SPECIFICITY OF PASSIVELY TRANSFERRED DELAYED HYPERSENSITIVITY*, ‡

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The passive transfer of delayed hypersensitivity by sensitized cells, as originally described by Landsteiner and Chase (1) and by Chase (2), has provided an excellent model to study the cellular events in the site of the specific reaction. Until the advent of H_3-thymidine, however, the donor and host components of the cellular infiltrate could not be certainly separated. With this isotope, it was possible to mark the donor cells permanently so that they could be followed, identified in the recipient, and distinguished from host cells (3).

In recent reports on the passive transfer of delayed hypersensitivity using H_3-thymidine–labeled lymphoid cells, it has been suggested that specificity of reaction was lacking, *i.e.* that the accumulation of donor-labeled cells at the skin test site was no greater than at control sites and that the "stickiness" of sensitized cells caused the transferred elements to congregate at any locus of injury (4–6). This problem was examined by passively transferring into a single recipient guinea pig cells sensitized to tubercle bacilli and cells sensitized to a simple chemical, dinitrofluorobenzene (DNFB), and by examining the skin sites tested with PPD and DNFB. The results have indicated that, despite the small numbers of cells involved, there was specificity to each type of reaction.

Materials and Methods

Design of Experiment.—Homologous untreated male guinea pigs were recipients of cells sensitized to tubercle bacilli (TBC cells) and of cells sensitized to DNFB (DNFB cells). In any one experiment, the TBC cells were labeled with H_3-thymidine (TBC cells*) or the DNFB cells were marked with the isotope (DNFB cells*). 24 hours after skin testing with PPD and DNFB in two different loci, the two lesions were removed for histologic and autoradiographic analysis, and for determination of total quantity of isotope present within the entire test sites.

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Donor Guinea Pigs.—Tuberculin sensitization was accomplished by injecting a total of 0.3 mg of tubercle bacilli[1] in a water-and-oil emulsion into the foot-pads and the nape of the neck of stock guinea pigs, each site receiving 0.2 ml. The antigenic materal was prepared by emulsing 1 part of saline (1 ml contained 0.6 mg of dead tubercle bacilli) with 0.15 part of arlacel C and 0.85 part of bayol F. 5 weeks later, the animals were skin tested with 0.2 μg PPD, and all guinea pigs showing a skin reaction at 24 hours of 15 mm in diameter or more, and central necrosis, were selected to be donors (about 75 per cent). H_3-thymidine, 0.3 μc/gm of body weight (specific activity 2 mc/mmole), was injected 2 days later into the selected donors every 12 hours for 9 injections. On the second day of isotope labeling, each donor received an enhancing dose of 0.2 μg of PPD in the right ear which was removed 24 hours later.

DNFB[2] sensitization of guinea pigs was accomplished by injecting a total of 50 μg of DNFB in adjuvant into the four foot-pads and the nape of the neck, each site receiving 0.2 ml. The antigen was prepared by emulsing 1 part of saline (1 ml contained 100 μg of DNFB dissolved in absolute alcohol) with 0.15 part of arlacel C and 0.85 part of bayol F. 10 mg of *Shigella*[3] was added to each ml of the materials to be emulsified. To enhance sensitization, 0.25 per cent DNFB in corn oil and acetone (1:2) was gently rubbed over a 25 mm circular area of flank skin with a glass applicator 1 week after the initial injections. To estimate sensitivity, a similar skin test on the opposite flank was done 2 weeks after beginning sensitization to DNFB. Only 4+ reactors (about 60 per cent) were selected for donors and these were labeled with H_3-thymidine according to the schedule and dosage described above.

Two other groups of guinea pigs were sensitized to DNFB in two ways. In one group 1 per cent DNFB dissolved in 1 part acetone and 1 part corn oil was painted over alternate shaved flanks 3 times weekly for 6 weeks. The regional lymph nodes were stimulated by injecting 0.3 ml of a 50 per cent suspension of sheep red cell stromata into each foot-pad 6 weeks after initiation of the sensitization procedure. 2 weeks later, the animals were labeled with H_3-thymidine as described before. A second group received a total of 50 μg DNFB in a water-and-oil emulsion, 0.2 ml in each foot-pad and 0.2 ml in the nape of the neck. 3 weeks later, after an enhancing DNFB skin test (see above), the best reactors were labeled with H_3-thymidine as described. Neither of these two methods of sensitization provided a good transfer of hypersensitivity. The recipient guinea pigs showed usually 1 to 2+ skin test responses and occasionally a 3+. Hence, these means of sensitization were abandoned in favor of the method using *Shigella* and DNFB in a water-and-oil emulsion.

Preparation of Sensitized Cells for Transfer.—The donor guinea pigs were killed 6 hours after the last injection of H_3-thymidine. The spleens and regional lymph nodes (axillary, cervical, inguinal, popliteal) were removed, cleaned of adventitial tissue, and raked in Hanks' solution containing 20 per cent polyvinylpyrrolidone (PVP) and 3 units of heparin per ml. Separate suspensions of spleen and lymph node cells were passed through a 100 mesh nylon gauze, centrifuged at 1000 RPM, and resuspended in Hanks'-PVP-heparin medium so that each 1 ml contained 1×10^8 lymphoid cells. Aliquots of the suspensions were taken to count total number of cells, to determine the percentage of labeled cells by autoradiography, and to determine by a combustion method (7) the total quantity of radioactivity injected. TBC cells and DNFB cells were injected intravenously into homologous recipients, 400 to 600 gm in body weight (see Tables I and II for number of cells, per cent label in transfer, and total radioactivity injected).

Examination of Test Sites.—Immediately after cell transfer, each recipient was injected in his right ear with 10 μg PPD, and received 2 drops of 0.25 per cent DNFB in corn oil and acetone (1:2) gently rubbed over a 25 mm circle of flank skin cleared of hair. After 24 hours,

[1] Dried, non-viable *Mycobacterium tuberculosis*, Lot 04784, Parke, Davis & Co., Detroit.

[2] Fluoro-2,4-dinitrobenzene, Eastman Organic Chemicals, Rochester, New York.

[3] Lyophilized *Shigella flexneri*, type 3, prepared by Dr. W. O. Weigle.

the two lesions were measured. For recovery of total isotope, each gross lesion was carefully cut at its periphery, weighed, and then prepared for combustion and counting as described elsewhere (7). To permit comparison of the two types of lesions, independent of lesion weight and of dose of radioactivity injected, results were expressed as c/m/gm of skin, per cent of dose. In addition, since the PPD and DNFB lesions were elicited by intradermal injection and by contact application, respectively, and since the DNFB test sites were generally larger than the PPD test sites, results were also expressed simply as c/m, per cent of dose, thus permitting a comparison of total radioactivity in the lesions.

For autoradiographic studies, the gross lesions were removed, cut into 3 to 5 parallel strips, and processed for histology and autoradiography (3). The histology of the PPD lesion was different from that of the DNFB lesion and a strict comparison was not possible. The data, therefore, were collected in several ways. In the first way, the total number of labeled cells per cross-section of lesion was recorded and then averaged for each lesion (see columns 2 and 10 of Table III). This was equivalent to estimating total radioactivity per lesion but did not take into account the density of cellular infiltrate. A second approach estimated concentration of donor cells; *i.e.*, the proportion of labeled cells to total infiltrating mononuclear elements (see columns 5 and 13 of Table III). It was obvious, however, that this type of comparison, labeled cells/total infiltrating mononuclear cells, overlooked the histologic differences of the two lesions. The PPD test reaction was characterized by perivenular and perineural clusters of mononuclear cells; between these clusters there was a relatively sparse dispersion of mononuclear elements. In autoradiographs, there was a greater density of labeled cells in the perivenular and perineural clusters than elsewhere. The DNFB test reaction was characterized by a diffuse infiltration of mononuclear cells in the upper dermis, the epidermis, and the hair follicles of the upper dermis. There was no clustering effect. In autoradiographs, labeled cells were distributed in the infiltrate just below the epidermis, in the epidermis, and in hair follicles.

To obviate these histologic differences, the proportion of labeled cells was recorded in a perivenular or perineural cluster of mononuclear cells framed by an ocular grid 0.25 mm^2 in area. Similarly, the grid was used to outline a DNFB infiltrate wherever labeled cells were seen. In this fashion, the concentrations of tagged cells in a unit area of the most characteristic parts of the lesions were compared (see columns 4 and 12, Table III).

Passive Transfer of DNFB Cells into Guinea Pigs Actively Sensitized to Tubercle Bacilli.*—Eight guinea pigs were sensitized to tubercle bacilli as described above for donor animals. 6 weeks later, they were injected intravenously with 8 to 15 $\times$ 10^8 lymphoid cells from either spleen or lymph nodes of guinea pigs sensitized to DNFB (see Table IV). Immediately thereafter, each recipient was tested in its right ear with 0.2μg of PPD and on its clipped flank with 2 drops of 0.25 per cent DNFB in corn oil and acetone. 24 hours later, the two lesions were cut at their peripheries, weighed, and prepared for determination of total isotope by combustion.

RESULTS

I. Passive Transfer of TBC Cells and DNFB Cells.*—In Tables I and II, part A, the radioactivity of the test skin lesions is recorded 24 hours after transfer of TBC cells* and DNFB cells, and after application of PPD and DNFB to two sites. Both the total radioactivity per lesion and the concentration of isotope were significantly greater in the PPD skin reactions than in the DNFB sites. In Table III, part A, are presented the autoradiographic data. It is apparent that in the PPD test sites, the total number of labeled cells, their concentration per total infiltrate of section, and their concentration in perivenular

and perineural clusters were greater than in the DNFB lesions. It should be noted that in PPD lesions the concentration of labeled cells in a 0.25 mm^2 area of perivenular infiltrating mononuclear elements was over 2 times greater when TBC cells* were used than when DNFB cells* were transfused (see Table III).

TABLE I

Total Radioactivity in Skin Lesions 24 Hours after Transfer

C/m, per cent of dose.‡

Guinea pig No.	Cell transfer				DNFB sites		PPD sites	
	c/m $\times 10^3$	TBC cells $\times 10^6$	DNFB cells $\times 10^6$	Per cent label	Wet weight of lesion	c/m per cent dose	Wet weight of lesion	c/m per cent dose
A. TBC cells§ and DNFB cells*‖								
					mg		*mg*	
1–2	440	10	6	22	366	0.005	388	0.095
1–3	177	4	6	22	354	0.000	286	0.069
1–4	441	10	7	26	424	0.000	397	0.102
1–5	441	10	5	26	269	0.009	252	0.033
1–6	406	10	6	16	383	0.017	313	0.108
1–7	464	10	6	23	288	0.008	104	0.016
Mean					347	0.007	290	0.071
B. DNFB cells¶ and TBC cells*‡‡								
6–5	320	7	12	14	382	0.313	406	0.011
6–6	320	7	12	14	333	0.168	430	0.003
6–7	230	8	6.7	26	582	0.315	384	0.019
6–8	230	8	6.7	26	256	0.144	262	0.002
Mean					388	0.235	370	0.009

‡ Calculated by subtracting radioactivity in equivalent weights of flank skin from that in skin lesions.

§ Sensitized to tubercle bacilli and labeled with H_3-thymidine.

‖ Sensitized to DNFB, no label.

¶ Sensitized to DNFB and labeled with H_3-thymidine.

‡‡ Sensitized to TBC, no label.

Figs. 1 and 2 illustrate the microscopic cellular infiltrate characteristic of tuberculin sensitivity. Fig. 3 demonstrates the accumulation of labeled donor cells in perivenular and perineural clusters when TBC cells* and DNFB cells were transfused. The proportion of labeled cells to infiltrating mononuclear cells is high. Fig. 4 is an autoradiograph of a dermal cluster of mononuclear cells in a PPD test site. The proportion of labeled donor cells to total mononuclear

cell infiltrate is low. This latter figure represents the greatest concentration of labeled elements found when DNFB cells* and TBC cells were transferred.

Based on the total radioactivity, the number of cells, and the per cent label transferred, on the radioactivity in the lesion and the autoradiographic data,

TABLE II

Total Radioactivity in Skin Sites 24 Hours after Transfer

C/m/gm of skin, per cent of dose.

Guinea pig No.	Skin sites			Skin sites corrected‖	
	Untreated flank	DNFB	PPD	DNFB	PPD
A. TBC cells‡ and DNFB cells§*					
1–2	0.06	0.07	0.30	0.01	0.24
1–3	0.11	0.05	0.35	0.00	0.24
1–4	0.13	0.01	0.39	0.00	0.26
1–5	0.02	0.05	0.15	0.03	0.13
1–6	0.04	0.09	0.39	0.05	0.35
1–7	0.02	0.05	0.17	0.03	0.15
Mean				0.02	0.23
B. DNFB cells¶ and TBC cells‡‡*					
6–5	0.07	0.89	0.10	0.82	0.03
6–6	0.03	0.53	0.04	0.50	0.01
6–7	0.00	0.54	0.05	0.54	0.05
6–8	0.01	0.58	0.02	0.57	0.01
Mean				0.61	0.02

‡ Sensitized to tubercle bacilli and labeled with H_3-thymidine.

§ Sensitized to DNFB, no label.

‖ Calculated by subtracting the radioactivity in equivalent weights of flank skin from that in skin lesions.

¶ Sensitized to DNFB and labeled with H_3-thymidine.

‡‡ Sensitized to tubercle bacilli, no label.

the number of donor and host cells in the specific and non-specific lesions was calculated. A mean of 6.5×10^5 donor cells was present 24 hours after initiation of the specific PPD lesion and this represented about 18 per cent of the mononuclear elements infiltrating the test site at this time. The host contributed better than 80 per cent of the mobile cellular mass. The control DNFB lesion contained 0.7×10^5 mononuclear cells at 24 hours. This latter figure was, therefore, a measure of the number of donor labeled cells which accumulated at a

TABLE III

Autoradiographic Data in 24-Hour Skin Test Sites

Guinea pig No.	PPD site					No. of cells transferred			DNFB site				
	Avg. No. of mononuclear cells/section		Per cent labeled cells in						Avg. No. of mononuclear cells/section		Per cent labeled cells in		
	Labeled	Total infiltrate	0.25 mm²‖ clusters	Total infiltrate	Epidermis and hair follicles	TBC cells × 10^8	Per cent label	DNFB cells × 10^8	Labeled	Total infiltrate	0.25 mm²‖	Total infiltrate	Epidermis and hair follicles
A. Transfer of TBC cells ‡ and DNFB cells§*													
5	60 (5)¶	8460	3.3	0.7	5	8	13.8	6	33 (5)	4961	1.2	0.6	10
6	82 (4)	9020	3.0	0.9	9	5	13.5	7	16 (3)	2480	1.8	0.6	14
7	83 (4)	8798	2.5	0.9	12	5	16.2	4	36 (4)	5863	1.5	0.6	16
8	27 (4)	4954	2.4	0.5	10	7	17.4	6	9 (5)	2727	1.1	0.3	11
9	Poor histologic reaction								Poor histologic reaction				
Mean	63	7808	2.8 ± 0.21‡‡	0.8 ± 0.10	9	6.2	15.2	5.7	27	4008	1.4 ± 0.16‡‡	0.5 % 0.08	13
B. Transfer of DNFB cells §§ and TBC cells‖ ‖*													
1-0	10 (4)	3575	1.4	0.5	0	8	30.2	12	27 (4)	3230	2.9	0.8	38
1-1	28 (2)	4950	2.0	0.5	10	9	29.4	9	47 (4)	2228	3.8	2.1	30
1-2	9 (2)	5389	0.5	0.2	0	8	29.4	9	18 (4)	2439	3.2	0.7	35
1-3	20 (3)	8268	1.7	0.3	7	7.5	24.0	12.5	53 (3)	3721	2.7	1.4	36
1-4	11 (3)	5562	1.2	0.2	21	7.5	24.0	12.5	20 (3)	3900	2.4	0.5	24
1-5	28 (2)	9770	1.2	0.3	0	8.5	26.4	6	84 (3)	3366	3.8	2.5	56
1-6	5 (3)	2160	2.0	0.2	0	8.5	26.4	6	30 (3)	4371	3.0	0.7	28
1-7	8 (3)	6882	—¶¶	0.1	0	7.0	35.5	8.5	19 (3)	3250	2.7	0.6	21
1-8	4 (4)	2808	—¶¶	0.1	0	7.0	35.5	8.5	27 (3)	3109	3.5	0.9	18
1-9	6 (3)	1998	1.1	0.3	12	7.5	43.0	9	70 (4)	4352	4.0	1.6	62
2-0	7 (2)	2547	0.8	0.3	0	7.5	43.0	7.5	53 (2)	3834	2.8	1.4	37
Mean	12	4901	1.3 ± 0.17‡‡	0.3 ± 0.04‡‡	4.5	8.0	31.5	9.0	41	3436	3.2 ± 0.15‡‡	1.2 ± 0.19‡‡	35

‡ Sensitized to tubercle bacilli and labeled with H_3-thymidine. § Sensitized to DNFB, no label. ‖ See text for explanation. ¶ Figures in parentheses indicate number of autoradiographs examined. ‡‡ Significantly different from other lesion at 1 per cent level. §§ Sensitized to DNFB and labeled with H_3-thymidine. ‖‖ sensitized to tubercle bacilli, no label. ¶¶ No labeled cells found in perivenular or perineural clusters.

site of injury without specificity. If subtracted from the number of donor cells in the specific reaction, the resultant figure of some 6×10^5 cells might have represented the number of cells carrying the specific message, which was a very small fraction of the cells transferred.

II. Passive Transfer of DNFB Cells and TBC Cells.*—The reciprocal observations are shown in Tables I to III, part B. Under these circumstances, in which DNFB cells* and TBC cells were transferred to a homologous host, the results were the opposite of those obtained when TBC cells* and DNFB

TABLE IV

Total Radioactivity in Skin Lesions 24 Hours after Transfer of DNFB Cells‡ to Tuberculin-Sensitized Guinea Pigs*

C/m, per cent of dose.§

Guinea pig No.	Cell transfer			PPD sites		DNFB sites	
	c/m $\times 10^3$	DNFB cells* $\times 10^8$	Per cent label	Wet weight of lesion	c/m per cent dose	Wet weight of lesion	c/m per cent dose
				mg		*mg*	
6–9	207	15	12	294	0.003	818	0.088
7–0	207	15	12	408	0.011	344	0.046
7–1	96	9.5	15	462	0.021	498	0.123
7–2	96	9.5	15	207	0.000	384	0.146
7–3	340	10	23	248	0.067	255	0.055
7–4	340	10	23	216	0.024	292	0.118
7–5	71	8	23	317	0.012	218	0.132
7–6	71	8	23	402	0.118	462	0.467
Mean				319	0.032	409	0.147

‡ Sensitized to DNFB and labeled with H_3-thymidine.

§ Calculated by subtracting the radioactivity in equivalent weights of flank skin from that in skin lesions.

cells were transferred. The concentration of isotope and the total radioactivity per lesion were this time significantly greater in the DNFB reactions than in the PPD lesions. And the autoradiographic observations also showed that the total number of H_3-tagged cells per section, their concentration in 0.25 mm^2, and their concentration in the entire mononuclear cell infiltrate per section were also significantly greater in the DNFB test sites than in the PPD reactions. It should be noted that 35 per cent of the labeled cells was seen in the epidermis and hair follicles in the DNFB lesion when DNFB cells* were used and only 13 per cent when TBC cells* were transfused.

Figs. 5 and 6 illustrate, at two levels of magnification, the DNFB lesion, which was obviously different from the PPD lesion. Figs. 7 and 8 show the prominent distribution of labeled cells in the epidermis and around hair follicles

in a DNFB test site. In Fig. 7, when DNFB cells* and TBC cells were transfused, the number and proportion of labeled cells to total mononuclear infiltrate are high. In Fig. 8, when TBC cells* and DNFB cells were transfused, the number and proportion of labeled cells to total mobile cell mass are low.

Calculations of the number of donor and host cells in the specific DNFB and control PPD lesions revealed that a mean of 2.2×10^6 donor cells was present at 24 hours in the specific reaction and less than 0.1×10^6 cells was present in the control site. The number of donor cells again was a very small fraction

TABLE V

Total Radioactivity in Skin Sites 24 Hours after Transfer of DNFB Cells‡ to Tuberculin-Sensitized Guinea Pigs*

C/m/gm of skin, per cent of dose.

Guinea pig No.	Skin sites			Skin sites corrected§	
	Untreated flank	PPD	DNFB	PPD	DNFB
6–9	0.00	0.01	0.11	0.01	0.11
7–0	0.03	0.05	0.16	0.02	0.13
7–1	0.00	0.04	0.25	0.04	0.25
7–2	0.01	0.01	0.38	0.00	0.37
7–3	0.03	0.30	0.24	0.27	0.21
7–4	0.04	0.14	0.44	0.10	0.40
7–5	0.00	0.04	0.61	0.04	0.61
7–6	0.00	0.29	1.01	0.29	1.01
Mean				0.10	0.39

‡ Sensitized to DNFB and labeled with H_3-thymidine.

§ Calculated by subtracting the radioactivity in equivalent weights of flank skin from that in skin lesions.

of the total number transfused and represented less than 10 per cent of all the infiltrating mononuclear cells in the lesion.

III. Transfer of DNFB Cells to TBC-Sensitized Guinea Pigs.*—The data of this experiment are presented in Tables IV and V. When DNFB cells* were transferred to TBC-sensitized guinea pigs and PPD and DNFB were applied to skin sites, more radioactivity, *i.e.* cells, arrived at the specific DNFB lesion than at the PPD lesion, with one exception. In guinea pig 7–3, for unknown reasons, there was more radioactivity in the PPD site than in the DNFB lesion. These data were confirmatory of those obtained by passively transferring two kinds of sensitized cells.

DISCUSSION

The original problem posed a simple question: Do sensitized H_3-labeled lymphoid cells accumulate at the site of antigen deposition as the result of a

specific interaction or because of non-specific "stickiness"? The observations of these experiments have clearly demonstrated a specificity of response. After transfer of TBC cells* and DNFB cells to a homologous guinea pig the number and concentration of labeled elements in the 24 hour test skin sites was greater in the PPD lesion than in the DNFB lesion, when calculated from the total radioactivity in the lesions or from the autoradiographic data. After transfer of DNFB cells* and TBC cells, the number and concentration of labeled elements was greater in the DNFB lesion, by the two methods of examination.

The recovery of radioactivity from the whole lesion was a measure of the total donor contribution. With this method it was possible to calculate the number of donor cells in the entire lesion or their concentration per gram of skin tissue. By either calculation, the absolute number of donor cells was significantly higher in the specific lesion. It was apparent that size or weight of lesion did not influence the results.

However, a measure of total radioactivity within the test sites was not a measure of the total cellular infiltrate, but only of donor elements. If sensitized cells were unusually sticky, it might be postulated that the greater the number of mononuclear cells which arrived at the test site, the greater would be the number of tagged donor cells among them. By autoradiography, it was possible to separate the donor and host mononuclear cells within the lesion. With this method, of course, the total lesion was not examined, but only random samples as represented by the sections taken from the lesions. Again, the data (Table III) clearly disclosed that the total number of donor cells per section, and also the concentration of donor cells, were greater in the appropriate test site.

An additional telling observation was revealed in the microscopic distribution of the labeled cells. When TBC cells* were used in the transfer, the tagged elements appeared in relatively high proportions in the perivenular and perineural clusters of mononuclear cells (2.8 per cent), but were less than half this concentration in similar areas of the PPD lesions when DNFB cells* were transfused (1.3 per cent). In the other direction, when DNFB cells* were transfused to recipients, 35 per cent of the labeled cells was in the epidermis and hair follicles, but only 13 per cent was found in epidermis and hair follicles when TBC cells* were transferred.

There was evidence, nonetheless, that sensitized lymphoid cells were "sticky." In other experiments (3), non-sensitized labeled lymphoid cells were transferred to guinea pigs which were tested with PPD. The accumulation of tagged elements at a PPD test site was less than the lowest number found in PPD test sites when DNFB cells* were transfused by at least a factor of 2. Stated differently, more sensitized (or inflamed) lymphoid cells arrived at or remained in a non-specific focus of injury than did non-sensitized cells.

The density of cellular infiltrate did not seem to affect the accumulation of labeled donor cells. In general, there were more mononuclear elements per section in PPD lesions than in DNFB lesions. This might have been due to the

fact that the DNFB lesion was larger than the PPD lesion and hence the infiltrating cells were dispersed over a greater area. Nevertheless, when DNFB cells* were transferred, fewer labeled cells appeared in the PPD reactions despite the greater number of infiltrating mononuclear elements in this lesion. If simple non-specific "stickiness" were the only factor in determining the presence of labeled donor cells, then more should have appeared in the PPD test sites.

On the other hand, to obtain a considerable number of labeled cells at the specific test site, a high order of delayed hypersensitivity must be transferred. This was a difficult, if not impossible, factor to measure quantitatively. It was observed, however, that in donor guinea pigs, sensitized to tubercle bacilli, a skin test with 0.2 μg of PPD should have been at least 15 mm or more in diameter and should also have exhibited central necrosis. In recipients with test lesions less than 10 mm in diameter, using 10 μg of PPD, the microscopic infiltrate was sparse and the number of labeled cells was too small for accurate study. Similarly, with DNFB sensitization, only 4+ donors were capable of transferring adequately delayed hypersensitivity and even these failed unless adjuvant was incorporated into the hapten–water-and-oil emulsion. Tubercle bacilli were by far the best adjuvants. Passive transfer from donors sensitized with DNFB in complete Freund's adjuvant was most successful and recipients from these donors showed the most extensive cellular infiltrate and the greatest number of labeled cells (8). Since mycobacteria could not be used in developing chemical sensitivity to test its specificity against tuberculin sensitivity, *Shigella* was used, and though effective, it was not so effective as mycobacteria. DNFB in simple water-and-oil emulsion or with red cell stromata incorporated into the emulsion did not sensitize donors sufficiently to achieve a good passive transfer.

Even when the transfer of delayed hypersensitivity was highly successful, as measured by macro- and microscopic reactions, the total number of donor cells within the lesion was a minor component of all the infiltrating mononuclear elements and was a very small fraction of the cells transferred. By calculation, the donor cells ranged from one-half million to several million cells in their specific lesions, and host cells were 5 to 10 times this amount. The dynamics and quantitation of this type of reaction have been reported in another paper (7).

The data which have been collected concerning the passive transfer of delayed hypersensitivity with H_3-labeled cells have provided a concept of the development of the specific lesion. Following the intravenous injection of sensitized donor lymphoid cells, the transfused elements quite rapidly leave the blood stream and reside in a variety of organs, but especially the lymphoid organs. After some 12 hours, the level of circulating donor cells is low and remains so. When specific antigen is deposited in skin, host and donor leukocytes reach the site of injury. At first, the dominant infiltrating cells are polymorpho-

nuclear neutrophils, but after several hours, lymphoid cells begin to appear. There need be nothing specific up to this point about the arrival of leukocytes in the test locus. However, a small number of cells which reach the test site, by chance or otherwise, hold the specific message of hypersensitivity. These sensitized cells may interact with the antigen and be preferentially retained in the developing lesion, as shown by the present data. The antigen-sensitized–cell interaction may also induce a specific inflammation which would continue the non-specific accumulation of leukocytes, both host and donor, with further preferential retention of the sensitized cells. This process becomes maximal in 18 to 24 hours and then subsides. In such a circular series of events, specificity would be unrelated to the arrival of sensitized cells at the test site but it would presumably be responsible for the interaction of sensitized cells and antigen. At all times, the number of sensitized cells which initiate and accumulate in the developing specific lesion is small.

SUMMARY

Guinea pigs were injected intravenously with lymphoid cells sensitized to tubercle bacilli (TBC cells) and with lymphoid cells sensitized by contact to a simple chemical, dinitrofluorobenzene (DNFB cells). In each transfer, either the TBC cells or the DNFB cells were labeled with H_3-thymidine. Immediately after transfusion, each recipient was skin tested with PPD and DNFB. 24 hours later these lesions were removed for determination of total radioactivity and for autoradiographic analysis. When TBC cells labeled with H_3-thymidine were transferred with DNFB cells without an isotopic marker, the total radioactivity and the concentration per gram of skin lesion were greater in the PPD test sites. In the reciprocal arrangement, when DNFB cells labeled with H^3-thymidine were transfused with TBC cells without an isotopic tag, the total radioactivity and the concentration per gram of skin lesion were greater in the DNFB test site. Similar results were obtained in guinea pigs which were actively immunized by tubercle bacilli and passively by transfer of DNFB cells. Autoradiographic analysis of test sites from guinea pigs passively transferred with both types of sensitized cells confirmed these findings.

By calculation, only a very small number of transferred sensitized cells reached the specific test lesion. Most of the cellular infiltrate was derived from the responding host. The specificity of the reaction of delayed hypersensitivity was apparently achieved by retention of the sensitized cells after they had arrived by chance at the specific antigen depot and was not due to a non-specific stickiness of sensitized or inflamed lymphoid cells.

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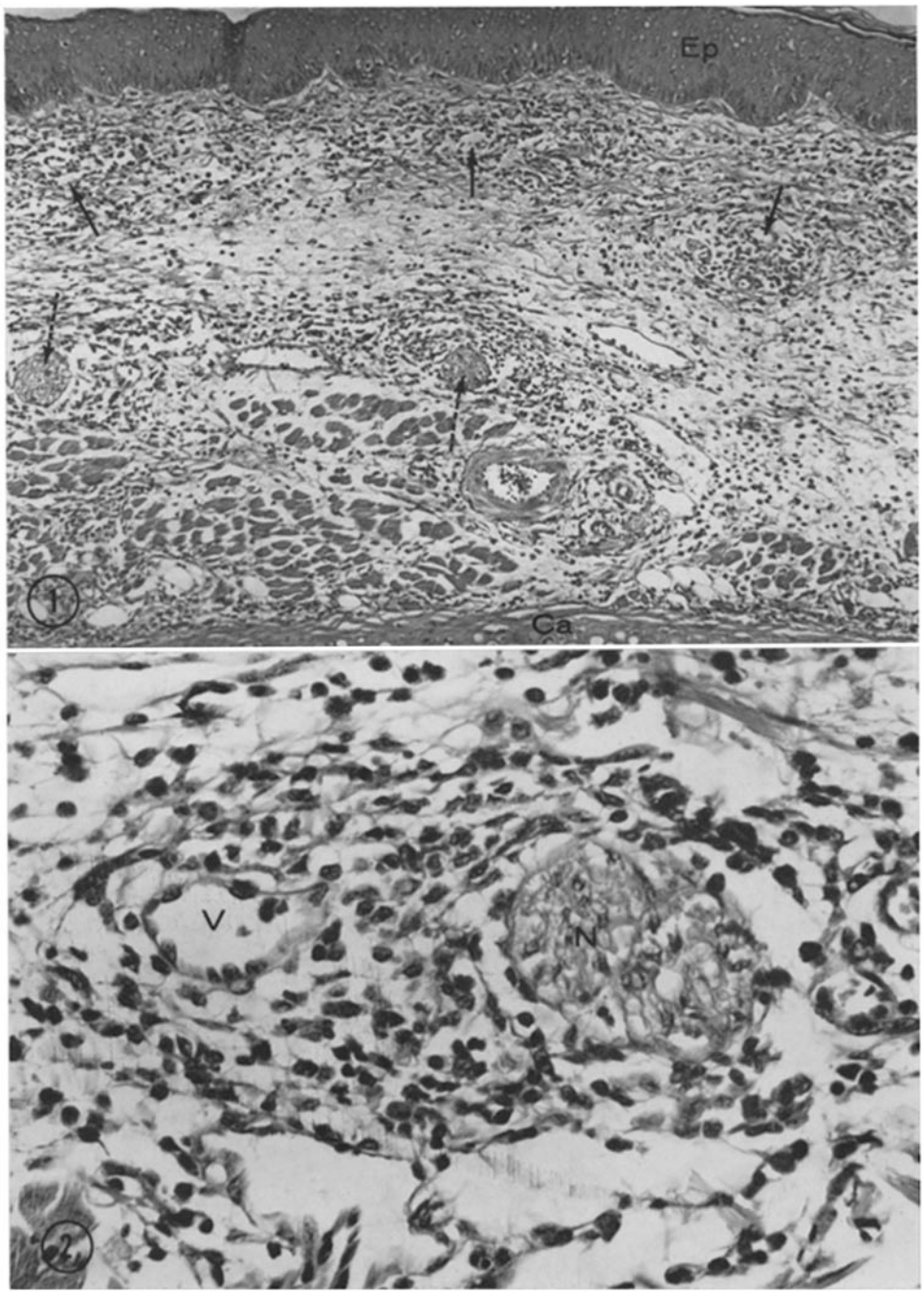
EXPLANATION OF PLATES

PLATE 36

FIG. 1. PPD reaction in the ear of a guinea pig injected with TBC cells* and DNFB cells. There is clustering of mononuclear cells around vessels (solid arrows) and nerves (broken arrows). Between the clusters is a relatively sparse dispersion of inflammatory cells. *Ep*, epidermis; *Ca*, cartilage. × 95.

FIG. 2. PPD reaction from a guinea pig transfused with TBC cells and DNFB cells*. The micrograph illustrates the characteristic histologic arrangement and types of mononuclear cells which accumulate around a vein (*V*) and nerve (*N*) in the dermis of the ear. × 440.

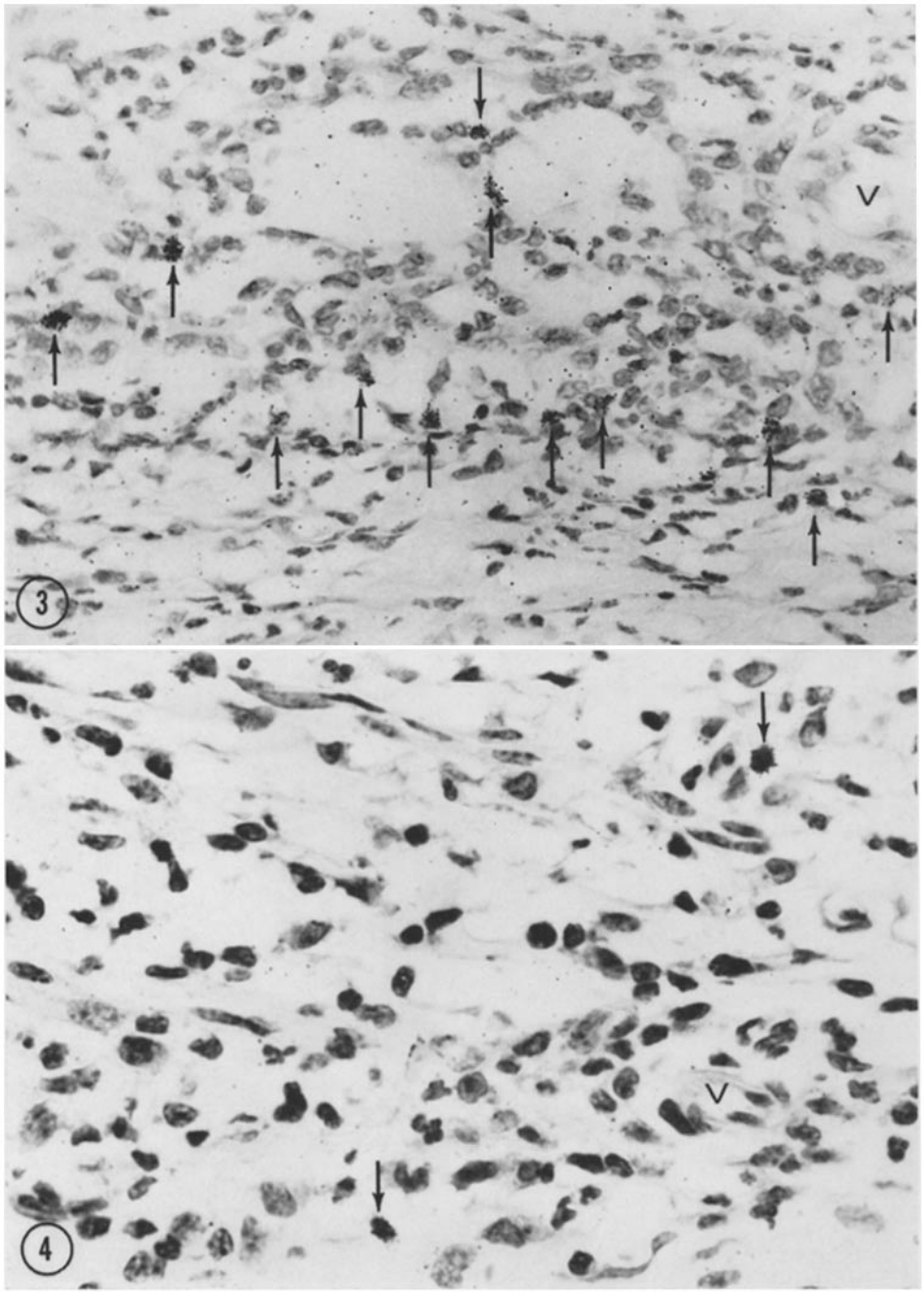


(Najarian and Feldman: Passively transferred delayed hypersensitivity)

PLATE 37

FIG. 3. Autoradiograph of a PPD reaction from a guinea pig transfused with TBC cells* and DNFB cells. There is a perivascular collection of mononuclear cells in the dermis of the ear. Arrows point to 12 labeled cells near vessel (*V*). × 500.

FIG. 4. Autoradiograph of PPD site from a guinea pig injected with DNFB cells* and TBC cells. There are 2 labeled cells (arrows) in the loose cluster eccentric to a vessel (*V*). This was the maximum number seen in a 0.25 mm^2 area of a cluster when DNFB cells* and TBC cells were transferred. Compare with Fig. 3. × 640.

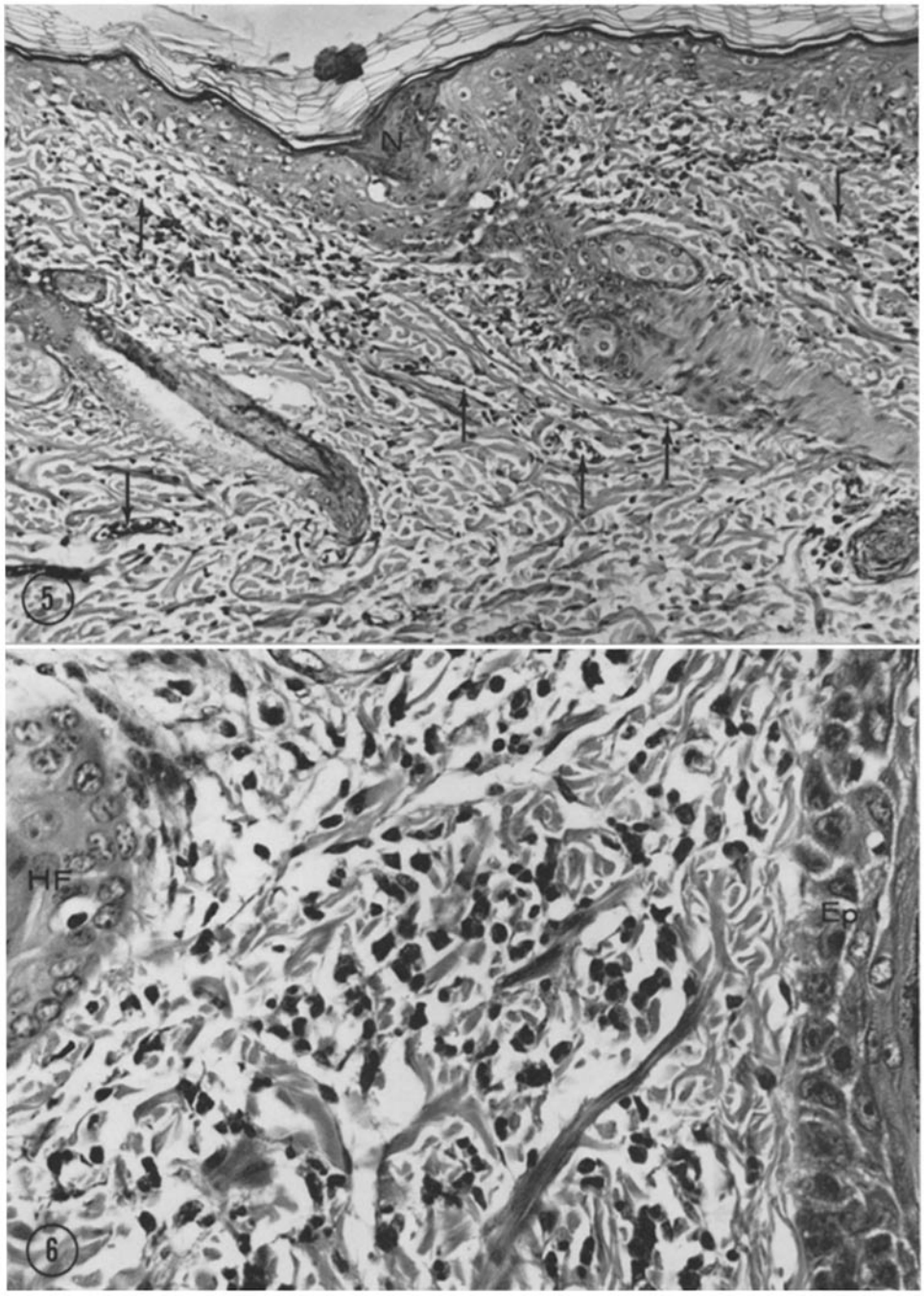


(Najarian and Feldman: Passively transferred delayed hypersensitivity)

PLATE 38

FIG. 5. DNFB reaction in the flank skin of a guinea pig injected with DNFB cells and TBC cells*. There is a diffuse patternless infiltration of mononuclear and polymorphonuclear cells in the upper dermis and epidermis. Some epidermal cells are swollen and a zone of necrosis is present at *N*. Several dilated vessels are visible in the dermis (arrows). × 95.

FIG. 6. DNFB reaction at higher magnification to illustrate the types of mononuclear cells which have spread throughout the upper dermis. From a guinea pig transfused with DNFB cells* and TBC cells. *Ep*, epidermis; *HF*, hair follicle. × 440.

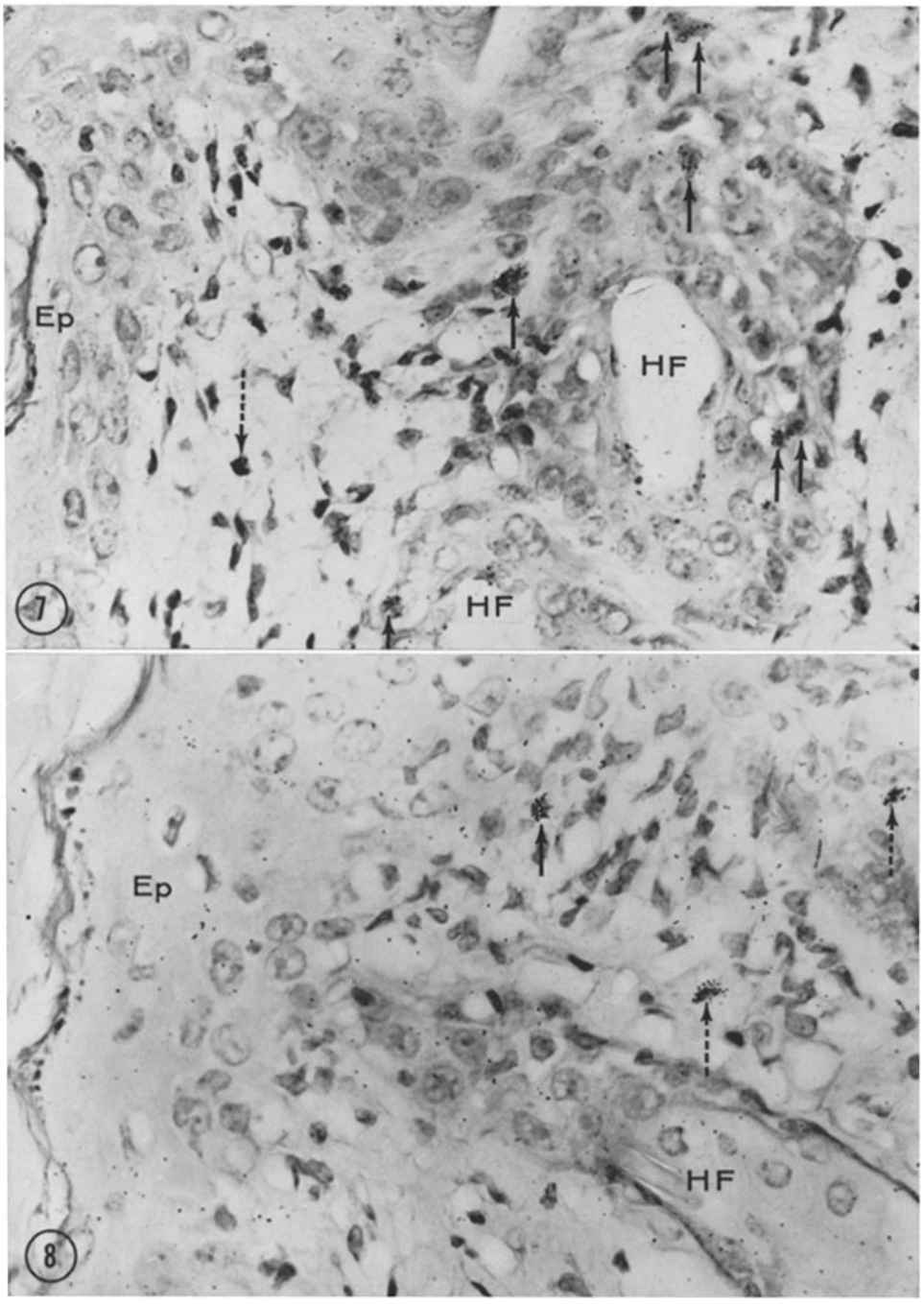


(Najarian and Feldman: Passively transferred delayed hypersensitivity)

PLATE 39

FIG. 7. Autoradiograph from a guinea pig injected with DNFB cells* and TBC cells. The epidermis (*Ep*) is to the left and two hair follicles (*HF*) are to the right. 7 labeled cells (solid arrows) are present in the hair follicles and 1 in the dermis (broken arrow). × 500.

FIG. 8. Autoradiograph of DNFB site from a guinea pig given TBC cells* and DNFB cells. 1 labeled cell lies in the epidermis (solid arrow) at its junction with the dermis. 2 other labeled cells (broken arrows) are in the dermis. *Ep*, epidermis; *HF*, hair follicle. × 530.



(Najarian and Feldman: Passively transferred delayed hypersensitivity)