TRANSFER OF ALLERGIC ENCEPHALOMYELITIS IN RATS BY MEANS OF LYMPH NODE CELLS

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PLATES 3 TO 6

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Allergic encephalomyelitis may be induced in rats and several other species of laboratory animals by single or repeated injections of mammalian brain or spinal cord emulsified in Freund's adjuvant (1–9). Clinical signs of central nervous system disease are displayed by animals 2 to 3 weeks after injection. The disease is identified pathologically by the presence of characteristic focal areas of vascular and perivascular inflammation confined to the brain, spinal cord, and their meningeal coverings. In some species of animals, these lesions may be accompanied by perivascular demyelination. For convenience, allergic encephalomyelitis will be referred to here as AE.

Numerous studies of AE have suggested that this disease has an immunological mechanism. Paterson (10) and Waksman (11) have recently reviewed many of these studies. Direct evidence for an immunological mechanism, however, by transferring AE to animals by means of serum or cells has not been reported. Since 1947, attempts by several investigators (3–5, 12–16) to accomplish transfer in this manner, using rats, guinea pigs, and monkeys, have failed. Chase (17) has recently summarized the results of these, as well as previously undescribed, AE transfer attempts. Lipton and Freund (14) in 1953 did succeed in transferring AE in rats using the technique of parabiosis. Their experiment has provided the only evidence of the feasibility of AE transfer by serum or cells.

This paper describes the transfer of AE in rats by injection of lymph node cells from actively sensitized donors into recipients pretreated neonatally with normal rat spleen cells. The design of this study was based on the concept that extended survival of donor cells in recipients might be a key factor in transfer. In past work employing genetically dissimilar donors and recipients, transferred cells would not be expected to survive and function for very long because they would be rejected immunologically by the recipients (18). From the studies of Woodruff and his associates (19–21), confirmed by Egdahl *et al.* (22), it was known that rats injected neonatally with rat spleen cells may acquire immunological tolerance to skin homografts and later be unable to reject skin transplants derived from the spleen cell donors.

It seemed likely that cellular transfer of allergic encephalomyelitis might well be accomplished using recipient rats pretreated in this fashion. Such pretreated recipients would be expected to have some tolerance of sensitized donor lymph node cells and, consequently, permit these cells to survive and function longer after transfer. In this more favorable environment, the surviving transferred cells themselves or antibodies continually elaborated by them would have a better opportunity to react with antigen(s) in the brain and spinal cord of the recipients.

Experimental Procedures and Methods

Design of Transfer Experiments.—As shown diagrammatically in Text-Fig. 1, newborn rats were pretreated with pooled, normal spleen cells from splenectomized adult donor rats. Five to 6 weeks later, the donor rats were injected with guinea pig spinal cord in adjuvant. Lymph node cells were collected from the donors 7 or 12 days later. The lymph node cells were pooled, washed, and injected intravenously into the pretreated, and now adult, recipient rats. The recipients were observed for 6 to 8 days after lymph node cell transfer, then sacrificed, and their brains and spinal cords examined microscopically for neurological lesions. All recipient rats were deliberately sacrificed within 6, 7, or 8 days after lymph node cell transfers in order to minimize the possibility of lesions resulting from active sensitization to transferred spinal cord antigen. Comparable lymph node cell transfers were performed using control donors injected with guinea pig kidney-adjuvant and control recipients which were not pretreated with spleen cells.

Rats.—CFN rats and albino rats (designated as HLH rats) were purchased from two commercial breeders,[1] respectively. Both types of rats were derived from closed, random-bred, colonies of Wistar stock rats. They were not highly inbred. Adults weighed 100 to 175 gm. each. Litters of rats, born in the laboratory, were obtained from pregnant females purchased 4 to 10 days before term. Adult rats were housed in large wire cages. Neonatal rats were kept with their mothers in metal boxes containing wood shavings until 3 to 4 weeks old at which time they were weaned and then housed in wire cages. Food pellets and fresh tap water were provided daily.[2] Only females were used as donors in order to avoid sex-linked tissue incompatibility reported to occur in inbred strains of mice by Eichwald and Silmser (23), Adler (24), and other workers (25, 26)—an incompatibility postulated to occur owing to antigens determined by a gene, or genes, associated with the Y chromosome of males. Both male and female rats were used as recipients.

Splenectomy.—Groups of 4 adult rats each were etherized, abdominal hair removed with electric clippers, and exposed skin swabbed with tincture of merthiolate (1:1000) and then 70 per cent ethyl alcohol. Using aseptic technique, the spleen was delivered through an incision in the left upper abdominal quadrant and the spleen stump ligated with 2 silk sutures. The entire spleen was cut free with scissors and removed. The ligated stump was returned to the abdomen and incised muscle and skin were closed with interrupted silk sutures.

At the outset of this work, from 50 to 75 per cent of HLH rats displayed typical features of *Haemobartonella muris* infection about 6 days after splenectomy (27). These rats lost weight, appeared weak, and exhibited clinical signs of a severe hemolytic anemia (progressive pallor and hemoglobinuria) confirmed by hematocrit values and blood smears. Giemsa-stained blood

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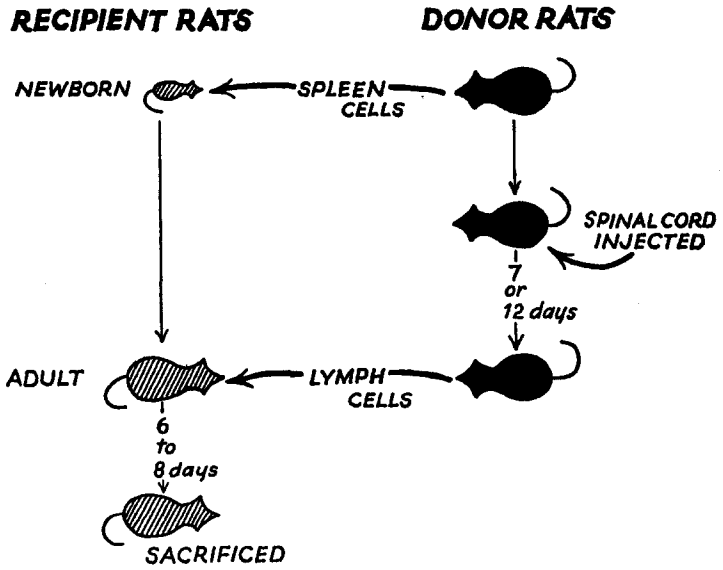
[1] CFN rats obtained from Carworth Farms, New City, New York; HLH rats obtained from Hemlock Hollow Farms, Paterson, New Jersey.

[2] Lab-blox, Wayne feeds, Boundbrook, New Jersey.

smears from 15 moribund rats were examined microscopically and 1 to 20 per cent of the erythrocytes in a given oil immersion field found to contain 1 to 15 minute coccobacillary bodies indistinguishable from *H. muris*. Neopeptone broth and thioglycolate media inoculated with blood from ill rats and incubated at 37°C. showed no evidence of bacterial growth over a period of 10 days. It was found that addition of approximately 20 mg. of aureomycin[3] to each 100 ml. of drinking water offered daily to HLH rats for 2 weeks after splenectomy, and periodically thereafter, suppressed clinical illness. Rats receiving this medicated water gained weight, appeared healthy in all respects, and could be used successfuly as donors for cell transfers. In contrast to HLH rats, the CFN rats, known to be "specific pathogen free," remained well after total splenectomy and examination of blood smears made periodically revealed no



TEXT-FIG. 1. Design of allergic encephalomyelitis transfer study.

erythrocyte parasitism. It should be stressed that there is no evidence that *H. muris* plays any role in the pathogenesis of AE in rats. AE may be induced actively in both HLH and CFN rats (see Table I) and comparable results were obtained in the transfer studies described here using either type of rat.

Spleen Cell Suspensions and Pretreatment of Rats.—One to 4 donor rat spleens, immediately after removal, were placed in chilled Hanks balanced salt solution (HBSS)[4] containing sodium bicarbonate, and cut into 2 or 3 pieces. The cells were expressed and isolated by repeatedly squeezing the spleen segments with thumb forceps and passing the spleen cell suspension through a 25 gauge needle. The cells in HBSS were pooled, transferred to a graduated conical tube, and centrifuged at 145 *G* for 5 minutes at 4°C. The washed cells were promptly resuspended in a sufficient volume of fresh, chilled, HBSS to give approximately the desired cell concentration.

[3] Chlortetracycline HCl, American Cyanamid Co., New York.

[4] Microbiological Associates, Bethesda, Maryland.

Twenty-four litters of HLH and CFN neonatal rats were pretreated with different spleen cell suspensions. One to 8 rats in a litter were injected intraperitoneally with 0.3 to 0.5 ml. of each cell suspension. Each rat received from 17 to 49 million viable nucleated spleen cells as determined by eosin-Y vital staining (28) and hemocytometer counts of aliquots of injected cell suspensions. Eighteen litters were injected 1 to 3 days after birth. The remaining 6 litters were injected 4, 6 (2 litters), 10, and 12 (2 litters) days after birth, respectively. Beginning about 10 days after injection, a variable proportion of rats in different litters showed clinical signs of disease. This disease was characterized clinically by failure to gain weight, sparse growth of hair particularly over the face and distal portions of extremities, roughened skin, progressive weakness, and variable pallor. Death occurred usually 14 to 22 days after injection of spleen cells. The disease was observed in both HLH and CFN rats and was related directly to the number of viable spleen cells injected neonatally. Brains of 9 rats injected with spleen cells when 1 day old and dying of this disease 18 to 25 days later showed neither gross nor microscopic histological abnormalities. It would appear that this disorder is similar to that known as "runt syndrome" described by Billingham and Brent (29) and other workers (30, 22) in mice, chickens, and rats, injected embryonically or neonatally with foreign, living, immunologically competent cells. Those pretreated rats appearing well when 5 to 6 weeks old and presumed to have acquired immunological tolerance to donor cells because of prior neonatal treatment were used as recipients for lymph node cell transfers.

Tissue-Adjuvant Inocula and Injection of Rats.—Spinal cord and kidneys were removed aseptically from Hartley guinea pigs[5] weighing 450 to 600 gm. each and stored at −25°C. for 1 to 6 days. Spinal cord was used promptly without freezing on one occasion and in two instances after storage at −25°C. for 25 and 29 days. Weighed portions of thawed tissues were homogenized in distilled water containing 0.25 per cent phenol using a TenBroeck tissue grinder. All homogenates contained 33 per cent tissue (wet weight). Complete adjuvant was prepared as described by Freund (31). Heat-killed, lyophilized, *Mycobacterium tuberculosis* (strain H37Rv)[6] cells were suspended in 9 parts paraffin oil[7] and 1 part emulsifying agent[8] in a mortar by stirring with a pestle. Each final milliliter of complete adjuvant contained 4 mg. of *M. tuberculosis*. Adjuvant preparations were autoclaved for 20 minutes at 120°C. and a pressure of 20 pounds and then stored at 4°C. Tissue homogenates were emulsified in equal volumes of adjuvant in a mortar by stirring with a pestle. Rats were etherized lightly, hair over the back removed by electric clippers, and the exposed skin swabbed with 70 per cent ethyl alcohol. Each animal was injected intracutaneously with 0.1 ml. of tissue-adjuvant emulsion in each of 6 sites on the upper one-third of the back and 1 site on the anterior surface of the neck. Each rat received approximately 115 mg of spinal cord or kidney (wet weight).

Donor Lymph Node Cell Suspensions.—Donor rats were sacrificed 7 or 12 days after injection of spinal cord or kidney in adjuvant. Using aseptic technique throughout, 3 to 5 anterior cervical and 2 to 3 axillary lymph nodes were removed from each of 1 to 4 donor rats. The nodes were immediately pooled in chilled HBSS, trimmed, and cut into small fragments with scissors. The fragments were then pressed through mesh screening ("cytosieve",[9] described by Snell (32)), using fresh HBSS, in order to obtain suspensions of dissociated cells.

[5] Guinea pigs of Hartley strain obtained from Tumblebrook Farms, Chestertown, New York.

[6] The strain of *Mycobacterium tuberculosis* was grown on medium prepared as described by Youmans, G. P., A method for the determination of the culture cycle and the growth rate of virulent human type tubercle bacilli, *J. Bact.*, 1946, **51**, 703.

[7] Light paraffin oil, called bayol-F, obtained from Esso Standard Oil Co., New York.

[8] Arlacel-A kindly provided by Atlas Powder Co., Wilmington.

[9] Cytosieves obtained from Macalaster Bicknell Co., Cambridge, Massachusetts.

The cells were then washed at 4°C. using 1 or 2 cycles of centrifugation at 145 *G* for 5 minutes and resuspended in fresh HBSS to give approximately the desired concentration of lymph node cells to be transferred. Aliquots of these cell suspensions were examined microscopically and were found to consist mainly of isolated cells with minimal clumping and little debris. As many as 36 lymph nodes from 4 donors could be removed and processed as described within 1 hour.

Transfer of Lymph Node Cells.—Recipient rats were etherized lightly, their tails warmed for 1 minute in tap water heated to 50°C. and injected in the lateral tail veins with 0.5 to 1.0 ml. of lymph cell suspensions. In 3 transfers of larger volumes of cells, 1.0 ml. was injected intravenously and 0.25 to 1.0 ml. was injected intraperitoneally. Each lymph node cell suspension was transferred to 1 to 4 recipients. Each recipient received from 10 to 244 million viable nucleated lymph node cells, based on eosin-Y vital staining (28) and hemocytometer counts of aliquots of lymph node cell suspensions transferred. Only an occasional animal exhibited respiratory distress and transitory cyanosis for a few minutes after injection. The recipients were observed daily for clinical signs, *sacrificed 6, 7, or 8 days after cell transfer*, and their brains and spinal cords removed for histological examination.

Histological Studies.—Brains, spinal cords, and other tissues (thyroid, heart, liver, lungs, spleen, kidneys, adrenals, testis, mesenteric and axillary lymph nodes) were fixed in 10 per cent formaldehyde in tap water. Blocks of tissues were processed through the stage of paraffin embedding in an autotechnicon. Sections 5 to 10 microns thick were stained with hematoxylin and eosin in the usual manner. Adjacent sections of brains and spinal cords were cut at 15 to 20 microns and stained using the luxol fast blue technique (33) for myelin studies. A minimum of 12 different hematoxylin and eosin stained sections of nervous tissue from each recipient rat were examined microscopically. These sections were cut through the thalamus, mesencephalon, cerebellum-pons, medulla, and cervical-thoracic spinal cord.

Complement Fixation (CF) Tests.—Blood samples were obtained aseptically by cardiac puncture from recipients when sacrificed, allowed to clot at 4°C. for 16 to 18 hours and sera collected by centrifugation. Sera were stored in sterile, cork-stoppered, glass tubes at −25°C. The brain antigen used in the CF tests was an ethanol extract of lyophilized normal rat brains prepared as previously described (2). Sheep blood in Alsever's solution, ampules of lyophilized guinea pig complement (C′) and rabbit anti-sheep red cell immune serum were obtained from one commercial source.[10] Quantitative CF tests were performed using the 50 per cent hemolytic technique as described by Kabat, Mayer, Osler, and associates (34–37) with certain modifications. A colorimeter[11] was used to standarize sheep red blood cell suspensions and to obtain optical densities (O.D.) of supernatants from final reaction mixtures. One volume of 5 per cent sheep red cell suspension lysed in 29 volumes of distilled water gave an O.D. of 0.342 at a wavelength of 540 millimicrons. Final values were corrected by proportion and represented the number of $C'H_{50}$ of exactly 100 $C'H_{50}$ available fixed by 0.25 ml. of rat serum in the presence of appropriately diluted rat brain antigen.

EXPERIMENTAL RESULTS

Paralytogenic Activity of Spinal Cord—Adjuvant Inocula.—Since the majority of donor rats were sacrificed 7 days after injection of spinal cord adjuvant, at a time when they would not yet be expected to exhibit clinical signs of neurological disease, groups of control rats were injected with the same spinal cord emulsions in order to be sure that the emulsions had paralytogenic activity.

[10] Certified Blood Donor Service, New York.

[11] Bausch and Lomb "spectronic 20" colorimeter.

The occurrence of AE in these control rats is shown in Table I and is representative of AE induced actively in more than 150 other rats in this laboratory during the past 2 years. Each of the 12 inocula was paralytogenic and induced AE in from two-thirds to all of the HLH or CFN rats in each group. A total of 35 of the 43 rats (81 per cent) developed AE. Twenty-one of these 35 rats exhibited ataxia and hindleg weakness or paralysis and were subsequently

TABLE I

Allergic Encephalomyelitis (AE) in Control Rats Injected with Same Spinal Cord Adjuvant Emulsions Used to Inject Donor Rats

Rat group No.*	No. of rats	No. of rats with		Occurrence of AE
		AE lesions only	Both clinical signs and AE lesions	
1	3	0	2	2/3‡
2	4	2	2	4/4
3	5	1	2	3/5
4	3	1	2	3/3
5	3	1	1	2/3
6	3	1	1	2/3
7	3	2	1	3/3
8	3	1	1	2/3
9	2	0	2	2/2
10	8	0	6	6/8
11	3	2	1	3/3
12	3	3	0	3/3
Total.....	43	14/43	21/43	35/43

* CFN rats used for groups 1 to 4 and HLH rats used for groups 5 to 11. Group 12 consisted of 1 HLH and 2 CFN rats splenectomized 8 to 11 weeks previously. Rats observed for 21 to 26 days after injection, then sacrificed, and brains—spinal cords examined for AE lesions.

† Numerator, No. of rats with AE lesions; denominator, No. of rats in group.

found to have AE lesions in their brains and spinal cords. These clinical signs in 16 of the 21 animals were first seen 12 to 15 days after injection. Signs appeared initially in the other 5 rats 11, 16, 17, 18, and 19 days after injection, respectively. The remaining 14 animals displayed no clinical signs but were found to have typical AE lesions. It should be noted that AE occurred in each of the splenectomized rats in group 12, indicating that presence of the spleen is not essential for induction of disease.

It can also be mentioned that neonatal pretreatment of rats with normal rat spleen cells does not alter the capacity of such animals to develop AE. Eight rats injected 2 to 8 days after birth with 26 to 44 million spleen cells were

injected with spinal cord adjuvant 5 to 10 weeks later. Seven of these 8 animals developed typical AE.

Transfer of Lymph Node Cells to Recipient Rats Pretreated with Spleen Cells.— Thirteen lymph node cell transfers were carried out using donors injected with

TABLE II

Allergic Encephalomyelitis (AE) in Recipient Rats after Transfer of Lymph Node Cells

Donor rats providing lymph node cells injected with spinal cord adjuvant. Recipient rats pretreated neonatally with normal spleen cells *from same donor rats* (see text)

Transfer No.*	No. of donor rats*	No. of donor lymph node cells transferred ($\times 10^6$)	Recipient rats‡		
			No. of rats	Days after transfer sacrifice	Occurrence of AE lesions
1	1	10	2	8	1/2§
2	2	32	2	8	1/2
3	1	32	1	7	1/1
4	3	37	3	8	1/3
5	1	55	1	8	1/1
6	1	59	1	8	1/1
7	1	61	1	7	1/1
8	2	64	1	8	1/1
9	3	92	1	7	1/1
10	2	106‖	1	7	0/1
11	4	108	2	8	1/2
12	3	108	1	8	1/1
13	4	156	5	6	3/5
Total.....	28		22		14/22

* Donors in transfers 5, 8, 11 and 12 sacrificed 12 days after injection; although none of these 10 donors displayed clinical signs of AE, 2 of them had AE lesions. The 18 donors in the remaining 9 transfers were sacrificed 7 days after injection; none showed clinical signs of AE and only 1 of 13 examined histologically had AE lesions.

‡ Recipients pretreated with from 14 to 49 million viable spleen cells when 1 to 3 days old except those used in transfers 5, 8 and 10 which were pretreated when 10, 12, and 6 days old, respectively. Lymph node cells transferred to recipients 5 to 6 weeks after pretreatment.

§ Numerator, No. of recipients with AE lesions; denominator, No. of recipients used in transfer.

‖ Injected into recipient solely by intraperitoneal route; intravenous route used in other transfers.

spinal cord and *recipients pretreated neonatally with spleen cells from these same donor rats*. The results of these transfers are shown in Table II. A total of 14 of 22 recipients were found to have AE lesions after transfer of 10 to 156 million viable donor lymph node cells. The number of lesions demonstrable in each of these 14 positive recipients varied from a minimum of 2 to a maximum of 15.

Both the number and intensity of lesions in these recipients appeared to be related directly to the number of donor lymph node cells transferred.

In the 12 successful transfers, lymph node cells were collected from donors either 7 or 12 days after injection of spinal cord adjuvant and the donor: recipient ratios ranged from 0.5 to 3. In the single unsuccessful transfer, No. 10, the recipient was injected with donor cells solely by the intraperitoneal route after repeated failures to enter the tail vein. This observation may be important in view of the fact that all other recipients received intravenous injections of at least half or, in most instances, all of the transferred lymph node cells.

Clinical neurological signs were observed in the 2 recipients in transfers 5 and 7. These animals exhibited ataxia and hindleg weakness 4 and 5 days after cell transfer, respectively. These signs were still present when the animals were sacrificed 4 and 2 days later, respectively. Both animals were subsequently found to have intense neurological lesions. None of the other recipients displayed clinical signs.

The central nervous system (CNS) lesions in the 14 recipients consisted of discrete collections of inflammatory cells within the walls and perivascular spaces of small blood vessels, usually veins and venules (Figs. 1 to 4). The inflammatory cells were predominantly mononuclear in type; polymorphonuclear leukocytes and plasma cells were present in very small numbers (Fig. 5). Vascular lesions were almost always confined to white matter and were particularly prone to occur beneath the ependymal lining of the lateral ventricles, in the dorsal portion of the mesencephalon, and below the floor of the fourth ventricle. Spinal cord lesions were present only in those 2 recipients displaying clinical signs (Fig. 6). Perivascular demyelination of any significant degree was not found. Astrocyte proliferation was either minimal or not present. Neurons, axons, and other cellular elements of the parenchymal nervous tissue showed no conspicuous histological alterations. Sparse, focal collections of mononuclear cells were occasionally present in the leptomeninges, usually oriented around a vessel, and were always accompanied by 1 or more vascular lesions in the immediately underlying nervous tissue. The morphology and distribution of the CNS lesions in recipients were indistinguishable from the AE lesions induced actively in rats by injection of nervous tissue adjuvant. The recipient lesions differed only by being usually less intense and less numerous.

Non-neural tissues (liver, spleen, kidneys, and cervical-axillary lymph nodes) were collected from 6 recipient rats with CNS lesions. These tissues revealed neither gross nor microscopic histological changes of significance. In addition, brains, spinal cords, thyroid, heart, lungs, livers, spleens, kidneys, adrenals, testis, and lymph nodes were obtained from 24 rats injected neonatally with comparable numbers of normal rat spleen cells and sacrificed 5 to 7 weeks later without receiving donor lymph node cells. These tissues showed no conspicuous microscopic abnormalities.

Additional lymph node cell transfers were performed using recipients pretreated neonatally with *spleen cells from rats other than those providing the transferred lymph cells.* The results of 10 transfers of this type are shown in Table III. A total of 6 of 29 recipients had AE lesions. These 6 positive recipients were confined to transfers 17, 18, and 19 and received 68, 83, and 118 million lymph

TABLE III

Allergic Encephalomyelitis (AE) in Recipient Rats after Transfer of Lymph Node Cells

Donor rats providing lymph node cells injected with spinal cord-adjuvant. Recipients pre-treated neonatally with normal spleen cells *from rats other than these donor rats* (see text).

Transfer No.*	No. of donor rats*	No. of donor lymph node cells transferred ($\times 10^6$)	Recipient rats‡		
			No. of rats	Days after transfer sacrifice	Occurrence of AE lesions
14	3	33	4	8	0/4§
15	3	41	3	8	0/3
16	3	43	3	8	0/3
17	4	68	2	7	2/2
18	3	83	3	8	3/3
19	3	118	2	8	1/2
20	4	118	1	7	0/1
21	4	138	7	6	0/7
22	4	?	1	7	0/1
23	3	?	3	7	0/3
Total.....	34		29		6/29

? Clumping precluded accurate count; probably more than 100×10^6 cell transferred.

* Donors in transfers 18 and 19 sacrificed 12 days after injection; 3 of these 6 donors exhibited paralysis on day sacrificed and had AE lesions, as did 1 of the 3 remaining donors. The 28 donors in the other transfers were sacrificed 7 days after injection; none showed clinical disease and only 1 of 20 examined histologically had AE lesions.

‡ Recipients pretreated with from 14 to 25 million viable spleen cells when 1 to 3 days old except those used in transfers 14 and 21 which were pretreated when 4 and 7 days old, respectively. Lymph node cells transferred to recipients 5 to 6 weeks after pretreatment.

§ Numerator, No. of recipients with AE lesions; denominator, No. of recipients used in transfer.

node cells, respectively. Four of these 6 recipients (transfers 18 and 19) received lymph node cells collected from donors 12 days after injection, at which time 3 of the 6 donors showed hindleg paralysis. Three of the 6 positive recipients (2 in transfer 18 and 1 in transfer 17) displayed ataxia and hindleg weakness 2, 4, and 5 days after cell transfer, respectively. The clinical signs were still present when the animals were sacrificed 2 to 6 days later. These 3 rats with clinical evidence of disease were subsequently found to have particularly

striking AE lesions in their brains and spinal cords. The CNS lesions present in all 6 positive recipients had all the features described above. Microscopic examination of sections of kidneys, spleen, liver, and lymph nodes from 2 of these 6 positive recipients revealed no important histological alterations.

TABLE IV

Lymph Node Cell Transfers Involving Control-Donor and Control-Recipient Rats

Transfer no.	Donor rats		No. of donor lymph node cells transferred‡ ($\times 10^6$)	Recipient rats§		
	No. of rats	Inj. with*		No. of rats	Pre-treated neonatally with	Occurrence of AE lesions
24	1	Kidney	41	1	Spleen cells of donors	0/1‖
25	1	"	43	1	" " " "	0/1
26	1	"	52	1	" " " "	0/1
27	1	"	54	1	" " " "	0/1
28	1	"	56	1	" " " "	0/1
29	1	"	59	1	" " " "	0/1
30	4	Kidney	135	2	Spleen cells of rats other than donors	0/2
31	4	"	180	2	" "	0/2
32	4	"	187	2	" "	0/2
33	4	"	244	2	" "	0/2
34	4	Spinal cord	49	2	Not pretreated	0/2
35	4	" "	118	2	" "	0/2
36	4	" "	130	4	" "	0/4
37	4	Not injected	50	2	Spleen cells of donors	0/2
38	4	" "	74	2	" " " "	0/2
Total....	42			26		0/26

* Indicated tissue emulsified in adjuvant (see text).

‡ Indicated No. of viable donor lymph node cells collected 7 or 12 days after injection.

§ Littermates of recipients used in other transfers (Tables II and III) and pretreated as described; sacrificed 6, 7 or 8 days after lymph node cell transfers and examined for lesions of allergic encephalomyelitis (AE).

‖ Numerator, No. of recipients with AE lesions; No. of recipients in each group.

Of the 7 unsuccessful transfers shown in Table III Nos. 14, 15, 16, and 20 were technically satisfactory and consisted respectively of 33, 41, 43, and 118 million lymph node cells collected from donors 7 days after injection. The remaining 3 unsuccessful transfers were not satisfactory from a technical standpoint. In 1 of them (No. 21) a very low donor:recipient ratio was used

and in the other 2 (Nos. 22 and 23) the donor cell preparations showed extensive clumping.

Lymph Node Cell Transfers Involving Control-Donors and Control-Recipients.—Donors were injected with kidney emulsified in complete adjuvant. Recipients were pretreated neonatally with normal spleen cells from either these same donors or from rats other than those providing the lymph node cells transferred. In addition, lymph node cells from uninjected donors were transferred to spleen cell pretreated recipients and lymph node cells from spinal cord-adjuvant donors were transferred to recipients not pretreated neonatally. The results of these transfers are shown in Table IV. None of the 26 recipients exhibited clinical signs and none were found to have CNS lesions when sacrificed 6 to 8 days after transfer of 41 to 244 million donor lymph node cells.

Antibodies against Nervous Tissue in Sera of Recipient Rats.—It was expected that lymph node cells collected from donor rats injected with spinal cord adjuvant would elaborate complement-fixing (CF) antibodies against nervous tissue for varying periods of time after their transfer to recipient rats. It was assumed that the levels of CF antibodies demonstrable in recipient sera would provide information as to how well the transferred donor cells survived and functioned in the recipients. For this reason quantitative CF tests were set up using sera obtained from recipients 6 to 8 days after transfer and rat brain antigen. Preliminary tests showed that sera from normal rats or rats injected with guinea pig kidney in adjuvant tested against rat brain antigen fixed less than 6 $C'H_{50}$, in most instances less than 3 $C'H_{50}$, of exactly 100 $C'H_{50}$ available. Fixation of more than 6 $C'H_{50}$ was considered, therefore, to indicate the presence of CF antibodies against brain.

Representative results of CF tests are shown in Table V. Eight recipients were pretreated with spleen cells; 4 recipients were not pretreated. All 12 animals received approximately the same number of viable donor lymph node cells. CF antibodies against brain were present in sera from 4 of the 5 recipients pretreated with spleen cells from the prospective lymph node cell donors; these 4 sera fixed from 9 to 19 $C'H_{50}$. CF antibodies were also present in serum of 1 of the 3 recipients pretreated with spleen cells from rats other than the lymph cell donors; this serum fixed 10 $C'H_{50}$. In contrast, none of the sera from the 4 recipients, which were not pretreated with spleen cells, had appreciable levels of antibodies; all 4 sera fixed less than 6 $C'H_{50}$. CF tests with sera from 25 additional recipient rats gave comparable results.

Judging from the levels of antibodies present in recipients of lymph node cells, it was apparent that donor cells survived and continued to elaborate antibodies for appreciable periods only in those recipients pretreated with spleen cells from the prospective lymph node cell donors. Most of the sera from such recipients contained circulating antibodies against brain, fixing from 6 to 22 $C'H_{50}$. As anticipated, there was no direct relationship between the presence

of CF antibodies and the occurrence of AE lesions in these individual rats. Previous studies (2, 8) showed that these CF antibodies, directed against alcohol soluble and non-paralytogenic, nervous tissue antigen(s), probably play no direct role in initiating AE. Only a few of the sera from recipients pretreated with spleen cells from rats other than those used as donors of lymph node cells contained antibodies. Sera from these few animals fixed from 7 to 11 $C'H_{50}$.

TABLE V

Fixation of Guinea Pig Complement by Sera of Recipient Rats, after lymph Node Cell Transfers, and Rat Brain Antigen

Recipient rat No.	Neonatal pretreatment of recipients*	No. of donor lymph node cells transferred‡ ($\times 10^6$)	No. of $C'H_{50}$ fixed with rat brain antigen§
C-775	Normal spleen cells of prospective lymph node cell donors	32	9
C-776	" "	32	1
C-780	" "	37	19
C-781	" "	37	10
C-782	" "	37	12
C-783	Normal spleen cells of rats other than lymph node cell donors	43	0
C-784	" "	43	10
C-785	" "	43	5
C-768	None	49	4
C-769	"	49	5
C-770	"	118	3
C-771	"	118	1

* Rats injected 1 to 3 days after birth with from 17 to 28 million spleen cells of indicated donor rats.

‡ Lymph node cells collected from donors 7 days after injection of spinal cord adjuvant and transferred to recipients 5 to 6 weeks old.

§ Maximum No. of 50 per cent hemolytic units of complement ($C'H_{50}$) fixed, of 100 $C'H_{50}$ available, by 0.25 ml. serum in presence of rat brain antigen diluted 1:250 or 1:1000. All sera collected from recipients 6 or 8 days after lymph cell transfers.

DISCUSSION

The results of this study (Tables II and III) indicate that cellular transfer of AE can be accomplished by injection of lymph node cells from spinal cord-injected donor rats into recipient rats pretreated neonatally with normal rat spleen cells. Transfer can be accomplished frequently if the recipients are pretreated with spleen cells of the prospective lymph node cell donors. Transfer is achieved occasionally if the recipients are pretreated with spleen cells from rats other than those providing the transferred lymph node cells.

The CNS lesions in positive recipients are indistinguishable from acute AE lesions found in rats injected with spinal cord adjuvant. Three lines of evidence support the conclusion that the recipient CNS lesions are the result of passive, rather than active, "sensitization" to spinal cord antigen(s). First, CNS lesions were present in recipients sacrificed 6 to 8 days after cell transfer. Previous work (14, 38) has shown that rats actively sensitized to nervous tissue rarely have demonstrable lesions until 9 days after injection. And in this study, only 2 of 45 donors (Tables II, III, and IV) sacrificed 7 days after injection and examined histologically had demonstrable AE lesions. Second, 5 of the 20 positive recipients (Tables II and III) displayed clinical signs of AE as soon as 2 to 5 days after cell transfer. Other studies (1, 2, 10, 14, 38 and Table I) have established that clinical signs rarely appear in actively sensitized rats until 10 days following injection. Third, the donor lymph node cells were transferred to recipients *via* the intravenous route. This particular route of injection, in our hands, has proven ineffective for induction of AE in rats using spinal cord emulsions.

The transfers of AE described here can best be attributed to the use of recipients which had acquired immunological tolerance of donor lymph node cells, to a varying degree, as a result of neonatal pretreatment with pooled, viable, normal rat spleen cells. The recipients were deliberately pretreated at an age and in a manner shown by several workers (19–22) to induce homograft tolerance in rats. More recently, Ashley *et al.* (39, 40) have found that newborn rats injected with pooled blood from a group of adult donor rats may later be tolerant of skin grafts from these same donors as well as other rats outside the donor group. Terasaki, Cannon, and Longmire (41, 42), and Hašek and Hašková (43) have obtained comparable results with chickens and ducks. These various studies indicate that by using pooled donor cells it is possible not only to simultaneously induce tolerance to skin grafts from more than 1 donor animal but also induce some degree of tolerance to skin transplants from other unrelated donors as well. This latter finding may be explained, particularly in the case of rats, by long term closed colony breeding with resultant sharing of strong histocompatibility genes by different animals (44). Although we did not test our pretreated recipient rats for homograft tolerance by skin grafting, the serological tests (Table V) provided indirect evidence that the recipients had less capacity to reject transferred donor lymph cells. Donor lymph node cells survived and produced antibodies against brain antigen(s) much better after transfer to spleen cell pretreated, in contrast to normal, recipient rats. This was particularly true when the spleen cells used for pretreatment were from the same donors providing the transferred lymph node cells, under the conditions that cellular transfer of AE was most often accomplished.

In contrast, attempts to transfer the disease in a comparable manner to recipients which were not pretreated failed (Table IV). This finding confirmed many previous transfer attempts of this type made in this laboratory with both

rats and guinea pigs. While it may well be possible to accomplish cellular transfer of AE using normal, genetically dissimilar, rats or other species of animals, the conditions for such transfer remain to be defined. Our data indicate that spleen cell pretreatment facilitates cellular transfer of the disease in the rat.

Extended survival of functioning donor cells, relatively unmolested by immunological attack by the recipient, may well be an important factor in AE transfers. Under such circumstances, the transferred cells themselves and/or antibodies elaborated by them would have a better opportunity to react with antigen(s) in the CNS of the recipient.

This concept of extended survival of donor cells is supported by the results of recent AE transfer attempts of other workers. Åstrom and Waksman (45) have found AE-like lesions in heavily x-irradiated recipient rabbits within 10 days after intravenous injection of large numbers of lymph node cells from spinal cord–injected donors. These workers have described an experiment involving 1 recipient in detail (46). Leonard and Thomas (47) have suggestive evidence that AE may be transferred to normal guinea pigs if the donor cells are injected *via* the intracerebral route. In both of these studies, the cells were transferred to an environment in which they could survive and function for appreciable periods of time.

While Lipton and Freund (14) have shown that AE in the rat can be transferred by parabiosis, our transfer studies indicate that a factor(s) intimately associated with washed, viable, lymph node cells of rats injected with nervous tissue adjuvant is capable of causing AE in recipients. The data do not indicate whether the tissue damage recognized as AE is initiated by antibodies released by these cells after their transfer or by the transferred cells themselves.

The relative importance of an immune factor(s) associated with living cells and conventional circulating antibodies in the production of this disease, thus, remains to be elucidated. The transfers reported here do provide direct evidence that allergic encephalomyelitis has an immunological mechanism. The transfers provide additional means for defining the sequence of events responsible for this disease in more precise terms.

SUMMARY

Transfer of allergic encephalomyelitis has been accomplished by injection of lymph node cells, obtained from donor rats sensitized to spinal cord, into recipient rats pretreated neonatally with normal rat spleen cells. Transfer of the disease may be achieved most frequently when the recipients are pretreated with spleen cells of the prospective lymph node cell donors. These transfers are attributed to the use of recipients which have acquired immunological tolerance to donor lymph node cells, as a result of the spleen cell pretreatment, and in which, therefore, the donor cells can survive and function longer after transfer.

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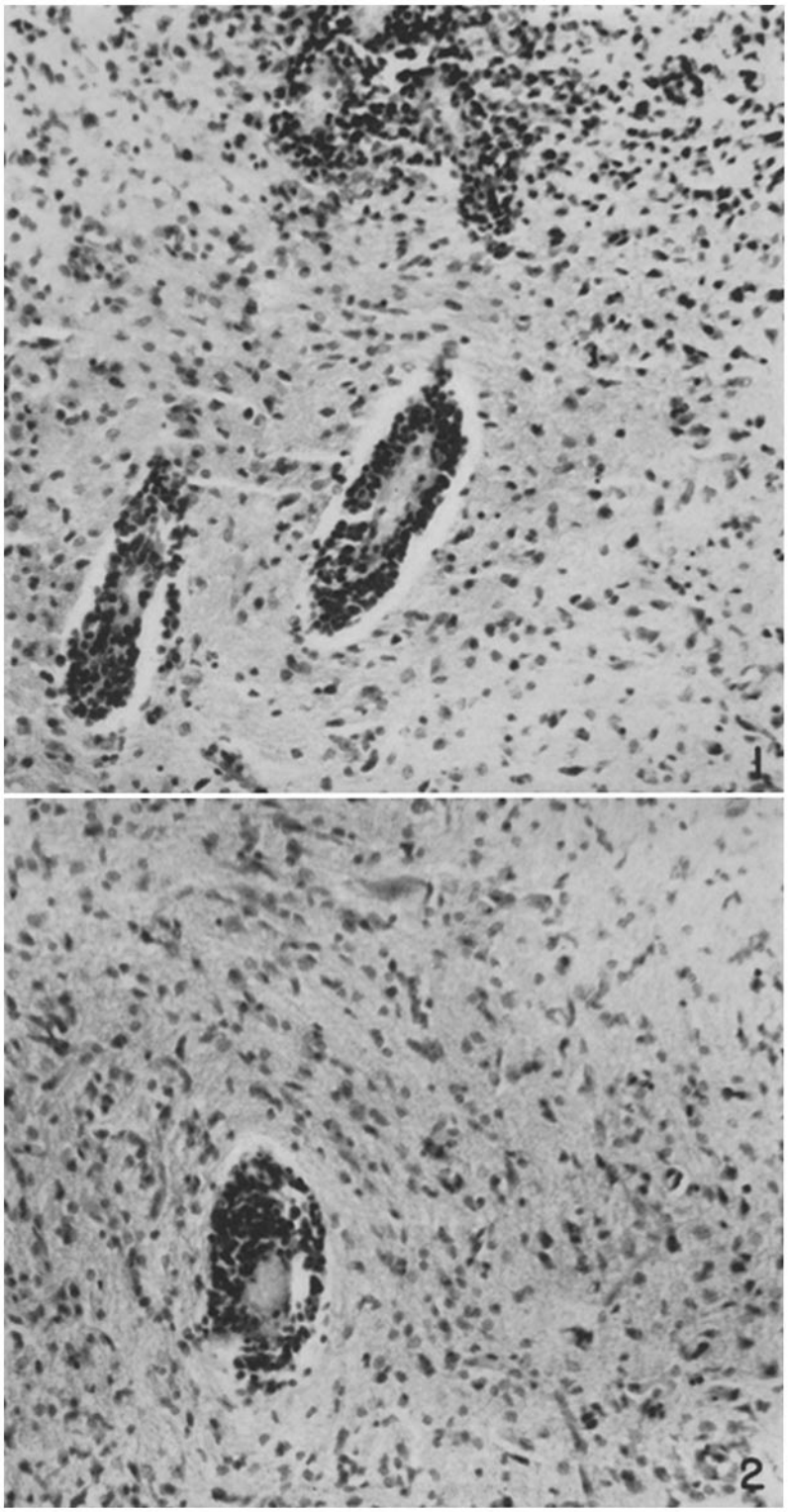
EXPLANATION OF PLATES

PLATE 3

FIG. 1. Recipient rat C830. Animal exhibited severe ataxia–hindleg weakness 5 days after transfer of donor lymph node cells; sacrificed 7 days after cell transfer. Two vascular-perivascular inflammatory lesions in mesencephalon. Hematoxylin and eosin. × 130.

FIG. 2. Recipient rat C839. No clinical signs; sacrificed 8 days after transfer of donor lymph node cells. Vascular lesion in cerebellum. Hematoxylin and eosin. × 130

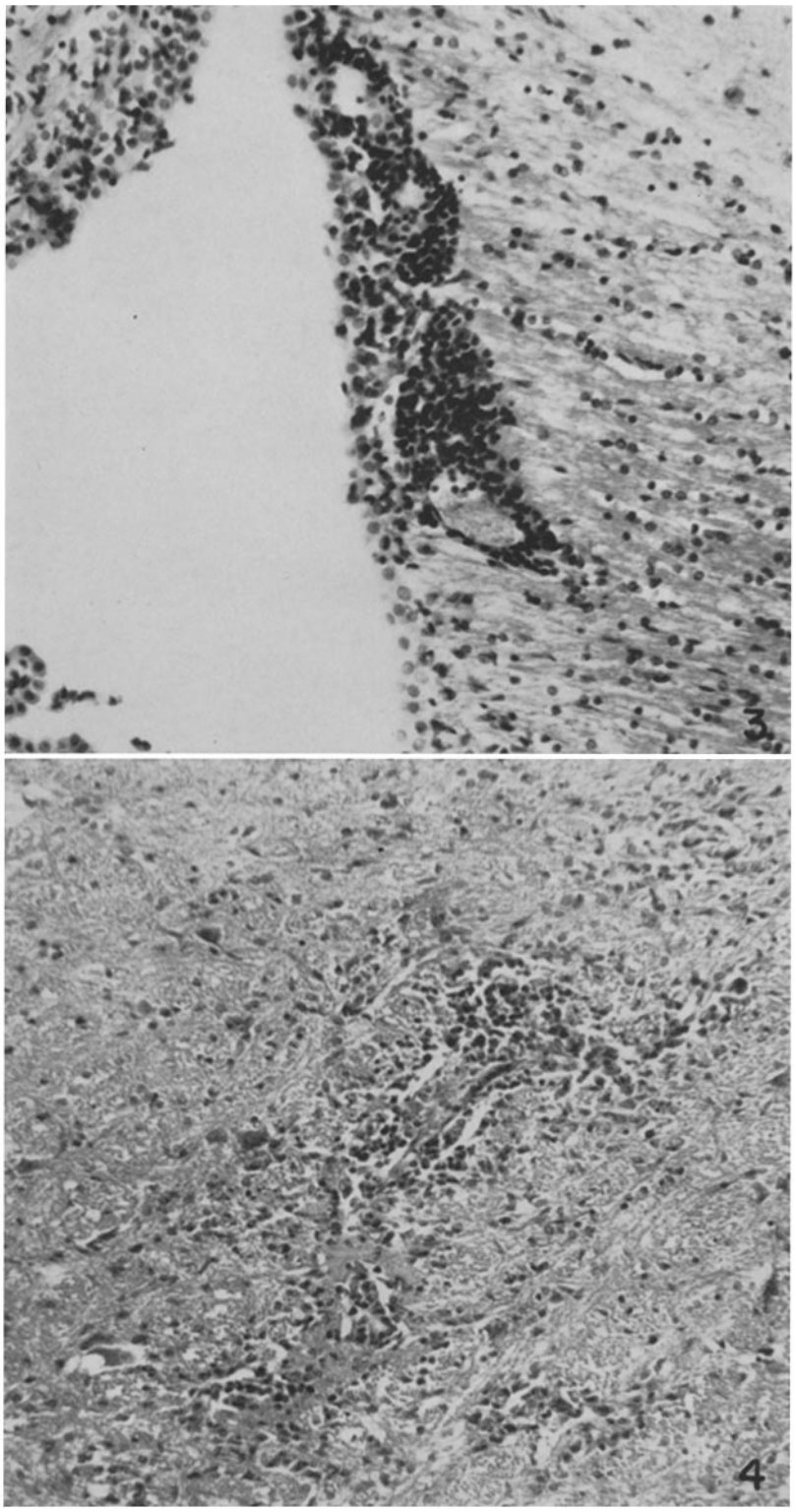


(Paterson: Transfer of allergic encephalomyelitis)

PLATE 4

FIG. 3. Recipient rat C836. Displayed severe ataxia-hindleg weakness 4 days after transfer of donor lymph node cells; sacrificed 8 days after transfer of cells. Two vascular lesions adjacent to ependymal lining of lateral ventricle. Hematoxylin and eosin. × 130.

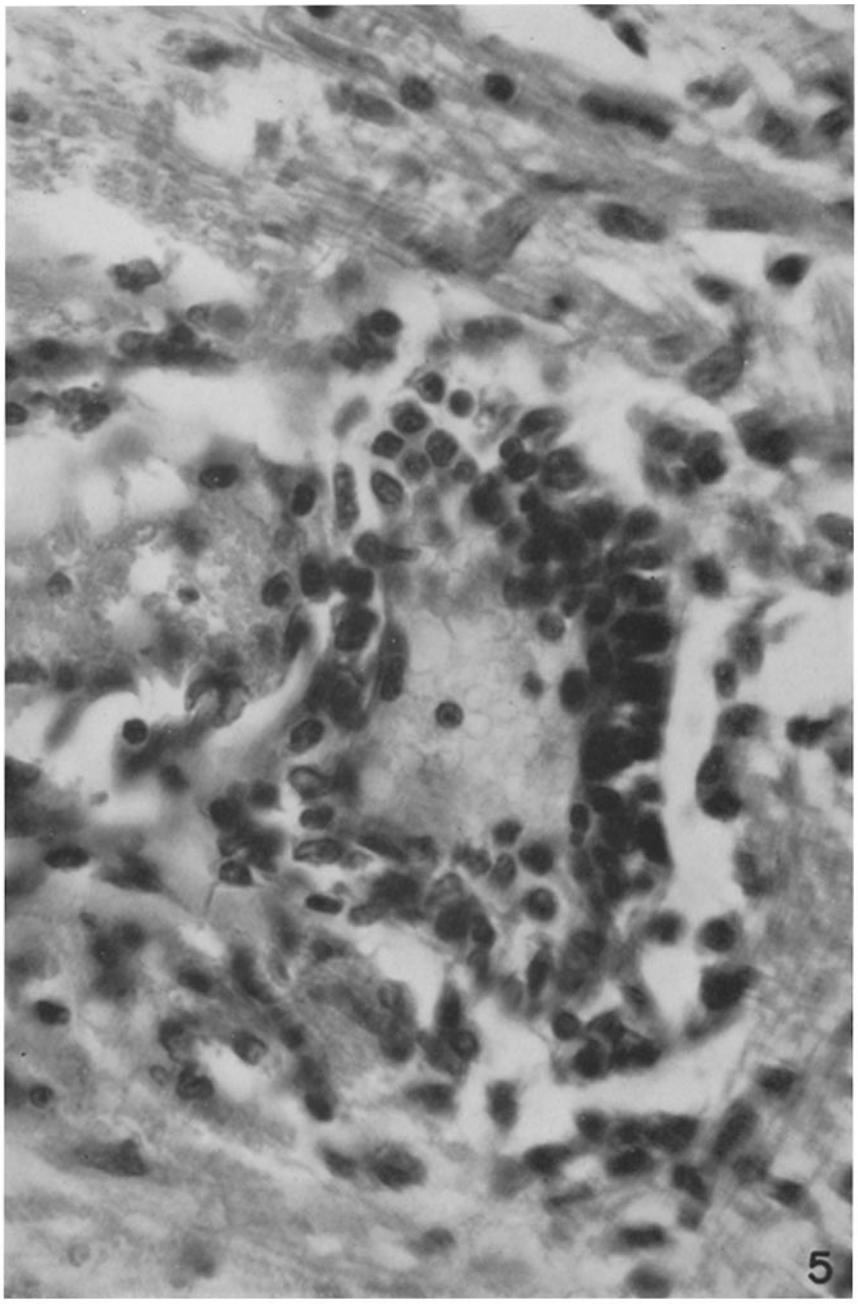
FIG. 4. Recipient rat C415. Sacrificed 6 days after transfer of donor lymph node cells without displaying clinical signs. Vascular lesions in pons. Hematoxylin and eosin. × 125.



(Paterson: Transfer of allergic encephalomyelitis)

PLATE 5

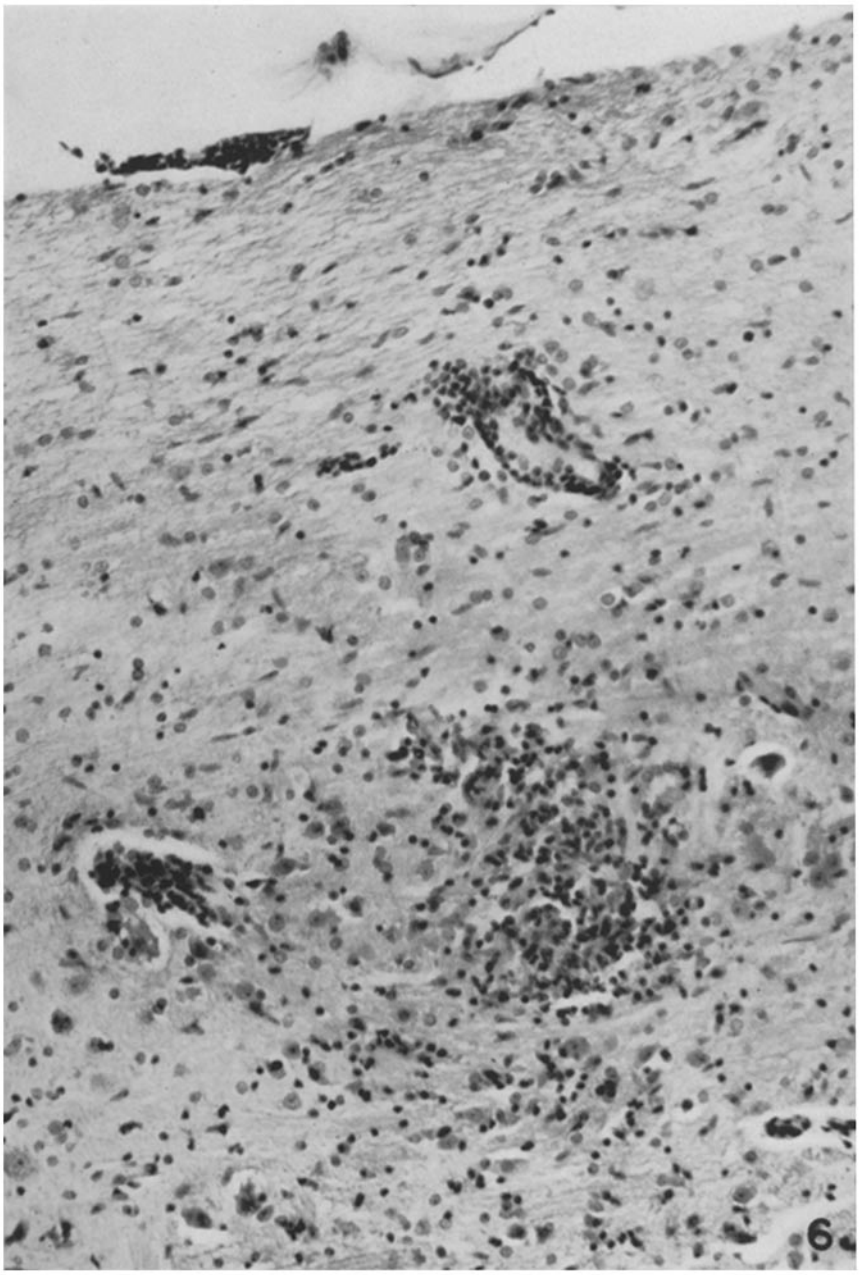
FIG. 5. Recipient rat C412. Sacrificed 6 days after cell transfer. Vascular lesion in pons. Hematoxylin and eosin. × 500.



(Paterson: Transfer of allergic encephalomyelitis)

PLATE 6

FIG. 6. Recipient rat C836. Appearance of hindleg weakness 4 days after donor lymph node cell transfer. Three lesions in longitudinal section of spinal cord. Hematoxylin and eosin. × 130.



(Paterson: Transfer of allergic encephalomyelitis)