag) to the regional lymph nodes. During the process of immunisation the immunocompetent lymphocyte carries the antigenic code. Depending on whether thymus dependent or independent it may or may not by transformation obtain the plasmocytic form. The plasma cell produces the circulating antibodies (36). Thus, during this stage of immunisation, one can find in the peripheral lymphoid tissues the following types of immunocompetent cells. The thymus dependent lymphocyte responsible for the delayed type of hypersensitivity (DTH), the thymus independent lymphocyte, responsible for the immediate type reaction (ITR), the plasma cell responsible for the production of circulating antibodies and their intermediary blastic forms (36). On the other hand it is known that during the secondary

immune reaction the lymphocyte transfers cellular antibodies or antibody-like substances while the plasma-cells release circulating antibodies.

Recent studies have tried to determine more precisely the role of the monocyte or macrophage (4, 36). It seems on one hand to transfer rapidly to the lymphocyte the information code through RNA exchange and on the other hand to recognize the specific antigen through the IgG and complement receptors, which it carries on the cell membrane (2). The activity of incomplete antibodies in the human can be explained in this way (11). Transformed lymphocytes with the appearance of monocytes may be included in this population of cell (30, 6, 4, 19, 31, 41).

Thus it is evident that during an Ag + Ab reaction the inflammatory cells and the Abs characteristic of a certain type of hypersensitivity will appear.

The Skin window Test (S.W.T.) developed by Rebuck & Crowley for the study of inflammation has been used by other research workers for the study of inflammatory exudate during an Ag + Ab reaction (1, 14, 18, 20, 21, 34, 50).

In our experiment we have tried to introduce elements which could further elucidate the significance of the S.W.T. and possibly contribute to its use in the field of allergy.

MATERIAL AND TECHNIQUE

We have applied 116 *Skin Window Tests* on adults of both sexes, either day-patients or patients from allergy wards. They almost all showed a polyvalent reactivity to the intradermal tests. The clinical manifestations of these patients were mostly of the immediate type hypersensitivity i.e. asthma, rhinitis, urticaria, Quincke's oedema, spasmodic cough and atopic dermatitis.

The antigens used for the S.W.T. were almost pure proteins (pollens) complex-proteins (dust, candida, moulds) and haptens (drugs). The antigen used for the S.W.T. had previously induced a positive Intradermal (I.D.) reaction on the patient. For the S.W.T. control we used physiologic saline. Thus, the S.W.T.s were applied on patients characterised by the history and I.D. reactions as sensitive.

The technique used was simple. A superficial abrasion was made on the inner surface of the arm with the dermabrasion apparatus. In this way, we

obtained a smooth denuded surface, of the same depth along the whole surface and without bleeding. It was covered with a sterile slide on which a drop of Ag was previously applied. The slides were changed every 2, 4, 6, 24, 48 hours, were subsequently stained by the May-Grünwald technique and the cells counted under ordinary light microscope.

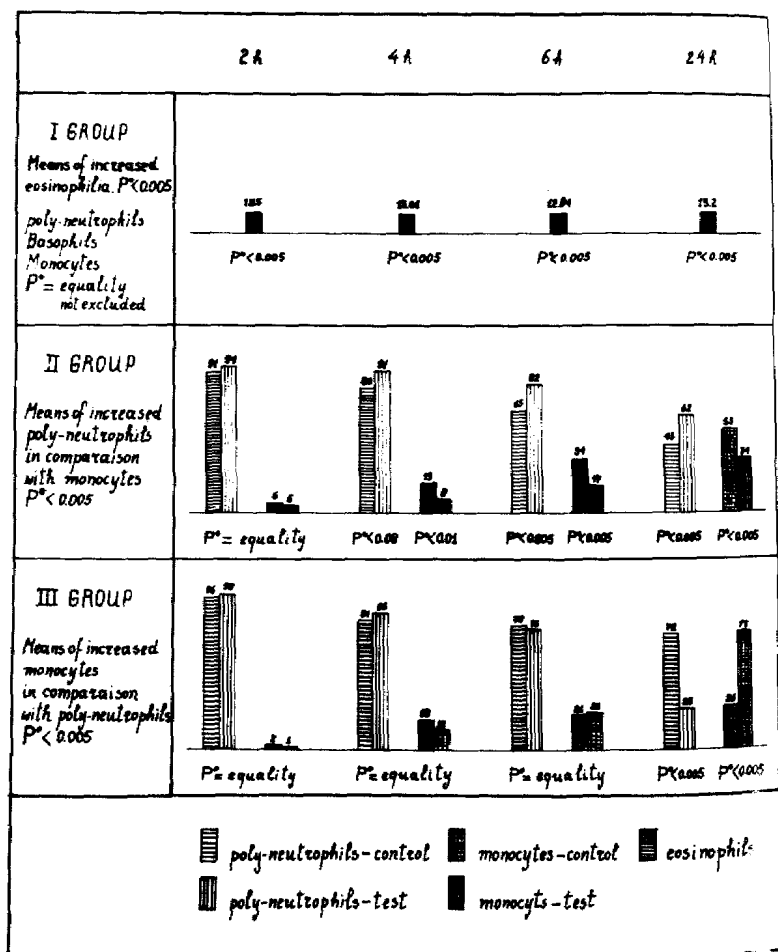
RESULTS

The results were obtained by a comparative study of the cells in the S.W.T. with Ag and in the S.W.T. control with isotonic saline. The cells were of the same nature and showed the same evolution in relation to time in the controls and the S.W.T.s with Ag. They differed only with regard to the percentage of the cells and with regard to certain cellular alterations, i.e. concerning the intensity of the inflammation. The cells which appeared were polynuclear neutrophils, eosinophils, basophils and mononuclear cells. It was not possible to study the lymphocytes since reading at the 9th and 12th hour were not available. The cellular evolution was such as could be expected, the polynuclears appearing in a large percentage during the first hours and diminishing during the last hours. However, exception must be made for the basophils which appeared from the first hours with increasing percentage during the last hours. Although there were some oscillations in the curves, they are not statistically significant since a subjective factor is inevitable during cell counting. What is evident and statistically significant, however, is the difference in the percentage of the different cells in the S.W.T. with Ag and the S.W.T. control.

According to these differences, we have distinguished three groups of S.W.T. with Ag. (Figure).

- 1st group - presenting an increase of eosinophils
- 2nd group - presenting an increase of poly-neutrophils
- 3rd group - presenting an increase of the monocytes.

In the first group, the increase of eosinophils is at least 3 per cent superior to that one of the control. The mean, of 13



per cent, presents a trend almost linear during the 24 hours and shows an important decrease (40 per cent) after (48th hour). In this group the difference between the polynuclear and mononuclear in the S.W.T.s with Ag and S.W.T. con-

trols is not statistically significant. The probability of error in the increase of eosinophils is $P^{\circ} < 0.005$).

In the 2nd group the poly-neutrophils increased with a $P^{\circ} < 0.005$ after 6 and 24 hours, while the mononuclear cells diminished the same hours with a $P^{\circ} < 0.005$. The equality of poly-eosinophils and basophils in the S.W.T. with Ag and S.W.T. control cannot be excluded.

In the 3rd group the cellular evolution shows appreciable particularities after 4 and 24 hours. After 4 hours the S.W.T. with Ag shows an eosinophilia superior to that of the control with a $P^{\circ} < 0.01$, while the monocytes are diminished with a $P^{\circ} < 0.005$. The equality of poly-neutrophils cannot be excluded.

After 24 hours, the S.W.T. with Ag shows in comparison to S.W.T. control an appreciable decrease in poly-neutrophils with a $P^{\circ} < 0.005$ and an increase of monocytes with a $P^{\circ} < 0.005$. The eosinophils and basophils can be present but their equality in S.W.T. with Ag and control cannot be excluded.

CONCLUSIONS

After the statistical study of our results we can say, that a S.W.T. (applied on a sensitive patient with a positive I.D. reaction with the same Ag) can present in comparison with the S.W.T. control with isotonic saline 1) an increase in eosinophilia which can appear from the 2nd hour 2) an increase of poly-neutrophils appearing after 6 and 24 hours and 3) an increase in monocytes appearing after 24 hours.

We define monocytes a population of cells of the same size or larger than polynuclear granulocytes with a kidney shaped round or oval nucleus and a basophilic cytoplasm. In certain slides we observed that these cells were very large, lightly coloured, and situated towards the periphery of the exudate. The eosinophils as well as the poly-neutrophils and the mononuclear cells showed a certain vacuolisation.

DISCUSSION

As a result of our study, we wish to suggest that an Ag which can produce a positive I.D. reaction on a sensitive patient can induce in a S.W.T. on the same patient a cellular exudate corresponding to a different type of hypersensitivity and consequently to a different type of Abs.

It is known from the histological studies on the immune reaction that an immediate type hypersensitivity induces in a few minutes a vasodilatation, diapedesis, oedema and eosinophilia. The Arthus's reaction which starts appearing about the 4th or 6th hour and is characterised by an acute inflammatory reaction with oedema and polynuclear infiltration, may be complicated by vascular lesions (13) haemorrhage and necrosis (9, 27, 30, 31, 42). On the contrary the delayed reaction is an intense inflammatory reaction with late appearance (6, 11, 16, 17, 31, 32, 33, 42, 45). Its peak appears the 24th and 48th hour and it is characterised by lymphocytic and monocytic infiltration.

Concerning Abs, the immediate reaction is mediated by complete, circulating Abs, while the delayed reaction is characterised by incomplete cellular Abs. Recently, *Zolov & Levine* (51) showed that blood eosinophilia is associated with reagins i.e. skin sensitizing IgE Abs, while IgG and IgM did not induce eosinophilia, although these immunoglobulins were present in the serum of patients who presented positive I.D. reactions and clinical symptoms of an immediate type hypersensitivity.

Thus one may suggest that as the different types of cells appeared in predominance in a S.W.T. with an Ag which induced a positive I.D. reaction on the patient, these cells are the expression of different types of Abs. In the 1st group the appearance of eosinophils in excess could signify the presence of skin-sensitizing Abs. In the 2nd group, the appearance of polynuclears in excess after 6 and 24 hours may express the presence of precipitating Abs, while the decrease of monocytes may be the result of the release of migration inhibitory

factor (M.I.F.) by the sensitized lymphocytes (5, 36), being the expression of a delayed type hypersensitivity. (One could even think that the immune process is not yet complete. Antibodies are produced against the hapten, against the complete Ag but not yet against the protein carrying the hapten).

In the 3rd group may be the participation of skin sensitizing Abs is expressed by the increased eosinophilia after 4 hours and that of incomplete cellular or cytophilic Abs by the increase of monocytes after 24 hours (11).

Although *Spector* (42) *Crowle & Patrucco* (11) accept that the delayed type hypersensitivity presents a biphasic evolution with a small peak at the 4th hour of polynuclears, this is considered as a non-specific reaction by the first as an expression of circulating Ab by the others. *Spector* considers 50 per cent of these mononuclear cells monocytes, the rest being, large lymphocytes. However there is no evidence that these large lymphocytes are not intermediary forms or resting forms of monocytes (4, 6, 19, 30, 31, 36, 41).

Consequently if the $P^o < 0.01$ of the statistical study does not permit one to accept that the eosinophilia after 4 hours is specific, the 3rd group must be assumed to represent incomplete cytophilic Abs (7, 8, 10, 26, 35, 37, 44, 46).

In conclusion a S.W.T. with Ag showing an increased eosinophilia represents the presence of skin sensitizing Abs (reagins, IgE), an increase in polynuclears precipitating Abs (IgG or IgM) and an increase of monocytes incomplete cytophilic Abs 7S γ_2 . On the other hand, the decrease of monocytes may represent the presence of a migration inhibitory factor released by the sensitized lymphocytes. This conclusion is supported by the correlation with the time factor.

The increased eosinophilia appeared after the 2nd hour. The poly-neutrophils increased at the 6th hour and the monocytes at the 24th hour i.e. the time involved correspond to an immediate type reaction, to an Arthus type reaction and to a delayed reaction respectively.

Consequently from 116 positive I.D. reactions 45 may be

precipitated from skin sensitizing Abs i.e. they represent an immediate reaginic type of hypersensitivity.

41 may be precipitated by a precipitating circulating Ab *i.e.* they are an immediate Arthus' type reaction possibly associated with a delayed type hypersensitivity.

28 may be precipitated by cytophilic Abs which have attracted monocytes carrying on their cell membrane specific receptors.

However, one cannot ignore the fact that even in this last group, the I.D. reaction was precipitated by the factor which produced the eosinophilia in the 4th hour. We advance the hypothesis that decreased monocytoxis and increased monocytoxis represents the existence of a delayed hypersensitivity given that both conditions are two different facets of the delayed type of hypersensitivity (36).

However, we cannot give a precise explanation of this hypothesis. A difference in the degree of the sensitivity, the quantity of the employed Ag, the nature of the available Ag are factors possibly influencing this disparity. The M.I.F. is released from sensitive lymphocytes only in the presence of the complete Ag (36) (protein + hapten) responsible for the sensitization. It is not released by the carrier protein. Could it be the cause of our results?

Studies of chemotaxis on the inflammatory cells have shown that the inflammation of an immune reaction differs from the ordinary inflammation only in the intensity. The presence of an Ag + Ab complex induces a rich influx of polynuclear cells in the anaphylactics reaction (30) and the presence of a specific Ag the exaggerated influx of the mononuclear cells in delayed type hypersensitivity (42).

The fact that the S.W.T.s were applied on sensitive persons who previously precipitated a positive I.D. reaction with the same Ag used for the S.W.T., allows to conclude that the intensity of the inflammation presented in the S.W.T. with Ag, compared with the control S.W.T., is due rather to an Ag + Ab complex than to a non-specific substance. This suggestion is also justified by the coincidence of the factor time

and the appearance of the inflammatory cells characteristic of a certain type of hypersensitivity.

From the practical point of view, we suggest:

a) that the eosinophilia by being indicative of reaginic activity can be used in the S.W.T. to detect the atopic background, difficultly detectable by the history.

b) that the S.W.T. may indicate the type of hypersensitivity i.e. the nature of the antibodies, and could therefore be applied before undertaking a specific desensitization.

c) that as the blocking Abs can inhibit the Ag + Ab reaction—the S.W.T. could be applied to follow the course of the specific desensitization.

S U M M A R Y

From the histo-pathological point of view the immediate hypersensitivity is characterized by polynuclear cells, the delayed type of hypersensitivity by mononuclear cells.

The immediate type reaction is mediated by reaginic and/or precipitating antibodies, the delayed type by cellular antibodies. The monocyte of haematogenic origin in the S.W.T. carrying specific receptors $7S \gamma_2$ may be the expression of cytophilic antibodies and consequently of delayed type hypersensitivity. Among the population of cells, we call monocytes, a small percentage could be intermediary forms of lymphocytes, i.e. medium and large lymphocytes, but the monocytes are by far more numerous.

116 S.W.T.s (applied on sensitive patients, who had clinical manifestations of immediate type hypersensitivity and a positive I.D. reaction to the same Ag used for the S.W.T.) can be divided in three groups according to the cellular exudate:

I group — with an eosinophilia superior by 3 per cent to that of the control S.W.T. ($P^\circ < 0.005$) during all the readings.

II group — with an increase of poly-neutrophils in comparison with the S.W.T. control ($P^\circ < 0.005$) after 6 and 24 hours and decrease of monocytes at the same hours ($P^\circ < 0.005$).

III group – with an increase of monocytes (compared to S.W.T. control) after 24 hours ($P^o < 0.005$) and eosinophilia after 4 hours ($P^o < 0.01$).

Zolov & Levine have shown that reagins IgE induce blood eosinophilia in contradistinction to IgG and IgM circulating antibodies. Thus we consider that the cellular exudate in a S.W.T. with an antigen in relation to S.W.T. control could represent the type of antibodies precipitated by the antigenic determinants of the antigen in question. In addition, though the clinical manifestations of the patients are of the immediate type, the same patient have both types of hypersensitivity, one being more predominant than the other.

From the practical point of view, after these results the Skin window Test could be used

- a) to detect the atopic background
- b) before undertaking a specific desensitization and
- c) to follow the evolution of the specific desensitization.

REFERENCES

1. *Archer, R.K.*: On the functions of Eosinophils in the Antigen-Antibody reaction. *Brit. J. Haematol.* 11, 123, 1965.
2. *Berken, A. & Benacerraf, B.*: Properties of Antibodies cytophilic for macrophages. *J. Exp. Med.* 123, 119, 1966.
3. *Benacerraf, B. & Levine, B.B.*: Immunological specificity of delayed and immediate hypersensitivity reactions. *J. Exp. Med.* 115, 1023, 1962.
4. *Bloom, W.*: The origin and nature of the monocyte. *Folia Haemat.* 37, 1, 1928.
5. *Bennett, B. & Bloom, B.R.*: Biological activity of a partially purified migration inhibitory factor (M.I.F.) associated with delayed type hypersensitivity. *Fed. Proc. (abst.)* 27, 263, 1968.
6. *Boughton, B. & Spector, W.C.*: Histology of the tuberculin reaction in guinea pigs. *J. Path. Bact.* 85, 371, 1963.
7. *Boydén, S.F. & Sorkin, E.*: The absorption of Ag by spleen cells previously treated with antiserum in vitro. *Immunology* 3, 272, 1960.
8. *Boydén, S.F. & Sorkin, E.*: The absorption of Antibody and Antigen by spleen cells in vitro. Some further experiments. *Immunology* 4, 244, 1961.
9. *Boydén, S.F.*: The chemotactic effect of mixtures of Antibody and Antigen on polymorphonuclear leucocytes. *J. Exp. Med.* 115, 453, 1962.

10. *Boyden, S. F.*: Cytophilic Antibody in guinea pigs with delayed hypersensitivity. *Immunology* 7, 474, 1964.
11. *Clozele, A. J. & Patrucco, A.*: Preferential development by mice of delayed hypersensitivity to purified basic proteins. *J. Allergy* 3, 141, 1968.
12. *Cohen, S., Fassali, P., McCluskey, R. C. & Benacerraf, B.*: Distribution of Ag in guinea pig lymph nodes during the primary and secondary immune response. *Fed. Proc.* 24, 696, 1965.
13. *Cochrane, C. G., Weigle, W. O. & Dixon, F. J.*: The role of polymorphonuclear leucocytes in the initiation and cessation of the Arthus vasculitis. *J. Exper. Med.* 110, 481, 1959.
14. *Cole, M. B. & Roberts, F. E.*: Inflammatory response to histamine in atopic and nonatopic individuals. *J. Allergy* 38, 26, 1966.
15. *Cooper, M. D., Peterson, R. D. A., South, M. A. & Good, R. A.*: The functions of thymus system and the bursa system in the chicken. *J. Exp. Med.* 123, 75, 1966.
16. *Dienes, L. & Mallory, T. B.*: Histological studies of hypersensitivity reaction. Part I. The contrast between the histological responses in the tuberculin (allergic) type and the anaphylactic type of skin reactions. *Amer. J. Path.* 8, 699, 1932.
17. *Dumonde, D. C.*: The role of the macrophage in delayed hypersensitivity. *British Medical Bull.* 23, 9, 1967.
18. *Eidinger, D., Wilkinson, R. & Rose, B.*: A study of cellular response in immune reactions utilizing the skin window technique. *J. Allergy* 35, 77, 1964.
19. *Elwes, M. W., Cough, J. & Israels, M. C. G.*: The relationship between the lymphocyte and polymorph during macrophage formation in vitro. *Exp. Cell. Res.* 44, 624, 1966.
20. *Feinberg, A. K. & Feinberg, S. M.*: Leucocytes and Hypersensitivity reactions. Eosinophil response in skin window, in skin window to ragweed extract, histamine and compound 48/80 in atopic and non atopic individuals. *J. Allergy* 40, 73, 1967.
21. *Fowler, J. W. & Lowell, F. C.*: The accumulation of eosinophils as an allergic response to allergen applied to the denuded skin surface. *J. Allergy* 37, 19, 1966.
22. *Gell, P. G. H. & Hinde, I. T.*: The histology of tuberculin reaction and its modification by cortisone. *British J. Exp. Path.* 32, 516, 1951.
23. *Gillman, T. & Wright, L. J.*: Probable in vivo origin of multinucleated giant cells from circulating mononuclears. *Nature, (London)* 209, 263, 1966.
24. *Gowans, J. L. & McGregor, D. D.*: The immunological activities of lymphocytes. *Prog. Allergy* 9, 1, 1965.
25. *Good, R. A., Dalmaso, A. P., Martinez, C., Archer, O. K., Pierce, J. C. & Papermaster, B. W.*: The role of the thymus in development of immunologic capacity in rabbits and mice. *J. Exp. Med.* 116, 773, 1962.

26. *Howard, J. G. & Benacerraf, B.*: Properties of macrophage receptors for cytophilic Antibodies. *British J. Exp. Path.* 47, 193, 1966.
27. *Humphrey, J. H.*: The mechanism of Arthus reaction. I the role of polymorphonuclear leucocytes and other factors in reversed passive Arthus reaction in rabbits. *British J. Exp. Path.* 36, 268, 1955.
28. *Huber, H., Polcy, M. Y., Linscott, W. D., Fudenberg, H. H. & Müller Eberhard, H. F.*: Human monocytes: distinct receptor sites for the third component of complement and for immunoglobulin G. *Science* 162, 1281, 1968.
29. *Keller, H. U. & Sorkin, E.*: Studies on chemotaxis, XIII differences in the chemotactic responses of neutrophil and eosinophil polymorphonuclear leucocytes. *Int. Arch. of Allergy* 35, 279, 1969.
30. *Keller, H. U. & Sorkin, E.*: Studies on chemotaxis VI. Specific chemotaxis in rabbit polymorphonuclear leucocytes and mononuclear cells. *Int. Arch. Allergy* 31, 575, 1967.
31. *Kolough, F.*: The lymphocyte in acute inflammation. *Am. J. Path.* 15, 413, 1939.
32. *Kosunen, T. U., Waksman, B. H., Flax, M. H. & Tihen, W. S.*: Delayed reactions to tuberculin and purified proteins in the rat and guinea pigs. *Immunology* 6, 276, 1963.
33. *Lenzini, L. & Barnabe, R.*: The nature of changes in the cellular infiltration during two weeks after tuberculin testing. *Int. Arch. Allergy* 35, 402, 1969.
34. *Litt, M.*: Eosinophils and Antigen + Antibody reactions. *Ann. N.Y. Acad. Sci.* 116, 969, 1964.
35. *Lobuglio, A. F., Cotran, R. S. & Jandl, J. H.*: Red cell coated with immunoglobulin G: Binding and shearing by mononuclear cells in man. *Science* 158, 1582, 1967.
36. *Meuwissen, H. J., Stutman, O. & Good, R. A.*: Functions of the lymphocytes. *Sem. haemat.* 6, 28, 1969.
37. *Nelson, D. S. & Boyden, S. V.*: Macrophage cytophilic antibodies and delayed hypersensitivity. *British Med. Bull.* 23, 15, 1967.
38. *Ohtaki, N., Grob, P. Y. & Inderbitzin, T. M.*: Delayed hypersensitivity (D.N.C.B. contact dermatitis) in panleucopenic guinea pigs. *Int. Arch. Allergy* 35, 134, 1969.
39. *Pazz, R. A. & Spector, W. G.*: The mononuclear cell response to injury. *J. Path. Bact.* 84, 85, 1962.
40. *Roberts, A. M.*: Rapid uptake of tritiated Ag by mouse eosinophils. *Nature*, 210, 266, 1966.
41. *Spector, W. G. & Lykke, A. W. J.*: The cellular evolution of inflammatory granulomata. *J. Path. Bact.* 92, 163, 1966.
42. *Spector, W. G.*: Histology of Allergic Inflammation. *British Med. Bull.* 23, 35, 1967.

43. *Speirs, R. S. & Speirs, C. E.*: Cellular localisation of radio-active antigen in immunized and non-immunized mice. *J. Immunol.* 90, 561, 1963.
44. *Tizard, I. R.*: Macrophage cytophilic antibody in mice. *Int. Arch. Allergy* 36, 332, 1969.
45. *Turk, J. L., Rudner, E. J. & Heather, G. J.*: A histochemical analysis of mononuclear cell infiltrates of the skin. II Delayed hypersensitivity in the human. *Int. Arch. Allergy* 30, 248, 1966.
46. *Turk, J. L.*: Cytology of the induction of hypersensitivity. *British Med. Bull.* 23, 3, 1967.
47. *Volkman, A. & Gowans, J. L.*: The origin of macrophages from bone marrow in the rat. *British J. Exp. Path.* 46, 62, 1965.
48. *Volkman, A. & Gowans, J. L.*: The production of macrophages in the rat. *British J. Exp. Path.* 46, 50, 1965.
49. *Willoughby, D. A.*: Mediators of Delayed hypersensitivity reactions. *Int. Arch. Allergy* 36, 22, 1969.
50. *Wolf-Jürgensen*: Basophilic leucocytes in delayed hypersensitivity. *Danish Med. Bull.* 9, 249, 1967.
51. *Zolov, D. M. & Levine, A. B.*: Correlation of blood eosinophilia with antibody classes. *Int. Arch. Allergy* 35, 179, 1969.

Authors' address: Service d'Allergie de la Fondation A. de Rothschild 29 rue Manin, Paris 19°, France.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.