Composition, Morphology, and Source of Cells in Delayed Skin Reactions

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THE CELLULAR EVENTS that occur in the pathogenesis of delayed hypersensitivity (DH) reactions have been reasonably established. At the site of antigen injection a relatively small number of sensitized lymphocytes [1-4] presumably interact with specific protein and generate one or more inflammatory reagents.[5,6] In vitro, at least, several reagents have been isolated—eg, macrophage inhibitory factor (MIF),[7-10] cytotoxin(s),[11-15] and permeability factor,[5,13,14,16,17] following the interaction of sensitized cells and specific antigen. Although conclusive proof that these reagents operate in vivo is still lacking, it is reasonable to assume that they, or similar substances, may also play a role in delayed skin reactions and induce the accumulation of numerous mononuclear cells over a period of hours.[10,18] The latter elements constitute a nonspecific component [1,3,19,20,21] derived chiefly, if not entirely, from bone marrow,[20-24] and at 24 or 48 hr they represent a majority of the cells present in the lesion.

The purposes of this paper were to examine the composition of the nonspecific elements of delayed skin reactions induced by specific antigen and nonspecific agents; to determine quantitatively the frequency of cell types among the aggregates; to study their fine structure; and finally, to analyze the kinetics and source of the cells. Light [25-29] and electron [30-33] microscopic studies of delayed hypersensitivity lesions have been often reported but have not clarified the facets of delayed reactions listed above.

Materials and Methods

Lewis rats were used as donors and recipients in all experiments (Microbiological Assoc., Inc., Bethesda, Md, and Simonsen Laboratories, Gilroy, Calif). Recipient animals received 650 r total body irradiation and 3 days later were infused intravenously with 2–4 $\times$ 10^8 lymph node cells of immunized donors. One day later they were again infused intravenously with 5–10 $\times$ 10^8 labeled cells of bone marrow (BM), lymph nodes (LN), spleen (S) or thymus (T) taken from

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virgin donors. Skin tests were made immediately with 10 μg of antigen in a volume of 0.05 ml and the rats were killed at 12, 18 or 24 hr later. At necropsy, skin test sites were removed for histologic and autoradiographic studies; blood samples were taken for leucocyte counts and autoradiographic analysis. Controls consisted of irradiated recipients of labeled cells from virgin donors, without infusions of sensitized cells; of irradiated rats infused with sensitized cells only; or of nonirradiated rats given sensitized cells but no labeled elements.

Immune cells were prepared in suspension from draining lymph nodes of donors inoculated intradermally with 1 ml of Freund's complete adjuvant (CFA) containing 1 mg of dried *M tuberculosis*, or with 1 ml of Freund's incomplete adjuvant containing 25 μg of bovine γ-globulin (BGG) (Reheis Chemical Company, Div. of Armour Pharmaceutical Co., Chicago, Ill). Only donors yielding positive skin tests to 1 μg of purified protein derivate (PPD) (Parke, Davis & Co., Detroit, Mich) or BGG, 10 days later, were used.

Labeled cells were prepared in suspension from virgin rats that had received, intraperitoneally, 0.5 μc of H_3-thymidine/g body weight three times daily for five days and were killed 6 hr after the final dose of isotope (Specific activity of methyl-thymidine 6.0c/mM, concentration 1 mc/ml Schwarz Bio-Research Laboratories, Orangeburg, NY). Smears of these suspensions were made for light microscopic autoradiography to determine percent of labeling, and centrifuged pellets were processed for electron microscopic autoradiography to determine the differential.

Skin test sites were dissected out and fixed flat in 2% buffered glutaraldehyde. One hr later, the skins were sliced into sections 1-mm thick. Under a dissecting microscope representative samples of dermal inflammation were selected, chopped into minute fragments, fixed for an additional 24 hr in glutaraldehyde and processed, after osmic acid fixation, for electron microscopic autoradiography. Other samples were additionally fixed in buffered formalin and processed for histologic and autoradiographic studies. Blood leucocytes of labeled donors were gently spun to form pellets, following hemolysis of red cells with 0.83 M NH_4Cl.[34] The pellets were used to make smears for light microscopic autoradiography and sections for electron microscopic autoradiography.

Grading of skin reactions was based upon the extent and intensity of inflammation found histologically in 4 slices of the test sites; the scale ranged from ±, a minimal reaction, to 3, the most severe.

Two sets of experiments were performed. In the first, sensitized lymph node cells and normal labeled cells were infused into irradiated recipients. The test antigens used were PPD and BGG and the purpose of the experiments was to record the morphology and relative proportion of labeled cells present in skin lesions elicited by specific and nonspecific antigens. In the second, irradiated and infused rats were tested with specific antigen (PPD) and the cellular composition of the lesion was compared with other test sites on the same animal injected intradermally with 0.1 ml of mineral oil, 0.1 ml of phytohemagglutinin M (PHA), and 0.1 ml of antilymphocyte serum (ALS). (PHA, powder of one vial dissolved in 5 ml of pyrogen-free distilled water. Difco Laboratories, Detroit, Mich. ALS, prepared in rabbits by injection, intravenously, of 5×10^8 viable rat thymus cells, 3 times, 1 week apart).

Results

Strongly positive lesions were elicited by specific antigen in irradiated recipients of sensitized lymph node cells and labeled BM cells or in nonirradiated recipients infused only with sensitized cells (Table 1).

Table 1. Skin Tests in Irradiated Recipients of Sensitized Lymphoid Cells and Normal Labeled Cells from Different Sources

No. of rats	Immunizing substance	Source of labeled cells	PPD skin test			BGG skin test		
			Histologic grade	Labeled/ total cells	Labeled cells (%)	Histologic grade	Labeled/ total cells	Labeled cells (%)
6	CFA	BM	3	855/3195	26.8	±*	7/474	1.5
4	CFA	S	1*	48/2865	1.7	0	0	0.0
4	CFA	LN	±	47/2134	2.2	0	0	0.0
4	CFA	T	0	0	0.0	0	0	0.0
2	CFA	†	0	0	0.0	0	0	0.0
2	—	BM‡	0	0	0.0	0	0	0.0
2	CFA	§	3	—	—	±	—	—
4	BGG	BM	1	67/1058	6.3	1	150/1072	14.0
2	BGG	S	0	0	0.0	±*	0	0.0
2	BGG	LN	±*	6/1033	0.6	±*	0	0.0
2	BGG	T	0	0	0.0	0	0	0.0

CFA represents Freund's complete adjuvant containing 1 mg tubercle bacilli per ml; BGG, 25 μg in 1 ml of Freund's incomplete adjuvant; BM, bone marrow; S, spleen; LN, lymph node; T, thymus.

* Numerous PMN's in lesions.

† Negative control; 600 r, infusion of sensitized cells only.

‡ Negative control; 600 r, infusion of labeled cells only.

§ Positive control; no radiation, infusion of sensitized cells only.

In the former, about 27% of the cells infiltrating the test site were labeled, while nonspecific antigen (BGG) produced a weak delayed reaction in which only 1.5% of the infiltrating cells were labeled. The results in Table 1 also show that in recipients of sensitized cells and labeled LN or S cells specific antigen elicited weak reactions with 1.7–2.2% of the infiltrating cells being labeled. When tagged thymus elements were used in place of bone marrow, no positive reactions could be obtained.

Table 1 also discloses that the positive skin tests were specific—ie, PPD produced good responses in rats infused with LN cells from donors inoculated with CFA; BGG in these same recipients elicited questionable or no reactions. Similarly, BGG produced a moderate response in irradiated rats restored with LN cells from donors immunized with BGG and labeled BM cells from virgin donors; 14% of the infiltrating elements were labeled. PPD in these same rats produced a moderate lesion of about the same severity, but only 6.3% of the infiltrating elements were tagged. In general, BGG contained in Freund's incomplete adjuvant was less potent for the production of DH than was CFA.

Results obtained from control rats disclosed the following: (1) irradiation of rats receiving only sensitized cells prevented the development of a positive skin test with specific antigen, (2) irradiated rats receiving only labeled BM cells were unable to provide positive reactions to specific antigen and no labeled cells were found in the test sites, and (3) nonirradiated recipients of sensitized cells only yielded strong reactions to PPD.

Table 2 presents a comparison of the number of labeled cells (light

Table 2. Number and Percent of Labeled Cells in Inoculums From Normal Donors

	Bone Marrow			Spleen			Lymph Node		
	Total Cells (No.)	Labeled cells		Total Cells (No.)	Labeled cells		Total Cells (No.)	Labeled cells	
		No.	%		No.	%		No.	%
Light Microscopic Autoradiography									
	2850	2586	90.7	3267	618	18.9	2594	359	13.8
Electron Microscopic Autoradiography									
Cell type									
Mono/Macro		10	6.5		10	14.6		10	8.4
Lymphocyte		14	9.3		27	39.7		65	55.5
Blast		21	13.9		15	22.0		18	15.3
Plasma		4	2.6		11	16.1		24	20.5
Myeloid		52	34.4		5	7.6		0	0
Erythroid		47	31.1		0	0		0	0
Other		3	2.0		0	0		0	0
Total		151			68			117	

microscopic autoradiography) and the differential of labeled cell types (electron microscopic autoradiography) obtained from BM, S and LN of virgin donors. Ninety per cent of the nucleated cells of BM were labeled at the time of infusion into recipients, and 19% and 14% of S and LN cell suspensions were tagged with tritium—ie, the percentage of labeled BM cells was 4.5–6.5 times greater than the percentage of labeled cells from lymphoid organs. However, in the lesions (Table 1) the percentage of labeled cells of the infiltrating elements was 16 and 12 times greater when bone marrow was the source than when S and LN provided the virgin labeled elements. It was therefore apparent that despite the greater number of labeled cells in bone marrow suspensions, bone marrow was significantly more efficacious in providing cells for the developing skin lesions.

The differential, determined by ultrastructural classification, showed

Table 3. Number and Percent of Labeled Cells in Blood of Irradiated Recipients After Infusion of Bone Marrow—Light Microscopic Autoradiography

Post infusion time (hr)	Counted cells (No.)	Labeled cells					
		Total		PMN		MN	
		No.	%	No.	%	No.	%
6	817	9	1.1	2	0.3	7	0.8
18	576	66	11.4	51	8.8	15	2.6
24	506	63	12.5	52	10.3	11	2.2

that 6.5%, 14.6%, and 8.4% of all the labeled elements belonged to the monocyte/macrophage category when derived from BM, S, and LN, respectively. By calculation the number of this cell class with label in bone marrow suspensions was 2 and 5 times greater than that of spleen and lymph node suspensions.

In Table 3, the levels of labeled cells in the circulation at necropsy are shown. It is apparent that the proportion of tagged circulating mononuclear cells was low, ranging from 0.8–2.6%.

Table 4 presents the comparative data of labeled cells present in lesions elicited by specific antigen and by other nonspecific reagents (Fig 1). The percentages of labeled cells in lesions elicited by all the reagents were similar and ranged around 25%. However, the differential of labeled cells, revealed by electron microscopic autoradiography, was quite different in the four test sites. With specific antigen, in positive delayed reactions, 80% of the labeled cells were of

Table 4. Number and Percent of Labeled Cells in Skin Test Sites of Irradiated Recipients Infused with Tuberculin Sensitive Lymphocytes and Labeled Bone Marrow Cells

	PPD			ALS			PHA			Oil		
	Total cells (No.)	Labeled cells		Total cells (No.)	Labeled cells		Total cells (No.)	Labeled cells		Total cells (No.)	Labeled cells	
		No.	%		No.	%		No.	%		No.	%
Light Microscopic Autoradiography												
	2189	614	28.0	1027	232	22.6	659	162	24.6	500	140	28.0
Electron Microscopic Autoradiography												
Cell type												
Mono/Macro		142	80.7		25	71.4		23	24.0		13	36.1
Blast		8	4.5		0	0		0	0		0	0
PMN		24	13.5		10	28.6		73	76.0		23	63.8
Other		2	1.1		0	0		0	0		0	0
Total		176			35			96			36	

the monocyte/macrophage category (Fig 2–5), while in lesions elicited by mineral oil and PHA the majority of labeled elements was in the polymorphonuclear (PMN) class. On the other hand, ALS produced histologic lesions that could not be distinguished from those induced by PPD (Fig 1), and the percentage of labeled monocytes/macrophages in the lesions was 71%, approaching the level obtained with PPD.

From the data of both Tables 3 and 4, it was apparent that in test sites affected by PPD and ALS there was a marked selection of monocyte/macrophage cells, and this category of cell types was concentrated 10–6 times over their levels in the circulation at 24 hrs. It should be noted, however, that mineral oil and PHA also produced lesions in which there was selective accumulation of monocytes/macrophages, being 4 and 2 times greater in test sites than in the blood. Similarly, these latter two agents produced lesions in which PMN's were concentrated almost 2 times over their blood levels. The data suggested that bone marrow was the source of a large number of inflammatory cells appearing in skin test sites elicited by a variety of noxious agents, that it provided both monocytes/macrophages and PMN's, and that in delayed lesions induced by specific antigen or by ALS, the predominant cell was of the monocyte/macrophage class.

An additional observation should be noted. The inoculum of bone marrow cells was 90% labeled, while in the skin lesions elicited by the four reagents the proportion of tagged elements was approximately 25%. Presumably, then, there was a loss of labeled cells in the test sites —about 3.5 times less than the proportion tagged in the original inoculum.

Discussion

The experiments reported here were devised to examine in the electron microscope the composition of the cellular infiltrate in specific lesions of delayed hypersensitivity and to compare this type of lesion with those induced by nonspecific reagents. The development of the delayed immunologic reaction [1–6] and the source of the infiltrating cells have already been clarified.[20–24] A small number of sensitized lymphocytes interact with specific antigen at the test site and this is accompanied or followed by an accumulation of host mononuclear cells, derived from bone marrow and reaching the test site via the circulation. The control manipulations shown in Table 1 confirm this concept.

In the specific 24-hr lesion of delayed hypersensitivity, the predominant cell type belonged to the category of monocytes/macrophages, constituting 80% of the labeled elements. There was no difficulty in

recognizing the fully-developed monocyte and the macrophage with ingested material. There were, however, a number of labeled cells that appeared to be more primitive or precursor forms (Fig 2 and 3) and these were clarified also as belonging to the monocyte/macrophage class, for the following cytologic reasons. First, from an examination of numerous electron micrographs, a series of gradations was found between these primordial elements and acceptable monocytes/macrophages. Second, their nuclei were large, had a thin rim of chromatin at the periphery and a relatively large amount of interchromatinic material—ie, they resembled the nuclei seen in monocytes. Lastly, their cytoplasm contained a variable number of vacuoles and surface invaginations of the type associated with phagocytic activity. These primitive elements resembled lymphocytes only in having a scanty cytoplasm and could not be confused with blast cells. We have attributed to them the quality of immaturity since they were capable of incorporating H_3-thymidine into their nuclei and presumably were capable of division. These are, most likely, the bone marrow elements that appear in a variety of inflammatory lesions [22,35] and in the stimulated peritoneal cavity.[36]

The monocytes/macrophages were selectively and highly concentrated from the blood when the bone marrow cells were infused into primed animals. The original bone marrow inoculum contained 6.5% of these labeled cells; 24 hr later the dermal infiltrate contained 80%, a 12-fold concentration. In the circulation at necropsy less than 3% of the labeled cells were mononuclear elements. The results indicated a highly selective process whereby this class of cells was permitted to accumulate while lymphocytes were excluded. A similar concentrating mechanism has been reported to occur in lesions of allergic contact dermatitis.[23] About 13% of the labeled cells in the test sites were PMN's and a similar proportion was found labeled in the circulation at necropsy —ie, there was no selective concentration of this cell type. PMN's have always been observed in delayed reactions and it is still unknown whether they play a necessary role in the development of the lesion of delayed hypersensitivity.[37]

The selective concentration of monocytes/macrophages in skin reactions was not restricted to those elicited by PPD. In test sites produced by ALS, PHA, and mineral oil there was a 4–10-fold concentration over the levels present in the original inoculums. As noted by others [20,36] then, the accumulation of a high proportion of monocytes/macrophages was characteristic of inflammatory lesions, whether elicited by specific antigen or by nonspecific agents. In the case of PHA and mineral oil, the predominant labeled cell was the PMN and this cell type consti-

tuted two-thirds to three-quarters of all the tagged infiltrating elements.

By contrast, the lesions induced by the intradermal injection of ALS were indistinguishable histologically and autoradiographically from those produced by specific antigen. The results point up the fact that the reaction of delayed hypersensitivity has a characteristic but nonspecific histology and cytology. The biologic mechanism by means of which ALS reproduced the lesion of delayed hypersensitivity remains unclarified. Presumably, it was due to the interaction of ALS with surface antigens of lymphocytes and not simply to agglutination, since PHA also agglutinated lymphocytes but did not elicit a characteristic delayed reaction.

The 4–12-fold concentrations of monocytes/macrophages were probably minimal estimations, for there was loss of labeled elements following infusion of bone marrow suspensions. In the original inoculums 90% of the nucleated elements were labeled by H_3-thymidine, while in the skin lesions elicited by the different reagents approximately 25% of the infiltrating cells were labeled. The loss could be accounted for in several ways. First, if division occurred with any significant frequency, the number of labeled cells that could be detected would decrease. At the light and electron microscope levels, no mitotic figures were encountered, so that divisions should have been an insignificant cause of loss of label. A second cause for loss of label might be simple elimination by the reticulo-endothelial system of injured cells, particularly those that were heavily irradiated by incorporated isotope. Lastly, the difference in the ratio of labeled cells of the original inoculums and the ratio of labeled cells in skin lesions might have been due to the selective concentration of monocytes/macrophages, which were labeled only to the extent of 6.5%, and the elimination from the test site of labeled lymphocytes and many labeled PMN's. It was also noted that the majority of unlabeled cells found in the skin test sites were monocytes/macrophages, an observation reported by others.[19,29,31]

The selective concentration of monocytes/macrophages in skin reactions of delayed hypersensitivity enhances the possibility that macrophage inhibitory factor (MIF) might operate in vivo. David and his colleagues [4,7,8] and Bloom and Bennett [9,10] have described and isolated a factor generated by the interaction of specific antigen with sensitized lymphoid cells. This substance (MIF) is capable of inhibiting movement of macrophages without killing them. Presumably, the same or similar material is generated in vivo at the skin test site where antigen is deposited, although there is no evidence at present that MIF is present in in vivo delayed reactions. Selective concentration of monocytes/

macrophages also occurred in lesions elicited by such nonspecific agents as ALS, PHA, and mineral oil. With the last agent it is unlikely that antigen-cell receptor interaction takes place and it would be difficult to ascribe the accumulation of monocytes/macrophages to the generation of MIF by mineral oil. Whether the response of lymphocytes to ALS and PHA reflect an antigen-cell receptor reaction which might generate MIF or a similar reagent remains to be elucidated.

Summary

The composition, morphology, and source of inflammatory cells in delayed skin lesions elicited by specific antigen, ALS, PHA, and mineral oil, were studied by light and electron microscopic autoradiography. In specific reactions, 80% of the infiltrating cells belonged to the monocyte/macrophage class. ALS produced skin lesions that were indistinguishable from those produced by specific antigen (PPD). PHA and mineral oil, by contrast, induced skin reactions in which PMN's were the predominant cell type. With all reagents, the monocytes/macrophages were selectively concentrated in the test sites. The preferential accumulation of this cell class was greatest in lesions of delayed hypersensitivity and ALS, and less in those induced by PHA and oil. Bone marrow was the primary source of the monocytes/macrophages and of many of the PMN's. The monocytes/macrophages seemed to be derived from a primordial cell present in bone marrow, capable of division, and cytologically different from lymphocytes and blast cells.

References

1. McCluskey, R. T., Benacerraf, B., and McCluskey, J. W. Studies on the specificity of the cellular infiltrate in delayed hypersensitivity reactions. *J Immun* 90:466–477, 1963.
2. Najarian, J. S., and Feldman, J. D. Passive transfer of tuberculin sensitivity by tritiated thymidine labeled cells. *J Exp Med* 114:779–790, 1961.
3. Feldman, J. D., and Najarian, J. S. Dynamics and quantitative analysis of passively transferred tuberculin hypersensitivity. *J Immun* 91:306–312, 1963.
4. David, J. R., Lawrence, H. S., and Thomas, L. Delayed hypersensitivity *in vitro*. II. Effect of sensitive cells on normal cells in the presence of antigen. *J Immun* 93:274–278, 1964.
5. Spector, W. G., and Willoughby, D. A. "Mediators of Delayed Hypersensitivity Reactions," In *Mechanism of Inflammation Induced by Immune Reactions*, Miescher, P., and Grabar, P., Eds. Schwabe, Basel, 1968. p 281.
6. Ruddle, N. H., and Waksman, B. H. Cytotoxicity mediated by soluble antigen and lymphocytes in delayed hypersensitivity. I. Characterization of the phenomenon. II. Correlation of the *in vitro* response with skin re-

activity. III. Analysis of the mechanism. *J Exp Med 128*:1237–1254, 1255–1263, 1267–1279, 1968.

7. DAVID, J. R., AL-ASKARI, S., LAWRENCE, H. S., and THOMAS, L. Delayed hypersensitivity *in vitro*. I. The specificity of inhibitional cell migration by antigens. *J Immun 93*:264–273, 1964.
8. DAVID, J. R. Delayed hypersensitivity *in vitro*. Its mediation by cell-free substance formed by lymphoid cells antigen interaction. *Proc Nat Acad Sci USA 56*:72–77, 1966.
9. BLOOM, B. R., and BENNETT, B. Mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. *Science 153*:80–82, 1966.
10. BENNETT, B., and BLOOM, B. R. Reactions *in vivo* and *in vitro* produced by a soluble substance associated with delayed-type hypersensitivity. *Proc Nat Acad Sci USA 59*:756–762, 1968.
11. GRANGER, G. A., and KOLB, W. P. Lymphocyte *in vitro* cytotoxicity: Mechanisms of immune and nonimmune small lymphocyte mediated target L cell destruction. *J Immun 101*:111–120, 1968.
12. GRANGER, G. A., and WILLIAMS, T. W. Lymphocyte cytotoxicity *in vitro:* Activation and release of a cytotoxic factor. *Nature (London) 218*:1253–1254, 1968.
13. SVEJCAR, J., PEKAREK, J., and JOHANOVSKY, J. Studies on production of biologically active substances which inhibit cell migration in supernatants and extracts of hypersensitive lymphoid cells incubated with specific antigen *in vitro. Immunology 15*:1–11, 1968.
14. KREJCI, J., PEKAREK, J., JOHANOVSKY, J., and SVEJCAR, J. Demonstration of the inflammatory activity of the supernatant of hypersensitive lymph node cells incubated with a high dose of antigen. *Immunology 16*:677–684, 1969.
15. PERLMANN, P., and HOLM, G. "Cytotoxic Effects of Lymphoid Cells *in vitro*." In *Advances in Immunology*, to be published.
16. WILLOUGHBY, D. A., Spector, W. G., and BOUGHTON, B. A lymph node permeability factor in the tuberculin reaction. *J Path Bact 87*:353–363, 1964.
17. INDERBITZEN, T., KEEL, A., and BLUMENTAL, G. The identity of the permeability factor from lymph node cells (LNPF) with the permeability increasing factor (PF). *Int Arch Allerg 29*:417–425, 1966.
18. YOSHIDA, T., BENACERRAF, B., MCCLUSKEY, R. T., and VASSALLI, P. The effect of intravenous antigen on circulating monocytes in animals with delayed hypersensitivity. *J Immun 102*:804–811, 1969.
19. COHEN, S., MCCLUSKEY, R. T., and BENACERRAF, B. Studies on the specificity of the cellular infiltrate of delayed hypersensitivity reactions. *J Immun 98*:269–273, 1967.
20. LUBAROFF, D. M., and WAKSMAN, B. H. Bone marrow as source of cells in reaction of cellular hypersensitivity. I. Passive transfer of tuberculin sensitivity in syngeneic systems. *J Exp Med 128*:1425–1435, 1968.
21. SPECTOR, W. G., and WILLOUGHBY, D. A. The origin of mononuclear cells in chronic inflammation and tuberculin reactions in the rat. *J Path Bact 96*:389–399, 1968.
22. VOLKMAN, A., and GOWANS, J. L. The origin of macrophages from bone marrow in the rat. *Brit J Exp Path 46*:62–70, 1965.
23. LIDEN, S. The mononuclear-cell infiltrate in allergic contact dermatitis. II. Selective accumulation of cells from the bone marrow. *Acta Path Microbiol Scand 70*:58–66, 1967.

24. LUBAROFF, D. M., and WAKSMAN, B. H. Bone marrow as source of cells in reactions of cellular hypersensitivity. I. Identification of allogeneic or hybrid cells by immunofluorescence in passively transferred tuberculin reactions. *J Exp Med 128*:1437–1449, 1968.

25. DIENES, L., and MALLORY, T. B. Histological studies of hypersensitive reactions. *Amer J Path 8*:689–710, 1932.

26. FOLLIS, R. H., JR. Studies on the cellular response in the early stages of the tuberculin reaction. *Bull John Hopkins Hosp 66*:245–252, 1940.

27. GELL, P. G. H. Experimental allergic lesions in animals with special reference to histological appearances. *Int Arch Allerg 13*:112–121, 1958.

28. WAKSMAN, B. H. "A Comparative Histopathological Study of Delayed Hypersensitivity Reactions." In *Ciba Foundation Symposium on Cellular Aspects of Immunity*, Wolstenholme, G. F. W., and O'Connor, M. Eds. Little, Boston, 1959, p 280.

29. BOUGHTON, B., and SPECTOR, W. G. Histology of the tuberculin reaction in guinea pigs. *J Path Bact 85*:371–381, 1963.

30. GOLDBERG, B., KANTOR, F. S., and BENACERRAF, B. An electron microscopic study of delayed sensitivity to ferritin in guinea pigs. *Brit J Exp Path 43*: 621–676, 1962.

31. FLAX, M. H., and CAULFIELD, J. B. Cellular and vascular components of allergic contact dermatitis. Light and electron microscopic observations. *Amer J Path 43*:1031–1053, 1963.

32. WYLLIE, J. C., MORE, R. H., and HAUST, M. D. Electron microscopy of epidermal lesions elicited during delayed hypersensitivity. *Lab Invest 13*:137–151, 1964.

33. WIENER, J., SPIRO, D., and ZUNKER, H. O. A cellular study of tuberculin sensitivity. *Amer J. Path 47*:723–763, 1965.

34. MARTELLI, M. Personal communication.

35. VOLKMAN, A., and GOWANS, J. L. The production of macrophages in the rat. *Brit J Exp Path 46*:50–61, 1965.

36. VOLKMAN, A. The origin and turnover of mononuclear cells in peritoneal exudates in rats. *J Exp Med 124*:241–254, 1966.

37. PAGE, A. R., and GOOD, R. A. A clinical and experimental study of the function of neutrophils in the inflammatory response. *Amer J Path 34*:645–669, 1958.

[Illustrations follow]

Legends for Figures

Fig 1 a. Mononuclear infiltrate of dermis in specific test site injected with PPD. **b.** Mononuclear infiltrate of dermis in test site injected with ALS. Note qualitative similarity of cellular elements in two lesions. **c.** Numerous polymorphonuclear cells in test site injected with PHA. Control for **a** above. **d.** Numerous polymorphonuclear cells in infiltrate of test site injected with mineral oil. Control for **a** above. H & E. × 500.

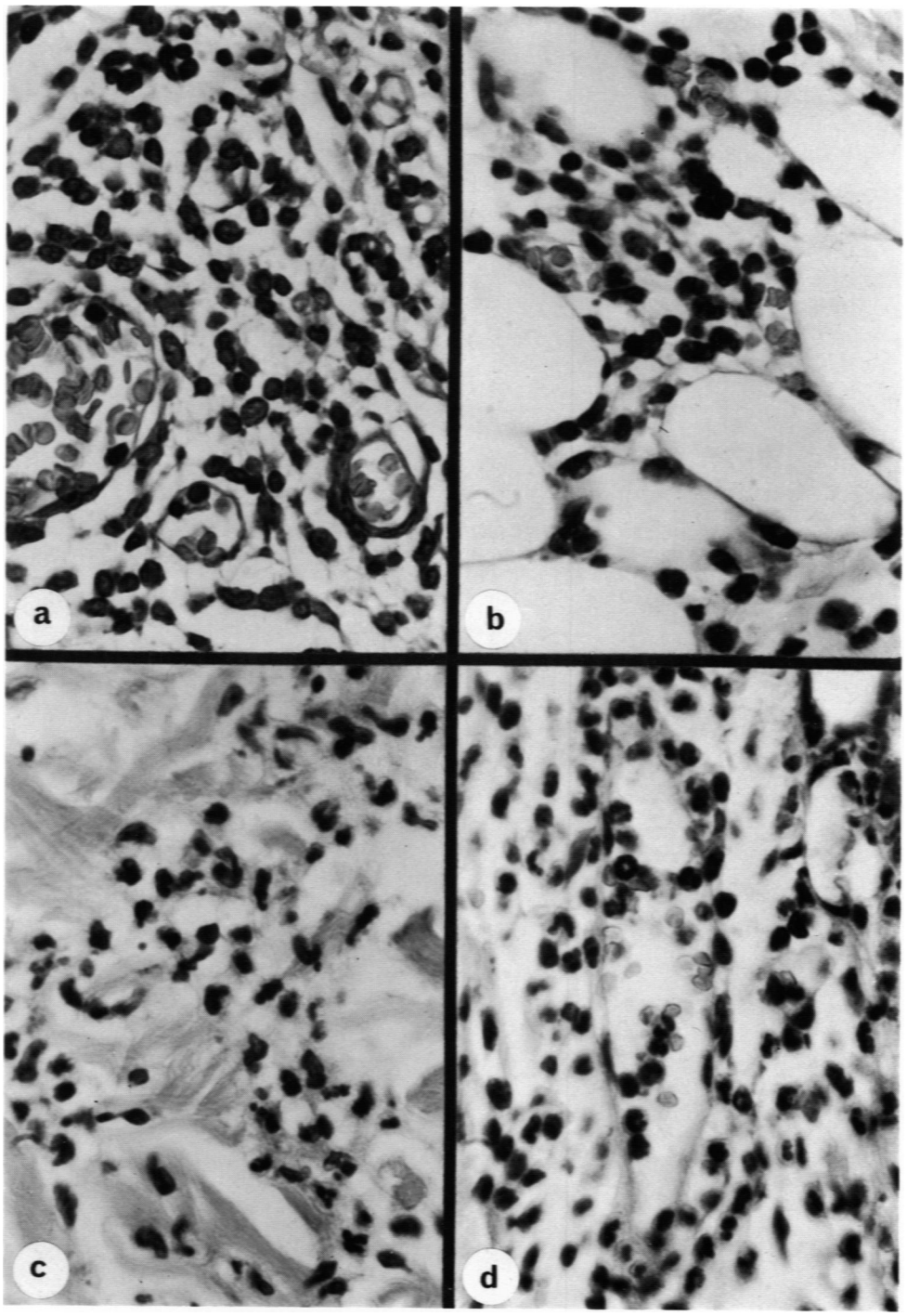
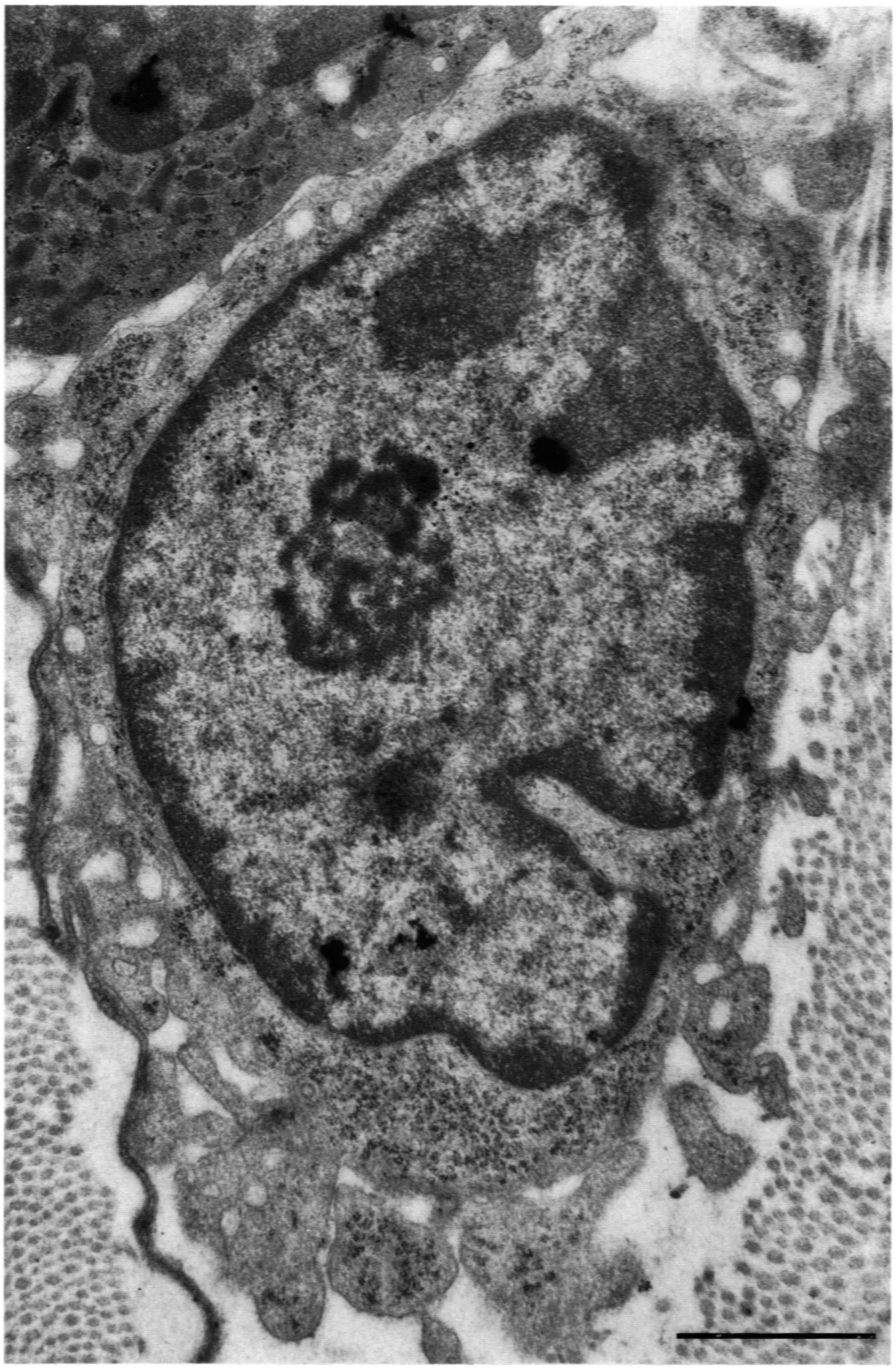

a
b
c
d

Fig. 2. Labeled bone marrow cell, in specific lesion of skin, with large notched nucleus resembling that seen in typical monocytes. Cytoplasm contains numerous polysomes and shows ruffled surface, close to which are numbers of vesicles. Portion of labeled polymorphonuclear cell is seen above. In this and succeeding micrographs, sections were stained with uranyl acetate followed by lead citrate. Horizontal line in each picture equals 1 μ. × 29,000.



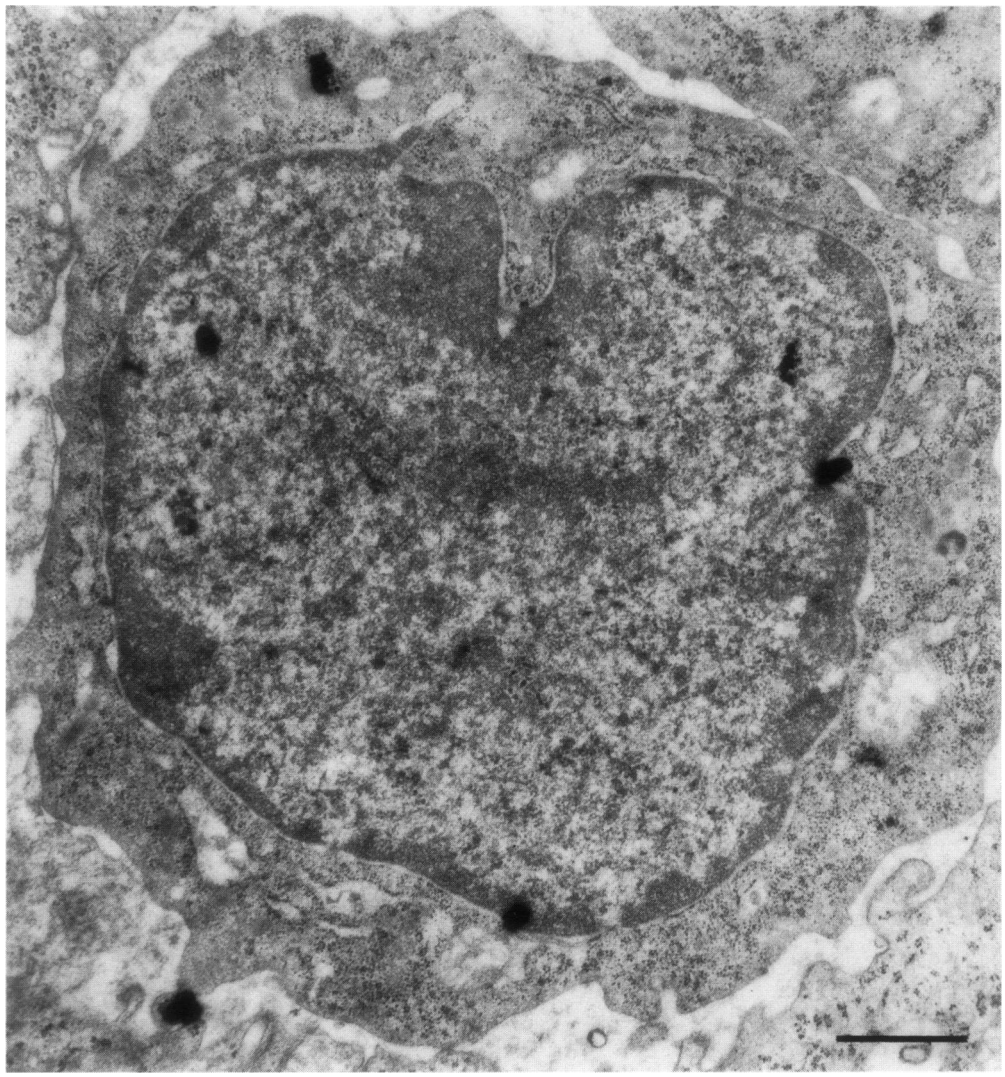


Fig. 3. Labeled bone marrow cell, in specific lesion of skin, with large, notched, relatively clear nucleus—ie, exhibiting dense chromatin as thin rim at periphery of nucleus. Cytoplasm contains free polysomes and ribosomes, a few profiles of rough endoplasmic reticulum, a few vesicles and dense granules. This cell and the one shown in Fig 2 are considered precursors of fully-developed monocytes and macrophages. × 17,000.

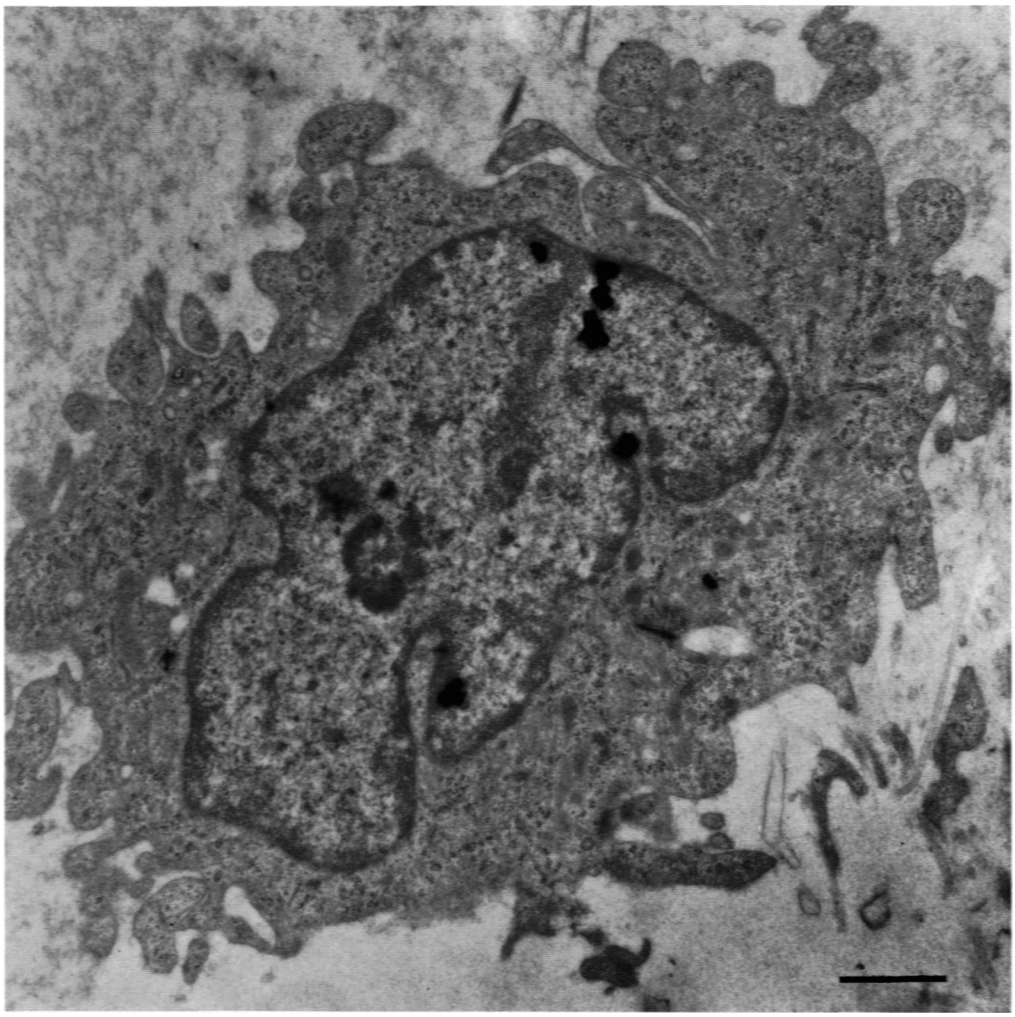


Fig. 4. Labeled bone marrow cell, in specific lesion of skin, showing typical features of monocyte—ie, relatively large, light nucleus, polysomes and ribosomes, a few profiles of rough endoplasmic reticulum, several vesicles and granules, and ruffled border. × 14,000.

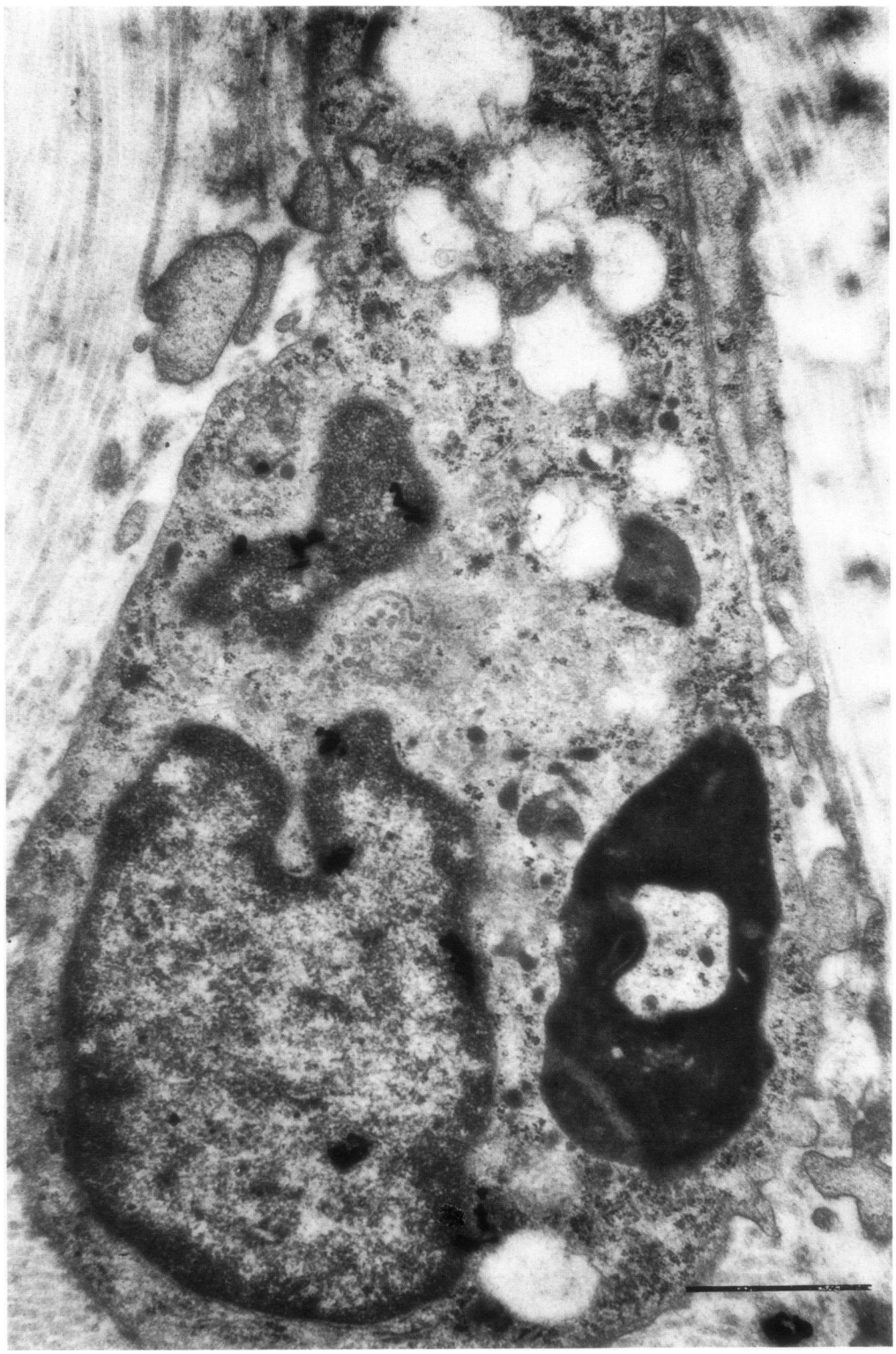


Fig 5. Labeled bone marrow cell, in specific lesion of skin, with typical features of macrophage. × 28,000.