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

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Plates 78 to 80

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The passive transfer of delayed sensitivity to simple chemicals (1) and bacterial antigens (2) and of tissue (3) or tumor (4) transplantation immunity by viable cells rather than humoral antibodies is now well established. Most of the studies of this model system have been primarily concerned with the visible and palpable lesions rather than with the associated histologic reactions. The precise role of the transferred cells within the host and their participation in the reaction site have remained undisclosed in the past because of the lack of a suitable and permanent cellular label. With the advent of tritiated thymidine, which can be incorporated into the nucleus of the cell during DNA synthesis as a permanent cellular label, the possibility of identifying donor cells within a transfused host became a reality.

This study was designed to observe tuberculin-sensitized lymphoid cells labeled with tritiated thymidine after transfer to a non-sensitized host and after initiation of a tuberculin skin test. If the transferred cells could be found in significant concentrations at a site of injection of specific antigen, then an identification of the cell types involved and their distribution within such loci could be ascertained by autoradiography of these tissues.

Materials and Methods

Donor guinea pigs averaging 600 gm body weight were immunized with a 2 to 3 week culture of BCG strain "Phipps" grown in liquid tween albumin media. Each guinea pig was injected with 1 ml of this suspension which contained approximately 10^9 viable bacteria. To provide a maximum yield of sensitized lymphoid cells, the injections were divided among 0.1 ml in each foot-pad, 0.1 ml in each subinguinal area, 0.2 ml in the nuchal region, and the final 0.2 ml intraperitoneally. Six weeks later these animals were skin-tested intradermally

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with 0.2 γ-PPD[1] (intermediate strength) on the shaved flank, and only those exhibiting reaction sites of 15 mm in diameter or more were used as donors. These latter animals were then given intraperitoneally 0.25 μc of tritiated thymidine per gm body weight every 8 hours for 3 days to assure maximum labeling. Six hours after the last isotope injection the popliteal, inguinal, axillary, cervical, and mesenteric lymph nodes, and the spleen, were removed and shredded with rakes in balanced salt solution (Hanks') containing 20 per cent PVP by volume. The resulting suspension was passed through an 80 gauge monel gauze filter. Smears were made for differential analysis and autoradiography. Total numbers were determined in a hemocytometer, and cell viability was measured by staining with 1:1000 trypan blue.

An average of 17.6 per cent of the cells in suspension was labeled. In Table I are shown the differential counts of the mononuclear cells, the percentage of each cell type labeled, and the proportion of each cell type labeled within the total number of tritiated elements. The number of gains overlying individual cell nuclei was quite variable, indicating either concentration of the isotope as a result of mitoses during the 3 day labeling procedure or dilution as a result

TABLE I

The Differential Counts and Per Cent of Labeled Cells in the Cell Transfer Suspensions

Mononuclear cell type	Per cent of total cells*	Per cent cell type labeled	Per cent cell type of total labeled cells
Small < 8 μ	38	10	22
Medium 8 to 12 μ	39	17	38
Large > 12 μ	22	32	40

* The remaining 1 per cent includes cell types other than mononuclear cells.

of mitoses after cessation of labeling. Grain counts, therefore, were not made. However, a minimal number of 7 grains per nucleus over background count was considered significant. Over 90 per cent of the transferred cells were viable as indicated by the trypan blue staining method.

Each recipient guinea pig was usually injected with lymphoid cells of one donor, except for guinea pig 2 which received lymphoid cells from two donors and which also received 400 r total body irradiation 48 hours before transfer. The actual number of transferred cells per recipient is shown in Table II. The cells were injected intravenously in a 1.5 to 2 ml volume. Immediately thereafter 0.1 ml of double second strength PPD (10 γ-protein) was injected intradermally into the right ear. Erythema and induration were measured just before removal of the ears at 6, 24, 48, 54, and 72 hours following PPD application. The ear reaction sites were sliced at several levels and prepared for histologic and autoradiographic examination. The ear was chosen because reactions were more easily read here than at other skin sites, and also because ear skin was much more easily handled for histologic and autoradiographic studies. A rough estimate of the intensity of the microscopic reaction based on the numbers of cells involved and the extent of the lesion was recorded from a minimum of + to a maximum of +++.

The left ears of two guinea pigs were nicked for blood smears at ½, 2, 4, 6, and 24 hours and 1, 3, 5, and 18 hours, respectively, after the intravenous injection of tritiated thymidine-labeled, activated lymphoid cells. One guinea pig, No. 18, died 8 minutes following intravenous

[1] Tuberculin, Purified Protein Derivative, Parke, Davis and Co., Detroit.

transfer of labeled cells and blood smears as well as tissue sections of his viscera were taken. Blood smears were stained with Wright's stain alone or, following the development of the track emulsions, with hematoxylin.

Four control situations were a necessary part of the experiment. In the first situation four

TABLE II

Summary of Data on Passive Transfer of Tuberculin-Sensitized Cells

Guinea pig	Time of PPD skin site biopsy after cell transfer	Cells transferred		Reaction		Infiltrating cells		
		Total	Labeled	Gross	Micro + to +++	Labeled	Total	Labeled
	hrs.	$\times 10^8$	*per cent*	*mm diameter*				*per cent*
2	24	6.54	18.7	13	++	136	1107	12.3
3	24	4.34	18.1	12	++	53	575	9.2
4	24	6.32	21.0	16	+++	156	2219	7.0
6*	24	3.08	16.4	22	+++	290	4190	6.9
	48			15	++	84	1887	4.5
	72‡			6	+	2	212	0.9
	120‡			3	±	0	105	0
10	24	4.65	15.8	11	++	34	1299	2.6
11	24	2.04	19.2	9	+	15	358	4.2
16	6	2.18	16.5	10	+	1	316§ 148	0.7
	72			6	+	0	279	0
17	6	2.73	23.0	12	++	13	2280§ 1268	1.0
	54			9	++	49	1520	3.3
18	8 min.	3.41	16.9			0	0	0
19	72	2.80	15.6	7	+	1	327	0.3

* Each PPD skin site biopsy was separate and was removed 24 hours after skin test.

‡ Abdominal skin biopsy.

§ Polymorphonuclear neutrophils.

recipient guinea pigs (Nos. 20–23, Table III) were injected with a mixed suspension of lymphoid cells derived from non-labeled BCG-infected animals and from labeled non-infected animals. (One of these animals, No. 22, died minutes after transfer of cells, and is not recorded in Table III). 0.1 ml of double second-strength PPD was then injected intradermally in the right ear. The reaction site was measured 24 hours later and removed for microscopic study. The purpose of this control was to induce a skin test site qualitatively and quantitatively

similar to that achieved in experimental guinea pigs and to observe whether labeled non-sensitized cells would appear in the infiltrate.

In the second control situation, two guinea pigs (Nos. 3 and 4), which were transfused with labeled sensitized cells and which were PPD-tested on their right ears (Table II), were also tested on their left ears with an injection of 0.1 ml of coccidioidin[2] given at the same time as the PPD (Table III). Coccidioidin was selected as a protein preparation unrelated to but comparable with PPD. Both of these materials when injected into normal untreated guinea pigs elicited a small macroscopic papule, usually 3 to 4 mm in diameter, and a microscopic

TABLE III

Summary of Data on Skin Reactions at 24 Hours in Control Animals

Guinea pig	Type of skin test material	Cells transferred			Reaction		Infiltrating cells		
		BCG-sensitized	Non-sensitized labeled	Labeled	Gross	Micro ± to +++	Labeled	Total	Labeled
		$\times 10^8$	$\times 10^8$	*per cent*	*mm (diameter)*				*per cent*
20	PPD	4.62	3.92	10.6	12	++	1	1961	0.05
21	PPD	2.58	4.64	9.1	14	++	2	2667	0.08
23	PPD	2.44	2.86	7.8	18	+++	0	3734	0
3	Cocci	4.34*		18.1	3	+	0	326	0
4	Cocci	6.32*		21.0	3	+	0	431	0
7	PPD		3.03	21.0	4	+	0	412	0
8	PPD		3.04	16.0	5	+	1	1382	0.07
	Cocci				3	+	0	221	0
9	PPD		0.98‡	8.0	4	+	0	376	0
	Cocci				3	+	0	158	0
24§	PPD				<2	±	0	256‖	0
25§	PPD				<2	±	0	178‖	0
26§	PPD				3	±	0	272‖	0

* Labeled.

‡ Lymph node cells only.

§ 3 ml serum transferred from BCG-sensitized hosts.

‖ Mostly polymorphonuclear cells and foreign body reaction.

reaction consisting of edema and scattered polymorphs and lymphocytes. None of the guinea pigs in this study was sensitive to coccidioidin nor infected with the parasite. The injection site of coccidioidin was therefore a non-specific control to determine whether labeled sensitized cells would appear in the reaction of non-specific inflammation.

Three guinea pigs (Nos. 7, 8, and 9) in the third control group were transfused with tritiated non-sensitized cells and were tested with PPD and coccidioidin. Again this was a control situation to observe whether labeled non-sensitized cells would appear in a reaction induced by PPD or coccidioidin.

In the fourth control situation each of three guinea pigs (Nos. 24, 25, and 26) received intravenously 3 ml of serum which had been taken from BCG infected donors, and each was tested with PPD. This control was run to examine the possibilities of transferring any reactants through serum.

[2] Coccidioidin 1:100 dilution, Cutter Laboratories, Berkeley.

Histological and autoradiographic sections were taken 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 azure B bromide or hematoxylin; blood smears with Wright's stain. For autoradiography the unstained tissues and smears were coated with liquid Kodak NTB-2 or NTB-3 track emulsion and placed into lightproof and vapor-tight plastic boxes. These boxes were stored in a cold room (20°C) during the 18 and 30 day exposure periods. The autoradiographs were then developed in Kodak D-19 developer and, after clearing in Kodak acid fixer, were stained with hematoxylin.

The number of tritiated 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. Polymorphonuclear leukocytes were also excluded, since this cell type represented less than 1 per cent of the cells in transfer suspensions and was not found with a label.

RESULTS

A summary of the data is presented in Tables II and III. From numerous preliminary cell transfer experiments, it was found that the 24 hour skin test reaction yielded the optimal gross and microscopic lesions and hence we shall emphasize this time period in our descriptions.

The transfer of BCG-sensitized and labeled lymphoid cells from donor guinea pigs to homologous recipients resulted in positive skin reaction when the ears were tested with PPD and examined 24 hours later. As can be seen from Table II, there was no correlation between the number of cells transferred and the size of the gross reaction. More severe and indurated macroscopic reactions might have been attained by using larger doses of PPD. The double second strength dose of 10 μg used in these experiments was found to yield a good local infiltrate of mononuclear cells with minimal or absent necrosis and polymorphonuclear accumulation. Control sites, except those following transfusion of mixed labeled non-sensitized, and non-labeled sensitized cells, were usually negative in the gross 24 hours after testing or occasionally exhibited a papular lesion up to 5 mm in diameter.

Microscopically the specific 24 hour test site showed an inhomogeneous infiltration of cells and edema (Fig. 1). The cells were predominantly mononuclear and appeared in clusters or masses. They were to be found around blood vessels, both arterioles and venules rather than capillaries, around hair follicles, or in the dermis without apparent relationship to the vascular tree (Figs. 2 and 3). In some areas of the focal skin reaction only the evidence of edema or hemorrhage or both was observed and cells were sparse. The types of cells making up the infiltrate were small and large lymphocytes, immature mononuclear cells, some polymorphonuclear leukocytes, and a small number of unknown cells. It was apparent that the mesenchymal tissue of the host had been activated, since many fibrocytes were plump with rounded nuclei, and endothelial cells were thickened.

The most significant finding was the presence of labeled lymphoid cells in the skin test sites (Figs. 4 and 5). As shown in Table II, the number of labeled

cells ranged from 2.6 to 12.3 per cent of the unfixed mononuclear cells present in the dermis. In all control sites the number of labeled cells did not exceed 0.08 per cent of the infiltrating mononuclear elements. The labeled cells in the PPD reaction sites were mostly small or medium sized lymphocytes. No quantitative differential was seriously attempted, since it was too difficult in autoradiographic tissue sections to study accurately the cytology of labeled cells. The tritiated elements were found among the clusters or masses of mononuclear cells, in vessel walls, in vessel lumina, in the dermis, in hair follicles, and in the epidermis (Figs. 6 and 7). The grain counts of the tagged cells varied considerably and could not be correlated with cell size or type.

Six hours after PPD testing, the lesion at the reaction site was greater than 10 mm in diameter. Microscopically the test site was characterized by the presence of abundant edema and numerous polymorphonuclear leukocytes (Fig. 8). The proportion of segmented to mononuclear elements was about 2:1. At this time the number of tritiated cells did not exceed 1 per cent of the infiltrating mononuclear elements.

In one animal, No. 17, the reaction was permitted to persist for 60 hours after cell transfer and 54 hours after PPD skin testing. The gross lesion was 9 mm in diameter at that time, and the microscopic reaction was vigorous. There were varying proportions of unfixed mononuclear cells and neutrophils in different areas of the lesion. The percentage of labeled cells was within the range seen at 24 hours and greater than the percentage calculated at 6 hours.

Test sites that were removed 3 and 4 days after injection of labeled, activated lymphoid cells and 24 hours after PPD injection were smaller in the gross and their microscopic reactions less than those observed at earlier time intervals. In like fashion, the number and percentage of labeled cells present at the test site were also diminished or completely absent.

The three control guinea pigs (Nos. 20, 21, and 23) which received mixtures of labeled non-sensitized and non-labeled sensitized cells yielded PPD test sites which were macroscopically and microscopically equivalent to the reactions obtained in the experimental animals (Figs. 9 and 10). The number of labeled cells found in these lesions did not exceed 0.08 per cent of infiltrating cells despite large accumulations of mononuclear cells. As shown also in Table III, the test sites of all the other control animals revealed at most one or no labeled cells.

The disposition of labeled cells, other than in skin sites, was searched for in the blood and other organs. Despite the intravenous injection of fairly large numbers of cells, the tritiated elements were found with difficulty in the blood even as soon as 8 minutes after transfusion. The highest number of labeled cells was seen in a 2 hour blood sample and consisted of one clump of four and three separate labeled mononuclear elements among 500 mononuclear cells. Smears at all other time intervals yielded no more than two tritiated

cells per 500 mononuclear elements. In the lymphoid organs such as lymph nodes and spleen, labeled cells were more readily detected and could be found as late as the 6th day after transfer. No labeled cells could be found in the liver, kidney, and other non-lymphoid organs.

DISCUSSION

This study has demonstrated that the passive transfer of tuberculin sensitivity was accompanied by the specific localization of sensitized lymphoid cells at the site of antigen application. The appearance of the activated elements at the skin reaction site was apparently the result of a specific attraction between these cells and the PPD and was not the result of a non-specific accumulation of mononuclear elements in an area of inflammation. Non-sensitized tritiated cells did not accumulate in sites of inflammation induced by PPD, nor did sensitized labeled cells appear in infiltrates elicited by coccidioidin. The reports of numerous and successful passive transfers of tuberculin sensitivity by transfusion of activated cells had certainly implicated the essentiality of the sensitized cell for the achievement of this reaction (2, 5–11). One could not be certain, however, that the activated cell was a direct participant in this phenomenon. In humans, Lawrence (12) has demonstrated successful passive transfer of tuberculin sensitivity by administration of extracts of activated lymphoid cells. It has also been postulated (13) that the transferred sensitized cells might transmit their specific reactivity to host cells. Our experiments have not eliminated this latter possibility nor the possibility that a soluble product of the transferred cells might be responsible for the positive skin tests. Our results, however, have shown that the BCG-sensitized cells themselves form a part of the test site.

The cells that contain the specific message are apparently lymphoid. In the skin reaction sites the tritiated cells were morphologically small or large lymphocytes or immature forms of this cell type. Whether there exists a special category of lymphocytes which can be sensitized in this fashion is unknown. Wesslen (11) was able to elicit positive reactions to PPD by the injection of sensitized lymphocytes into the skin. He used thoracic duct contents which contained almost a pure population of small lymphocytes. Others (2, 5–9) have passively transferred tuberculin sensitivity with peritoneal exudate cells. In this latter instance it is impossible to determine whether the activated cells belong to the majority of macrophages or to the minority of other cell types usually present in such exudates.

The host also participated in the reaction, but it was not possible to determine with any accuracy the proportion of its contribution. Six hours after testing with PPD in an animal transfused with activated lymphoid cells, the predominant cell was the polymorphonuclear cell which constituted between 60 and 80 per cent of the infiltrating elements. In the suspensions to be trans-

fused, the polymorphonuclear leukocytes were 1 per cent or less of the total number. Therefore, if the segmented leukocytes in the skin test site were derived entirely from the transferred suspensions, their numbers in the dermis would represent a remarkable concentration of this type of cell. It seemed more likely that the presence of the polymorphs was due to the injected foreign material, the PPD. Similar polymorphonuclear reactions could be observed in control reactions to PPD without cell transfer (guinea pigs 24, 25, and 26). At 24 hours the predominant cell in the skin test site was the mononuclear cell. Of these, an average of about 7 or 8 per cent were labeled with tritium, whereas in the suspensions about 15 to 20 per cent of the lymphoid cells were labeled. Assuming that the recipient host treated tritiated and non-tritiated cells alike, then the decrease in percentage of labeled cells at the test site represented a dilution by host lymphoid elements. It was also readily observed that the fixed mesenchymal tissue in the dermal lesion exhibited swelling and rounding up of fibrocytes and histiocytes, and endothelial cell thickening. Inasmuch as these evidences of host response were lacking at 24 hours in guinea pigs that received non-sensitized cells and PPD or coccidioidin, we concluded that the cellular contributions by the host animal in positive test sites were responses to the inflammation resulting from reaction of transferred sensitized cells and PPD.

The passive transfer of tuberculin sensitivity was effective for 3 days but not beyond this time. Most probably this was due to rejection of the transferred cells by the homologous host so that too few or no sensitized cells remained to support a positive skin test. Examination of the lymphoid organs also revealed the presence of fewer and fewer labeled cells with increasing time. The maximum duration of labeled cells in the spleen was 6 days in guinea pig 6. It is interesting to compare our results with those of Bauer and Stone (14). These latter authors studied the passive transfer of tuberculin sensitivity in isologous guinea pigs. They found that positive skin tests developed some 8 to 10 days after transfer and could be obtained throughout a period of several weeks. Their data would indicate that the total number of cells with specific reactivity was insufficient at first and later increased by proliferation to support a positive skin test. An effective level of activated cells was then maintained by continued reproduction for 20 to 30 days in the isologous host, as evidenced by significantly positive skin reactions to PPD until the 4th post-transfer week.

The morphologic observations recorded here permit a possible construction of the sequence of events which occurred in the homologous host following transfer of sensitized cells. Almost immediately after transfusion of leukocytes, the circulating blood is cleared of the foreign cells. These cells were apparently trapped in the lung, as described by others (15, 16), or in other capillary beds (17), or perhaps in bone marrow and lymphoid organs (18–21). Many of the

foreign cells were probably destroyed quickly. Following the mass removal of the homologous cells from the circulation there was a slow cell by cell return of injected elements back into the circulation. These tended to accumulate at the site of specific antigen injection over a period of 15 to 20 hours, thus providing a test lesion. What happened subsequently to the activated cell at the skin test site is unknown. In one guinea pig, the postauricular node draining the PPD reaction was removed after 24 hours and was found to contain numerous labeled lymphoid elements, 3 to 5 times as many as were present in lymph nodes elsewhere in the body. Hence, the activated mononuclear cells might have escaped from the test site to the regional lymph nodes and recirculated in the blood. They may also have died at the scene of conflict where their label was then lost and their burial place unrecorded.

In the actively immunized animal, one might visualize a similar process. The lymphoid reservoirs constantly feed small numbers of specifically sensitized cells into the circulation. These cells reach all parts of the body and, in the presence of specific antigen, would be specifically attracted to the exciting agent and would produce an inflammation.

Finally, this investigation has provoked several questions which are unanswerable at the present time. What is the specific attraction between antigen and activated cells that causes them to leave the blood stream and concentrate in the mesenchymal tissue? Does the antigen seek out the cell or *vice versa*? At what level in cellular development does a cell acquire its specific reactivity? In positive skin test sites a small number of labeled cells were stem or immature forms. These undifferentiated cells had accumulated specifically at this site and presumably contained the specific reactivity of tuberculin sensitivity. Such cells were never found at control sites of non-specific inflammation. Finally, is the delayed reaction of passively transferred tuberculin sensitivity the same as, or related to, the passively transferred homograft rejection phenomenon (22)? In isologous strains of mice (23), accelerated rejection of homografts were effected by the transfer of previously activated lymphoid cells. However, when tritiated activated cells were used for passive transfer, labeled cells were rarely found in the homograft rejection bed. These data would indicate differences between the behavior of lymphoid cells activated by bacterial protein and protein complexes and those activated by foreign grafts.

SUMMARY

Passive transfer of tritiated thymidine-labeled BCG sensitized lymphoid cells into homologous guinea pigs resulted in positive tuberculin skin reactions 24 hours after testing with PPD. Labeled cells were found specifically attracted to these sites. Labeled non-sensitized lymphoid cells did not appear at PPD injection sites, nor did labeled sensitized cells accumulate in non-specific inflammatory lesions. The specifically reacting tritiated cells were small,

medium, and large lymphocytes and stem or immature cells of the lymphoid series. In the homologous system employed, positive skin tests were either minimal or absent 3 days after transfusion of activated cells. The injected labeled sensitized cells were rapidly cleared from the circulating blood and lodged in the lungs, spleen, and lymph nodes. Upon application of specific antigen they reappeared at the skin test site at 6 hours and then increased in number for the next 18 hours.

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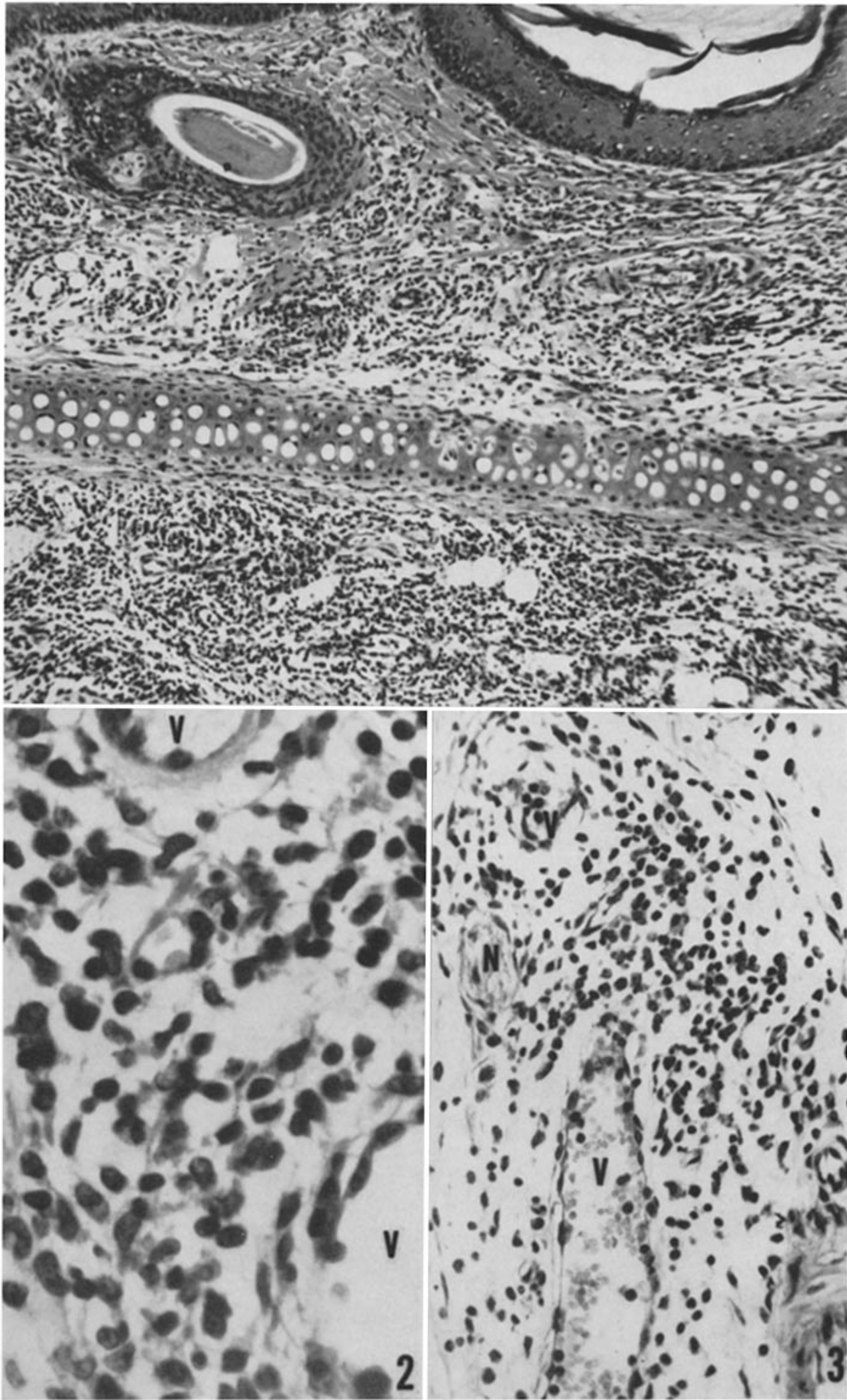
EXPLANATION OF PLATES

Plate 78

Fig. 1. Photomicrograph of 24 hour PPD ear test site from a guinea pig which received BCG-sensitized lymphoid cells. The lesion extends from surface to surface of the ear and consists predominantly of mononuclear cells. Compare with Fig. 9. ×180.

Fig. 2. Photomicrograph of 24 hour PPD ear test site from a guinea pig transfused with BCG sensitized lymphoid cells. The inflammatory cells are clustered about and between two vessels (*V*), larger than capillaries. At this magnification the majority of unfixed cells appear to be small and large lymphocytes. ×400.

Fig. 3. Photomicrograph of 24 hour PPD ear test site from a guinea pig transfused with BCG-sensitized cells. The infiltrating cells are disposed about a nerve (*N*) and several vessels (*V*) forming a loose nodule in the dermis. ×270.



(Najarian and Feldman: Passive transfer of tuberculin sensitivity)

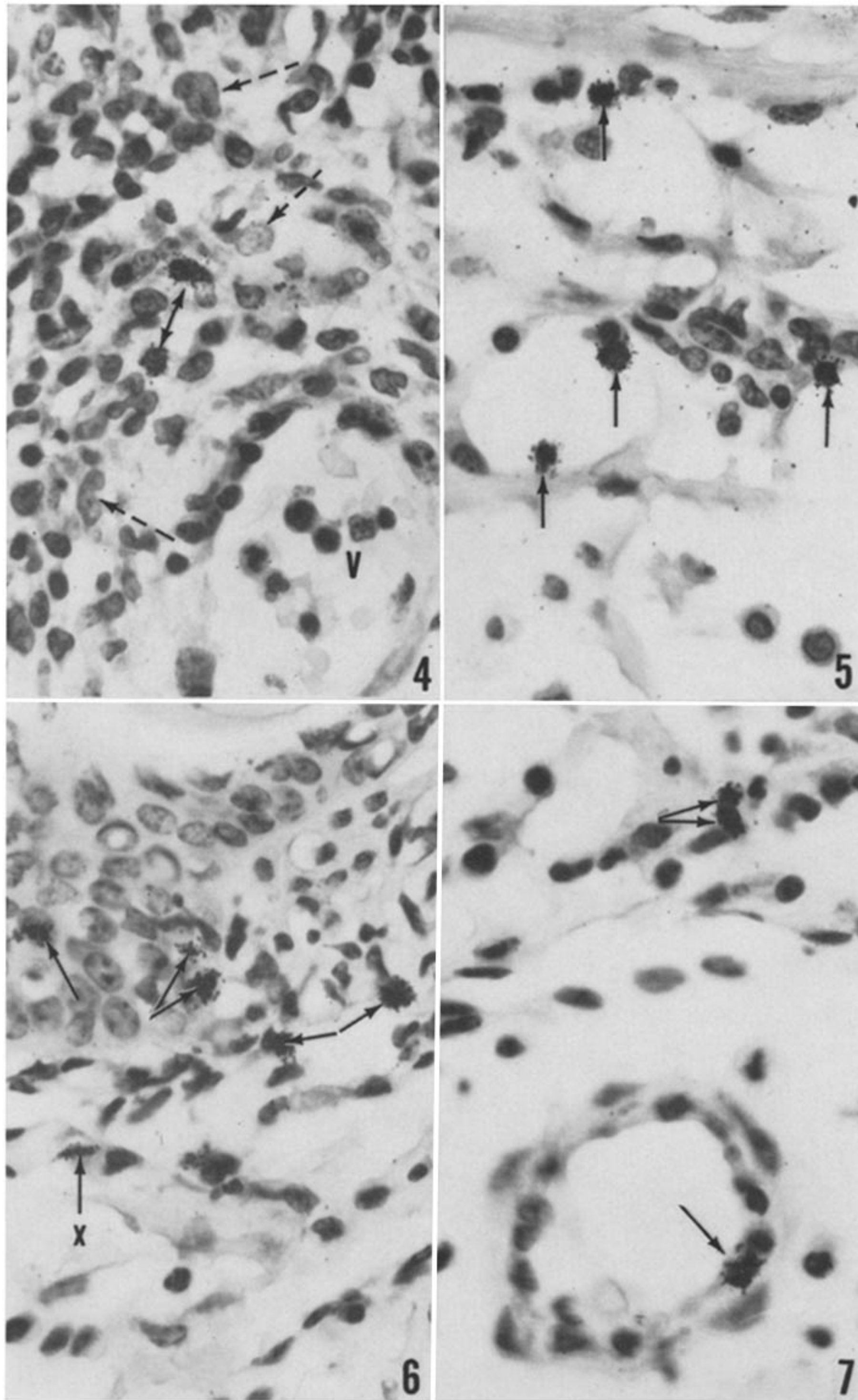
Plate 79

Fig. 4. Photomicrograph of an autoradiograph from a 24 hour PPD ear test site in an experimental animal. A number of mononuclear cells are aggregated close to a vessel (*V*). Grains are visible over two lymphocytes (arrows). Some of the cells in the aggregate are fixed host elements which have rounded plump nuclei (dotted arrows). ×630.

Fig. 5. Photomicrograph of an autoradiograph from a 24 hour positive PPD ear test site. Four labeled cells are present in the connective tissues of the dermis (arrows). ×630.

Fig. 6. Photomicrograph of an autoradiograph from a 24 hour positive PPD ear test site. Several labeled cells (arrows) infiltrate the base of a hair follicle. One labeled cell (*X*) shows an elongate nucleus resembling that of a fibrocyte. ×630.

Fig. 7. Photomicrograph of an autoradiograph from a 24 hour positive PPD ear test site. One lymphocyte with grains over it is in wall of vessel (arrow). Two small lymphocytes (arrows) are located in the connective tissues among fixed and unfixed cells. ×630.



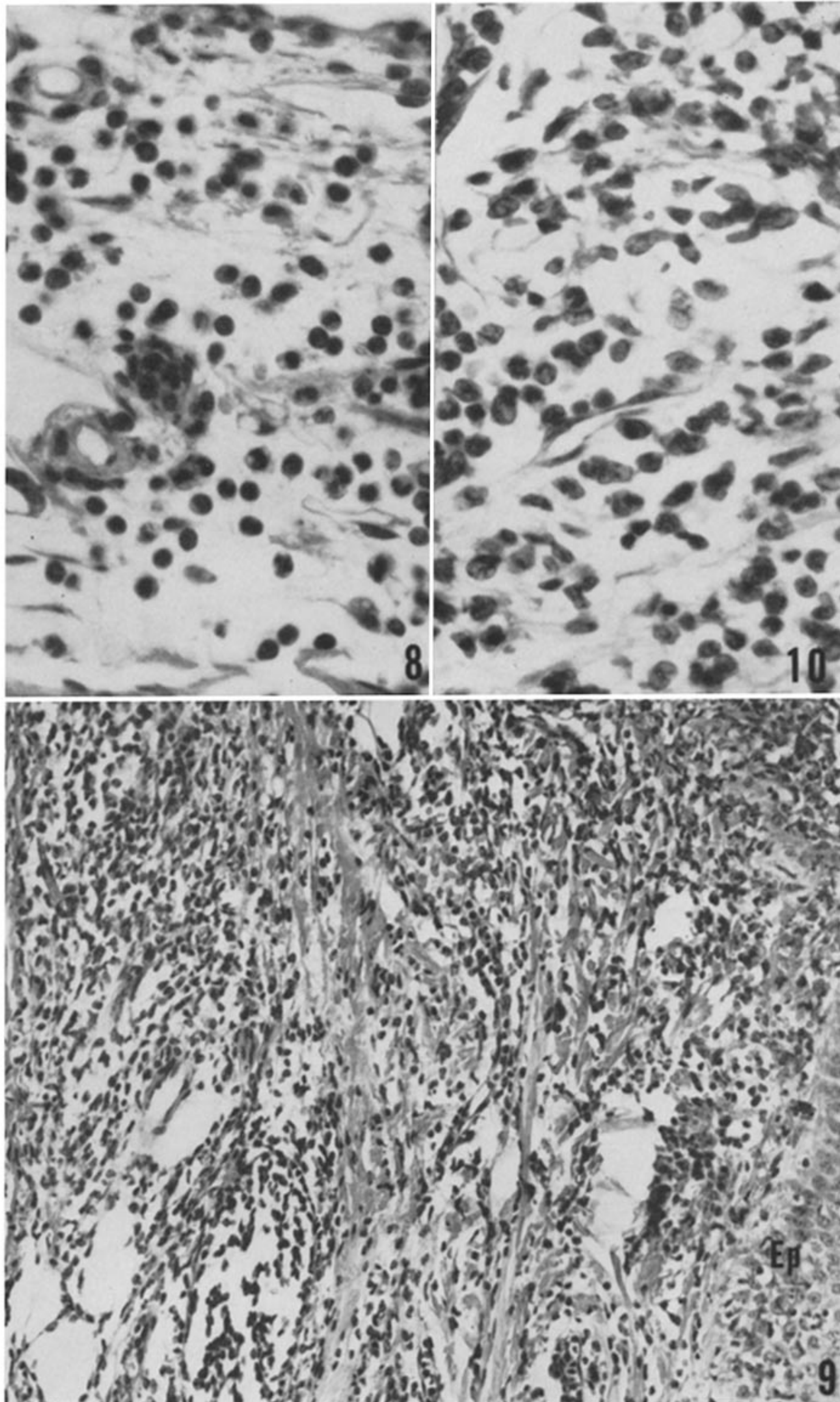
(Najarian and Feldman: Passive transfer of tuberculin sensitivity)

Plate 80

Fig. 8. Photomicrograph of a 6 hour positive PPD ear test site. The lesion consists of both edema and cells. The cells in majority are polymorphonuclear leukocytes. ×430.

Fig. 9. Photomicrograph of a 24 hour PPD ear test site in a control guinea pig. The animal received a mixture of non-labeled sensitized and labeled non-sensitized lymphoid cells. The reaction to PPD is similar in extent and in cellularity to the reactions of experimental guinea pigs. Only two labeled cells were found in this lesion. Compare with Fig. 1. ×200. *Ep* = epidermis.

Fig. 10. A higher magnification of a reaction site from an animal prepared as above. Most of the unfixed cells are lymphocytes, from small to large. A number of cells with large round pale nuclei are derived from the host's local tissues. ×430.



(Najarian and Feldman: Passive transfer of tuberculin sensitivity)