PASSIVE TRANSFER OF CONTACT SENSITIVITY BY TRITIATED THYMIDINE-LABELED LYMPHOID CELLS*, ‡

By JOHN S. NAJARIAN,§ M.D., AND JOSEPH D. FELDMAN, M.D.

(From the Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California)

PLATES 42 TO 44

(Received for publication, January 25, 1963)

When tuberculin sensitivity was passively transferred by lymphoid cells sensitized to tubercle bacilli and labeled with tritiated thymidine, relatively large numbers of labeled cells were found at the PPD skin test site (1). In contrast, when transplantation immunity was passively transferred in both mice (2) and guinea pigs (3) by lymphoid cells sensitized to homologous tissues and labeled with tritiated thymidine, the labeled cells were rarely found at the site of homograft rejection. These findings indicated that the homograft reaction and delayed sensitivity of the tuberculin type were achieved by different immunologic mechanisms.

This investigation was designed to observe whether cells sensitized to the simple chemical DNFB,[1] labeled with tritiated thymidine, and passively transferred to non-sensitized homologous guinea pigs could be found in considerable numbers at the skin test site of DNFB skin contact, and if so, what cell types they were.

Materials and Methods

Donor guinea pigs, ranging in weight from 400 to 600 gm, were immunized by injection of 50 μg DNFB in 1.0 ml of complete Freund's adjuvant, 0.2 ml in each foot-pad, and 0.2 ml subcutaneously in the posterior cervical region. 1 week later, the guinea pigs were skin-tested by contact with two drops of 0.25 per cent DNFB dissolved in one part corn oil and two parts acetone to enhance the immunization of the animal. The two drops of DNFB were applied to a dorsal skin site prepared with an electric clipper and were gently spread over a 25 mm area by means of a smooth glass stirring rod. The donor animals were skin-tested a second time 1 week later. All guinea pigs showing a ++++ contact reaction (approximately 50

* This is publication number 36 from the Division of Experimental Pathology, Scripps Clinic and Research Foundation, LaJolla, California.

‡ This work was supported by grants from the United States Public Health Service and The National Foundation.

§ Special Research Fellow of United States Public Health Service. Present address: Department of Surgery, University of California School of Medicine, San Francisco.

[1] Dinitrofluorobenzene, obtained from Eastman Kodak Co., Rochester, New York.

per cent of the donors) were used as lymphoid cell donors. These latter animals were given intraperitoneally ⅓ μc of tritiated thymidine per gram body weight every 12 hours for 4 days. 6 hours after the last isotope injection, the popliteal, femoral, inguinal, axillary, cervical, and mesenteric lymph nodes were removed and shredded with rakes in balanced salt solution (BSS) containing 20 per cent polyvinylpyrrolidone (PVP) by volume and 3 units of heparin per ml. The resulting suspension was passed through a 100 gauge nylon gauze filter and centrifuged for 10 minutes at 1000 RPM. The supernate was decanted and the pellet of cells resuspended in the BSS-PVP-heparin solution so that each milliliter contained 1×10^8 lymphoid cells. Smears were made for differential analysis and autoradiography. Cell viability was measured by staining with 1:1000 trypan blue. An average of 20 per cent of the cells in suspension was labeled and over 85 per cent were viable as indicated by the trypan blue staining method.

TABLE I

Summary of Data on Passive Transfer of DNFB-Sensitized Cells

Guinea pig No.	Time of DNFB skin site biopsy after cell transfer	Cells transferred		Gross reactions (+ to ++++)	Infiltrating cells			
		× 10^8 total	Per cent labeled		Labeled	Total	Per cent labeled	Per cent of labeled in epidermis
	hrs.							
1-0	24	7.20	21.8	+++	68	2014	3.4	23
1-5	24	4.21	17.2	++++	122	3218	3.8	20
1-6	24	8.72	17.5	++++	72	2246	3.2	30
1-8	24	5.77	22.4	++++	119	2756	4.3	22
1-9	24	6.24	20.8	++++	89	2866	3.1	32
2-2	24	6.13	16.7	+++	45	1669	2.7	18
2-0	48	7.79	21.2	+++	9	946	1.0	29
2-1	48	9.02	20.7	+++	14	1410	1.0	35
				Controls				
2-3	24	5.50 5.00*	16.3	+++	2	960	0.2	0
2-4	24	5.50 5.00*	16.3	++++	0	1642	0.0	0
2-5	24	6.25 6.00*	18.8	+++	2	1154	0.2	0
2-6	24	6.25 6.00*	18.8	+++	1	1267	0.1	0

* DNFB-sensitized non-labeled lymphoid cells.

Each recipient guinea pig was injected intravenously with lymph node cells of one donor. The actual number of transferred cells per recipient is shown in Table I. Immediately thereafter, the animals were skin-tested as described above. Gross reactions were recorded at 24 or 48 hours following contact applications of DNFB as follows: ++++, homogeneous, slightly raised erythema; +++, homogeneous pale erythema; ++, patchy erythema; +,

patchy pale erythema; and 0, no reaction. The contact skin sites were removed, sliced at several levels, and prepared for histologic and autoradiographic studies.

For controls, recipient guinea pigs (Nos. 2–3 to 2–6, Table I) were injected intravenously with a mixed suspension of lymphoid cells derived from non-labeled DNFB-sensitized animals and from labeled non-sensitized animals. Contact skin tests, recording of gross reactions, and preparation of tissue for histology and autoradiography were done as described above. This control resulted in a skin test site qualitatively and quantitatively similar to that observed in experimental guinea pigs and was used to determine whether labeled non-sensitized cells would appear non-specifically in the skin reaction of this type of delayed hypersensitivity.

Histological and autoradiographic sections were also made from the spleens and lymph nodes of both experimental and control animals. For histology, tissues were stained with hematoxylin and eosin. Smears of cell suspensions were stained with Giemsa or hematoxylin. For autoradiography, the unstained tissues and smears were coated with liquid Kodak NTB-2 track emulsion; after suitable exposure periods, the slides were developed and stained with hematoxylin.

The number of labeled cells in skin test sites was expressed as a percentage of all unfixed mononuclear cells in the dermis. Fibrocytes, fixed histiocytes, and endothelial cells were not regarded as infiltrating elements.

RESULTS

The transfer of DNFB-sensitized and labeled lymphoid cells from donor guinea pigs to homologous recipients resulted in positive skin reactions when the dorsal skin was contact-tested with DNFB and examined 24 hours later (Table I). No correlation was observed between the number of cells transferred and intensity of the gross reaction. There was, however, an inverse relationship between the size of the reaction and the intensity of the erythema and induration. Gross scores of control reaction sites, following transfer of a mixture of labeled non-sensitized and non-labeled sensitized cells, were comparable to the gross reactions observed in the experimental guinea pigs.

Microscopically, the 24 hour contact skin sites showed a diffuse cellular infiltration of the upper dermis, the epidermis, and the superficial hair follicles (Fig. 1). The infiltrating cells were chiefly lymphocytes, small and large, and also large immature lymphoid elements, macrophages and a minority of dispersed polymorphonuclear neutrophils (Figs. 2 and 3). The density of the cell population varied directly with the macroscopic severity of the lesions and with the extent of epidermal necrosis. In addition, the proportion of polymorphs in the total cellular infiltrate increased with increasing epidermal injury (Fig. 2). In contradistinction to tuberculin lesions, there was no perivenular and perineural clustering of cells, a relatively high proportion of mononuclear cells was present in the epidermis and hair follicles, and the infiltrate was densest just beneath the epidermis. Venular dilatation and congestion was prominent in the upper dermis, and arteriolar dilatation was more obvious in the deep connective tissues. Epidermal alterations ranged from simple intercellular and intracellular edema to liquefaction necrosis and blistering.

Autoradiographs of the contact skin test sites on the experimental animals at this time revealed that from 2.7 to 4.3 per cent of the infiltrating mononuclear

cells were labeled. The tritiated cells were dispersed in the upper dermis, among the epidermal cells, and in and around hair follicles (Figs. 4 to 6). About ¼ of the isotopically tagged cells were found in the epidermis. The labeled elements were chiefly small and large lymphocytes, and a small proportion consisted of large immature lymphoid cells and unclassified cell types.

The histology of the 48 hour contact skin sites was similar to that seen at 24 hours except for a diminution of total infiltrate and a decrease of polymorphonuclear neutrophils. In autoradiographs at this time there was a diminution of labeled cells to about 1 per cent of the infiltrating mononuclear elements and ⅓ of these were found in the epidermis.

The four control guinea pigs which received mixtures of labeled non-sensitized and non-labeled DNFB sensitized cells developed contact reactions that were quantitatively and qualitatively equivalent to the microscopic reactions in the experimental group. In autoradiographs of the control sites, only five labeled cells could be identified in the four control animals despite large accumulations of mononuclear cells; *i.e.*, less than 0.2 per cent of the mononuclear cell infiltrate. Despite the small number of labeled cells found in the skin test sites, donor cells could readily be identified in the lymph nodes and spleens of control recipients.

DISCUSSION

This study has shown that the passive transfer of DNFB-sensitized lymphoid cells resulted in a localization of the donor-sensitized cells at the site of the specific skin contact reaction. The accumulation of sensitized cells at the reaction site appeared to be specific; *i.e.*, the result of an interaction between sensitized cells and specific antigen. Non-sensitized labeled cells were rarely found in reaction sites induced by passive transfer of non-labeled sensitized cells. Moreover, autoradiographs of the experimental contact skin reaction sites revealed that between ¼ and ⅓ of all the labeled cells were infiltrating the epidermis and hair follicles, the primary locus of chemical antigen concentration (4). Too few labeled donor cells were found in control test sites to compare their distribution with that of the cells in experimental contact lesions.

The problem of non-specific "stickiness" of sensitized lymphoid cells (5) was not studied in these experiments which were designed to learn whether donor cells appeared in contact chemical test skin sites after passive transfer of labeled sensitized lymphoid elements. We have, however, examined this problem and in another communication (6) will report that sensitized (inflamed) lymphoid cells have exhibited a "stickiness" which exceeds that of non-sensitized cells; they also exhibited specificity as manifested by a greater accumulation of sensitized cells in the site of specific antigen deposition than in the site of non-specific antigen.

A calculation of the number of cells contributed by donor and by host in a homologous passive transfer has shown that even in the best reaction site

(guinea pig 1–8, Table I) only 20 per cent of the mononuclear infiltrate was of donor origin and 80 per cent of host origin. Thus, only a small minority of the cells at the reaction site had arrived at the lesion presumably because of immunologic specificity and the majority of infiltrating cells were of host origin and arrived non-specifically as a reaction to tissue injury.

The findings reported in this study for contact chemical sensitivity were similar to those reported for tuberculin sensitivity (1). Passive transfer of labeled sensitized lymphoid cells in both types of delayed hypersensitivity resulted in a considerable accumulation of sensitized cells at the site of specific antigen application. In addition, passive transfer of contact and tuberculin sensitivities was not accomplished when sensitized lymphoid cells were enclosed within cell-impenetrable Millipore chambers and transferred to non-sensitized hosts (6). In contrast, in passive transfer of transplantation immunity, the labeled sensitized cells were rarely found at the site of homograft rejection and were effective when transferred in Millipore chambers to mice (2), guinea pigs (3), and rabbits (7). Therefore, it would appear that the immunologic mechanisms of tuberculin and contact hypersensitivity differed from that of homograft rejection. In the former, the sensitized cells were apparently necessary for at least the initiation of the delayed hypersensitive skin reaction, while, in transplantation immunity, a soluble product was capable of rejecting specific homografts without the physical presence of the cell.

SUMMARY

Passive transfer of tritiated thymidine-labeled lymphoid cells sensitized to the simple chemical DNFB into homologous guinea pigs resulted in positive contact skin reactions 24 hours after skin testing with DNFB. Labeled sensitized cells were found to accumulate at these sites, whereas, labeled non-sensitized lymphoid cells did not appear non-specifically in contact skin reaction sites. The labeled cells were small and large lymphocytes and immature cells of the lymphoid series. The maximum reactions were obtained at 24 hours, with an average of 3.4 per cent of the infiltrating mononuclear cells showing a label. At 48 hours, the macro- and microscopic reactions were similar to the 24 hour reactions but diminished in intensity, and the number of labeled cells in the infiltrates had decreased to 1 per cent of the total infiltrating mononuclear cells. ¼ to ⅓ of the labeled cells were found within the epidermis in the test skin sites.

These data have indicated that contact sensitivity, like tuberculin sensitivity, required the sensitized cell to initiate the skin reaction and that the majority of the cellular infiltrate was the result of non-specific host response to injury.

BIBLIOGRAPHY

1. Najarian, J. S., and Feldman, J. D., Passive transfer of tuberculin sensitivity by tritiated thymidine-labeled lymphoid cells, *J. Exp. Med.*, 1961, **114**, 779.

2. Najarian, J. S., and Feldman, J. D., Passive transfer of transplantation immunity. I. Tritiated lymphoid cells. II. Lymphoid cells in Millipore chambers, *J. Exp. Med.*, 1962, **115,** 1083.
3. Najarian, J. S., and Feldman, J. D., Passive transfer of transplantation immunity. III. Inbred guinea pigs, *J. Exp. Med.*, 1963, **117,** 449.
4. Eisen, H. N., Hypersensitivity to simple chemicals, *in* Cellular and Humoral Aspects of the Hypersensitive States, (H. S. Lawrence, editor), New York, Hoeber-Harper Co., 1959, 89.
5. Lurie, M. B., Studies in the mechanism of immunity in tuberculosis: The mobilization of mononuclear phagocytes in normal and immunized animals and their relative capacities for division and phagocytosis, *J. Exp. Med.*, 1939, **75,** 247.
6. Najarian, J. S., and Feldman, J. D., unpublished observations.
7. Kretschmer, R. R., and Pérez-Tamayo, R., The role of humoral antibodies in rejection of skin homografts in rabbits. II. Passive transfer of transplantation immunity by sensitized lymph node cells within diffusion chambers, *J. Exp. Med.*, 1962, **116,** 879.

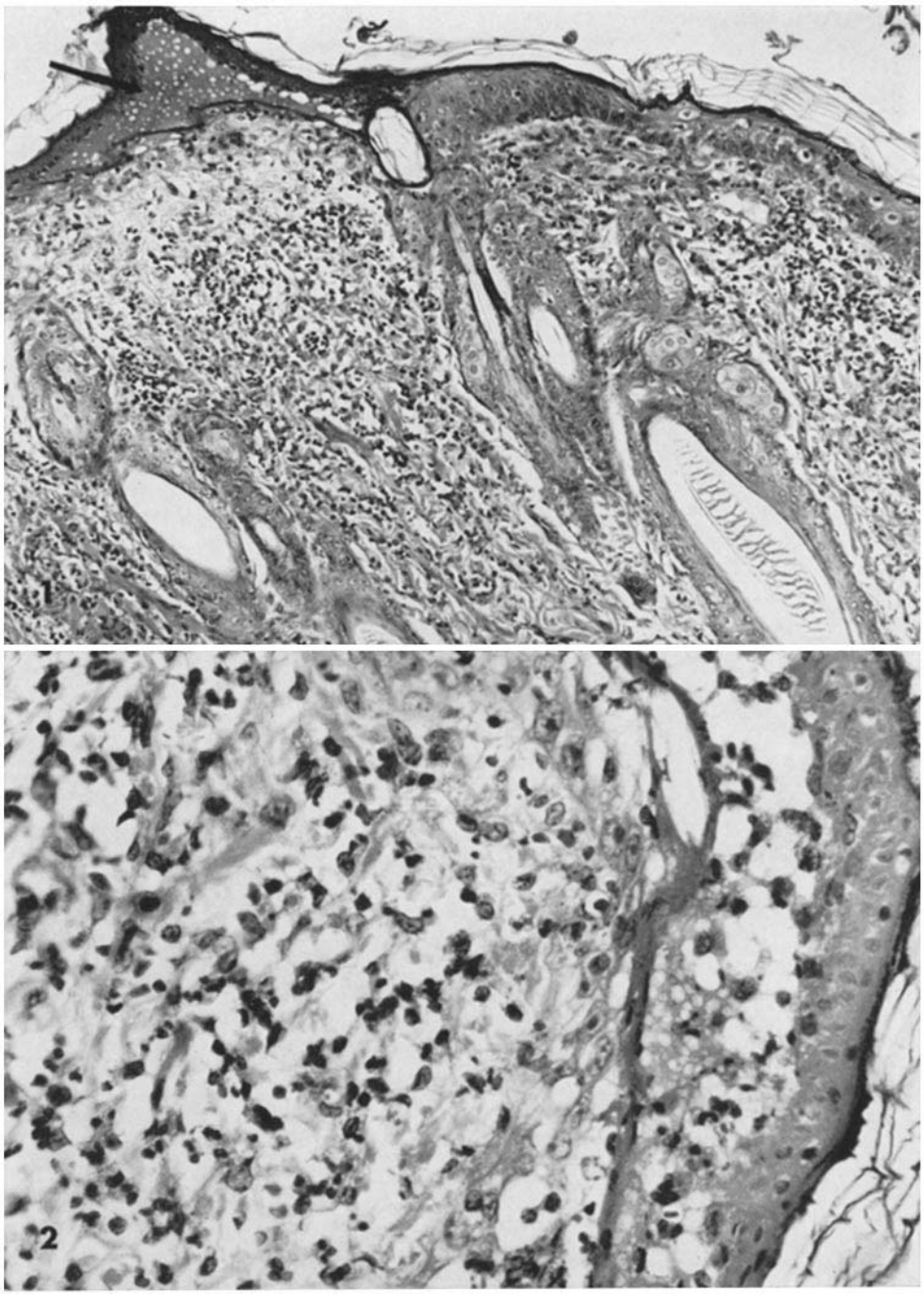
EXPLANATION OF PLATES

The slides were stained with hematoxylin.

PLATE 42

FIG. 1. Photomicrograph of DNFB test site from the flank skin of a guinea pig passively transferred with sensitized tritiated thymidine-labeled lymphoid cells. The cellular infiltrate is chiefly in the upper dermis and epidermis. In the epidermis to the left (arrow) fluid has accumulated to form a blister; to the right several clear cells (intracellular edema) are seen. × 120.

FIG. 2. Photomicrograph of DNFB test site from a guinea pig treated as in Fig. 1. The cellular infiltrate is a mixture of mononuclear cells and polymorphonuclear neutrophils. There is liquefaction necrosis of the epidermis and infiltrating cells can be seen in this area. × 400.

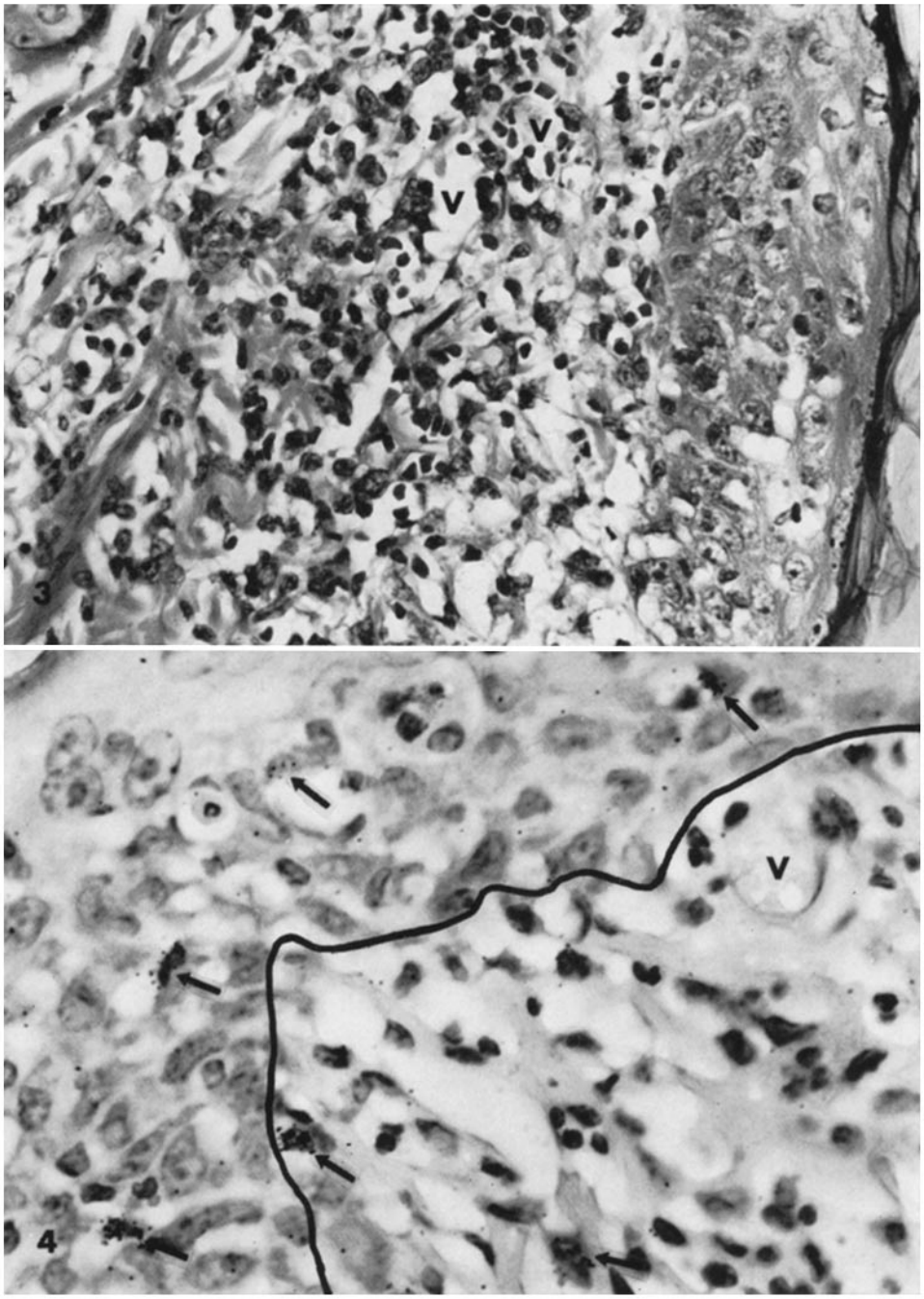


(Najarian and Feldman: Passive transfer of contact sensitivity)

PLATE 43

FIG. 3. Photomicrograph of DNFB test site from a guinea pig treated as in Fig. 1. The cells in the dermis are predominantly mononuclear elements. A dilated vessel is seen close to the epidermis (*V*). In the epidermis there are both intra- and intercellular edema. × 400.

FIG. 4. Photomicrograph of an autoradiograph of a DNFB test site from a guinea pig prepared as in Fig. 1. A number of labeled cells (arrows) are infiltrating both the dermis and epidermis (above and left of black line). A dilated vessel is seen at (*V*). × 500.

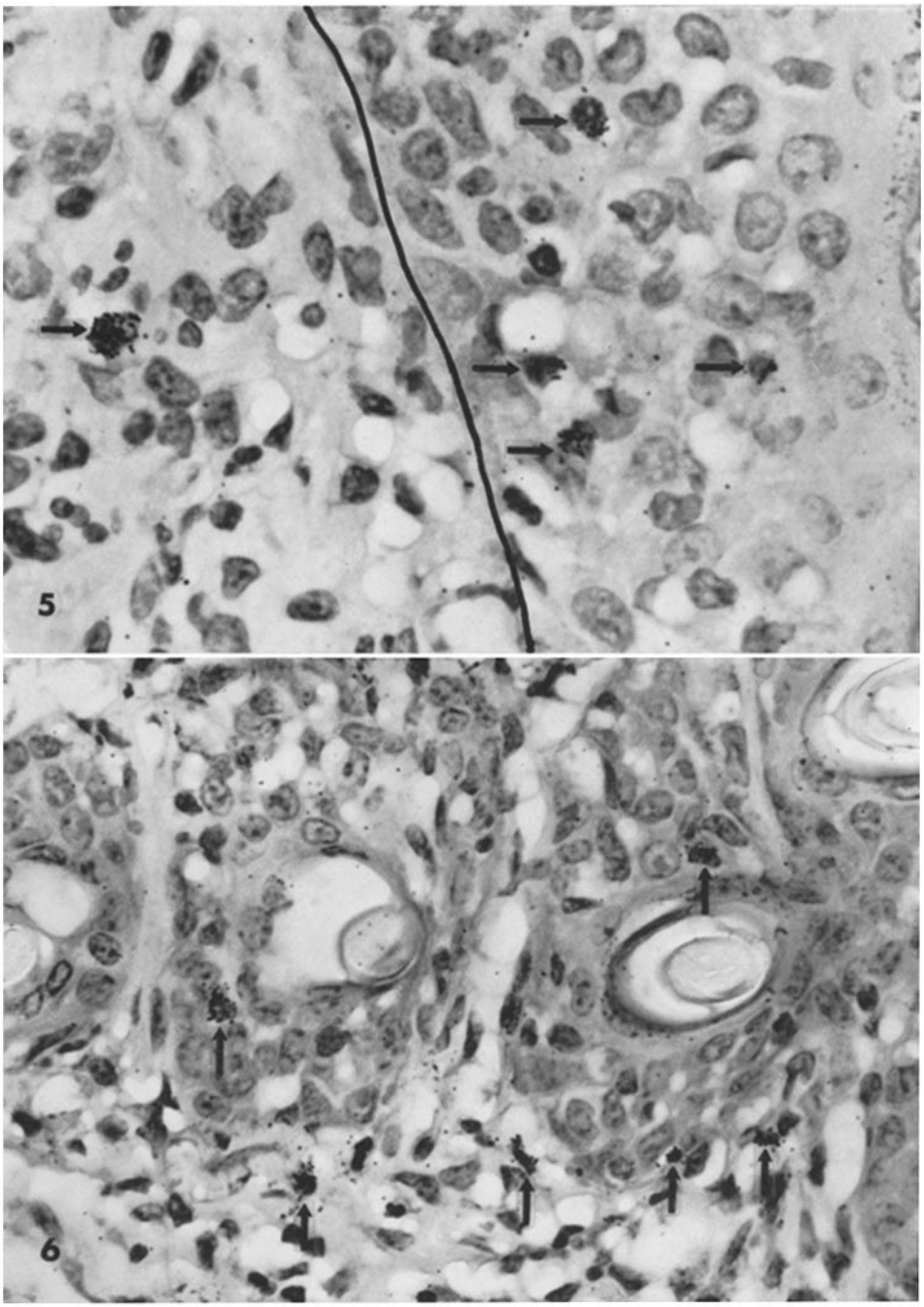


(Najarian and Feldman: Passive transfer of contact sensitivity)

PLATE 44

FIG. 5. Photomicrograph of an autoradiograph of a DNFB site showing infiltration by labeled cells (arrows) of the dermis and epidermis (to right of black line). × 800.

FIG. 6. Photomicrograph of an autoradiograph of a DNFB site showing labeled cells (arrows) in and around hair follicles. The epidermis is to the right. × 500.



(Najarian and Feldman: Passive transfer of contact sensitivity)