CELLULAR TRANSFER OF EXPERIMENTAL
ALLERGIC ENCEPHALOMYELITIS IN
OUTBRED GUINEA PIGS

by
Dorothy Ellen Lewis

A Thesis Submitted to the Faculty of the
DEPARTMENT OF MICROBIOLOGY AND MEDICAL TECHNOLOGY
In Partial-Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE
WITH A MAJOR IN MICROBIOLOGY

In the Graduate College
THE UNIVERSITY OF ARIZONA

1975
STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Dorothy Ellen Lewis

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

WAYBURN S. JETER
Professor of Microbiology and Medical Technology

Date
ACKNOWLEDGMENT

I want to thank my major professor, Dr. Wayburn S. Jeter, for the timely assistance and understanding given to me throughout this study. I also want to thank the entire "P.T. Team" and especially Dr. John Doll for help in the collection of the material. Lastly, I am indebted to both of my parents who instilled in me a desire to know.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vi</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>4</td>
</tr>
<tr>
<td>Animals</td>
<td>4</td>
</tr>
<tr>
<td>Sensitization</td>
<td>4</td>
</tr>
<tr>
<td>Collection of Peritoneal Exudative Cells</td>
<td>5</td>
</tr>
<tr>
<td>Collection of Lymph Node Cells</td>
<td>5</td>
</tr>
<tr>
<td>Passive Transfer Experiments</td>
<td>6</td>
</tr>
<tr>
<td>RESULTS</td>
<td>8</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>15</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>21</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Passive transfer of Experimental Allergic Encephalomyelitis in guinea pigs with peritoneal exudative and lymph node cells</td>
<td>11</td>
</tr>
<tr>
<td>2.</td>
<td>Passive transfer of Experimental Allergic Encephalomyelitis in guinea pigs with incubation fluids from peritoneal exudative and lymph node cells</td>
<td>12</td>
</tr>
<tr>
<td>3.</td>
<td>Passive transfer of Experimental Allergic Encephalomyelitis in guinea pigs with sonically disrupted peritoneal exudative and lymph node cells</td>
<td>13</td>
</tr>
<tr>
<td>4.</td>
<td>Passive transfer of Experimental Allergic Encephalomyelitis in guinea pigs with plasma from antilymphocyte serum treated donors</td>
<td>14</td>
</tr>
</tbody>
</table>
ABSTRACT

Experimental Allergic Encephalomyelitis was transferred passively in outbred guinea pigs by peritoneal and exudative and lymph node cells in 4 out of 7 trials. Attempts at passive transfer with fluids from incubated peritoneal exudative and lymph node cells as well as sonic lysates from these same cellular populations were unsuccessful. In addition, transfers using plasma from antilymphocyte serum treated sensitized donors failed. The possibility of transference of antigen to induce active sensitization is considered, as well as the evidence for the existence of a transfer factor in this system.
INTRODUCTION

Experimental Allergic Encephalomyelitis (EAE) is an autoimmune disease of the central nervous system, demonstrable in a variety of experimental animals, which is induced by single or repeated injections of purified myelin basic protein or whole central nervous system tissue emulsified in Freund's complete adjuvant (1-5).

Prior to 1947, the disease could be evoked in monkeys only by repetitive injections of nervous tissue over a period of months (6,7). When Freund's complete adjuvant was employed, however, investigators found that a single injection of the emulsified material produced rapid destruction of myelin accompanied by clinical signs including loss of weight, incontinence, paralysis, and death in a matter of weeks (2-4).

Transfer of the disease was attempted by employing hyperimmune sera or living lymphoid cells from sensitized animals. Transfer efforts with sera all proved unsuccessful (8-10). Lipton and Freund (11), however, in 1953, did successfully transfer the disease in para-biotic rats. But their experiments did not define the transfer as due to a humoral or cellular mechanism. In 1959, Chase (10) pointed out that EAE may not be transferred until great numbers of living lymphoid cells were used. He also suggested that the life of these cells might be prolonged in the recipient if the strains of animals used were highly inbred.
In 1960, Paterson (12) succeeded in transferring EAE in rats using lymph node cells injected into tolerant recipients prepared by injections of spleen cells in neonatal life. Then, in 1961, Stone (13) transferred the disease in inbred Wright Strain 13 guinea pigs with living lymphoid cells. However, transfer between the Strain 13 inbred animals and outbred Hartley Strain guinea pigs could not be accomplished. Both of these passive transfers were distinguished from actively induced EAE in that a shortened time course (as much as five days) for the development of EAE was observed. The disease has also been passively transferred in rabbits and in chickens (14, 15). The recipient rabbits received 400 rads of X-irradiation 1-8 days before transfer and large numbers of cells were used (14). Jankovic and Isvaneski (15) used approximately $10^8$ cells and transferred the disease in chickens by using spleen cells in homologous but genetically variant hosts.

More recently, Paterson and Weiss (16) accomplished transfer of the disease between Lewis and Wistar Strain rats by intracerebral injection of lymphoid cells. They concluded that this first transfer between two different rat strains was due to the prolonged survival of the cells. Levine and Wenk (17), in 1967, succeeded in the rapid passive transfer of EAE in Lewis Strain rats by increasing the number of cells used for transfer and by employing either adrenalectomy or hydrogen cyanide induced brain damage in the recipients before transfer. They found that brain lesions could be detected in as little as six hours after transfer. In 1967, Flad, Paterson, and Miescher (18) mixed
phytohemagglutinin (PHA) and brain antigen in vitro with EAE sensitized cells from rats to determine whether these substances might enhance cellular activity in the passive transfer of the disease. The PHA caused no apparent change, whereas incubation of the sensitized cells with brain antigen decreased successful transfers. Levine et al. (19), in 1970, using the immunosuppressive drug, cyclophosphamide, reported cellular transfer across a major histocompatibility barrier. The EAE was transferred from Lewis rat donors to BN rat recipients which had received the drug prior to the cell injections.

In 1965, Paterson (20) proposed three possible mechanisms for the transfer of EAE. One of these proposals was the existence of a transfer factor. In 1954, Jeter, Tremaine, and Seebohm (21) reported the transfer of delayed-type skin sensitivity to chemicals in guinea pigs by means of leukocytic extracts. Although some investigators were unable to confirm these results (22), several workers have been able to effect transfer of sensitivity to chemicals as well as in the tuberculin system (23-27).

Since transfer factors are active in other delayed hypersensitivity systems, and because EAE is considered to be a delayed-type manifestation of cell mediated immunity, the following study was undertaken to ascertain the existence of a transfer factor in leukocytic extracts from EAE sensitized lymphoid cells of guinea pigs.
MATERIALS AND METHODS

Animals

Albino guinea pigs of both sexes weighing from 500-800 g from three departmental colonies (Amana, Hartley, and Rockefeller strains) were used in all experiments. Original breeding stock for the Amana colony was obtained from a colony at Amana, Iowa. The original Hartley breeders were from a colony at Fort Dietrick, Md., and the original Rockefeller animals were obtained from Dr. J. R. Battisto, Albert Einstein College of Medicine, New York, New York. The animals were outbred and were maintained on Purina guinea pig diet, daily rations of fresh cabbage and water supplemented with 0.3% ascorbic acid ad libitum.

Sensitization

The antigen employed consisted of whole, fresh or frozen, guinea pig spinal cord (of the same strain), ground aseptically in a mortar with a pestle in a sterile solution of 0.1% phenol in 0.15 M sodium chloride. An equal volume of this spinal cord suspension was combined with a solution consisting of 8.5 parts sterile light mineral oil (Purepac Pharmaceutical Laboratories) and 1.5 parts Arlacel A (Atlas Chemical Industries, Inc.). To this was added 25 mg per ml of heat killed, dry Mycobacterium tuberculosis, strain H37RV. The spinal cord in 0.1% phenol and the Freund's complete adjuvant were combined slowly and were forced through a syringe until a stable emulsion...
resulted (2,28). All animals were sensitized by a single intracutaneous injection of 0.1 ml over a shaved area of skin on the nape of the neck. Each animal received approximately 2 mg wet weight of spinal cord. The suspension was injected either 5, 7, or 9 days before transfer of EAE was attempted (13).

**Collection of Peritoneal Exudative Cells**

Forty-eight hours before collection of peritoneal exudative cells, each animal received 20 ml of sterile light mineral oil intrabdominally. The injection site was cauterized with heat to prevent leakage. To collect the peritoneal exudative cells, each animal was anesthetized with ether, exsanguinated by cardiac puncture, and the abdominal wall opened aseptically. The cells were collected in two washes of the abdominal cavity with sterile Hanks' Balanced Salt Solution (HBSS) containing 10 units per ml of heparin and 10% normal guinea pig serum. The wash fluids were pooled and centrifuged at 500 x g for 15 minutes at room temperature. The cells were washed twice in HBSS plus 10% serum to remove all traces of oil and were then resuspended either HBSS plus 10% normal serum or in HBSS alone.

**Collection of Lymph Node Cells**

Suprascapular, axillary, and cervical lymph nodes were dissected from each animal and were trimmed of fat, minced, and the cells expressed through a stainless steel screen. The cells were centrifuged at 500 x g for 15 minutes at room temperature and washed twice in HBSS plus 10% normal serum. The peritoneal exudative cells and the lymph
node cells were combined and total and viable counts estimated by trypan blue exclusion (29).

**Passive Transfer Experiments**

Passive transfer using peritoneal exudative and lymph node cells combined was accomplished by injecting intraperitoneally at least $10^9$ washed, resuspended cells into a recipient of the same strain weighing between 300 and 500 grams.

In ether passive transfer experiments, combined peritoneal exudative and lymph node cell preparations were incubated with frequent gentle agitation for 4 hours at 37 C in HBSS. After this incubation period, the cells were sedimented at 500 x g for 20 minutes at room temperature and a viable count done. The supernatant fluid and the cell pellet were resuspended in HBSS and were injected intraperitoneally in equal volumes into separate recipients.

In the sonic oscillation experiments, peritoneal exudative and lymph node cell preparations combined were vibrated in a Bronwill Biosonic at a setting of 90 kilocycles in an ice bath for 10-15 one-minute exposures, interspaced by one-minute cooling periods. To assure complete breakage of the cells, a viable count was obtained after sonication. Then the suspension was centrifuged at 500 x g for 20 minutes at room temperature and the supernatant liquid and the cell debris injected intraperitoneally into separate recipients.

Passive transfer was also attempted using plasma from sensitized animals. At 5, 7, or 9 days after sensitization, animals were injected with 12 ml per kilogram body weight of either whole
antilymphocyte serum (ALS) or fractionated antilymphocyte globulin (ALG). The ALS was prepared in goats against guinea pig lymph node cells and its potency determined by the lymphocytotoxicity test (30, 31). The ALG was produced by fractionating the ALS with ammonium sulfate and the ALG was reconstituted to the original ALS volume (32). The ALS or ALG was injected into sensitized animals four hours before collection of plasma. The guinea pigs were anesthetized with ether and exsanguinated and the blood placed into centrifuge tubes containing 10 units per ml of heparin. The blood was then centrifuged at 500 x g for 20 minutes at room temperature. The plasma was drawn off and 20 ml were injected into each recipient.
RESULTS

To determine whether lymphoid cells from EAE sensitized donors could successfully transfer the disease to genetically variant recipients, transfer was attempted using combined peritoneal exudative and lymph node cell populations. Although most other investigators have used only lymph node cells, we felt that a greater number of sensitized cells would be collected by acquiring them from both sources. Accordingly, when possible, more than $10^9$ cells were used for transfer of the disease. The combination of peritoneal exudative and lymph node cells accomplished passive transfer of EAE in 4 out of 7 attempts (Table 1; see end of Results section for Tables 1 through 4). In all successful experiments, animals showed weight loss by day 5 and were near death from EAE when sacrificed. Death was imminent by day 9 in all successful experiments. In the unsuccessful transfers, no animal exhibited signs of the disease was a three-week period of observation. In all experiments a donor animal was allowed to develop EAE as a control on the sensitivity of the donor animals used for passive transfer.

Since transfer of EAE was accomplished among outbred animals in several experiments, transfer with fluids from EAE sensitized cells incubated for four hours at 37 C was next attempted to determine whether a transfer factor was responsible for the passive transfer of the disease. However, all transfers with such incubation fluids made on day 7 after sensitization of the donor animals were unsuccessful (Table 2).
On the other hand, in two of the six experiments, transfer was accomplished with the pellet of cells remaining after incubation.

Since transfers with incubation fluids were unsuccessful but were effected in two cases with the remaining cell pellet, we thought that the transfer factor might not have been released from the cells in the four-hour period of incubation. To test this hypothesis, sonic disruption of the cells was attempted on day 7 after the disease was initiated in the donors. Transfers with both supernatant fluid from sonically disrupted cells and the resuspended pellet failed to transfer the disease (Table 3). Viable cell counts of both the supernatant fluid and the sediment showed that complete disruption of cells occurred. In one experiment, transfer was attempted at nine days after sensitization to determine whether a longer incubation period in the donor might make more transfer factor available. However, the increased time of sensitization made no difference.

Since disruption of cells in vitro was unsuccessful, it was possible that in vivo release of lymphocytic products could be accomplished by using plasma from ALS treated sensitized donors to effect transfer. These attempts proved unsuccessful. To rule out the possibility of a temporal factor again, transfer was attempted at 5, 7, or 9 days after donor sensitization. All of these transfers with plasma at different intervals after sensitization failed to evoke EAE over a three-week observation period (Table 4). The use of either whole ALS or fractionated ALG made no difference in the efficacy of transfer. However, more plasma was recovered when ALG was used. When enough
plasma was available, dose responses using increasing amounts of plasma were also attempted. As much as 30 ml of plasma did not produce the disease. In one instance, a recipient died two days after transfer. However, this appeared to be due to the toxicity of the plasma rather than the development of EAE.
Table 1. Passive transfer of Experimental Allergic Encephalomyelitis in guinea pigs with peritoneal exudative and lymph node cells.

<table>
<thead>
<tr>
<th>No. of donors</th>
<th>Strain&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of cells x 10&lt;sup&gt;9&lt;/sup&gt;</th>
<th>Signs observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>H</td>
<td>3.0</td>
<td>Dead on day 8</td>
</tr>
<tr>
<td>6</td>
<td>H</td>
<td>0.03</td>
<td>Dead on day 7</td>
</tr>
<tr>
<td>17</td>
<td>R</td>
<td>3.6</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>A</td>
<td>3.15</td>
<td>Dead on day 8</td>
</tr>
<tr>
<td>9</td>
<td>H</td>
<td>1.025</td>
<td>Dead on day 9</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>0.0033</td>
<td>None</td>
</tr>
<tr>
<td>17</td>
<td>H</td>
<td>1.0</td>
<td>None</td>
</tr>
</tbody>
</table>

<sup>a</sup> H, Hartley strain; R, Rockefeller strain; A, Amana strain.
### Table 2. Passive transfer of Experimental Allergic Encephalomyelitis in guinea pigs with incubation fluids from peritoneal exudative and lymph node cells.*

<table>
<thead>
<tr>
<th>No. of donors</th>
<th>Strain ( a )</th>
<th>No. of cells ( x 10^9 )</th>
<th>Signs observed Fluid</th>
<th>Signs observed Pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>H</td>
<td>1.66</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>16</td>
<td>A</td>
<td>2.0</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>17</td>
<td>R</td>
<td>3.6</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>A</td>
<td>1.75</td>
<td>None</td>
<td>Dead on day 9</td>
</tr>
<tr>
<td>9</td>
<td>H</td>
<td>2.05</td>
<td>None</td>
<td>Dead on day 9</td>
</tr>
<tr>
<td>17</td>
<td>H</td>
<td>1.0</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

* Peritoneal exudative and lymph node cells were incubated at 37 °C for four hours. After centrifugation, the fluid and the pellet were injected into separate recipients.

\( a \). H, Hartley strain; R, Rockefeller strain; A, Amana strain.
Table 3. Passive transfer of Experimental Allergic Encephalomyelitis in guinea pigs with sonically disrupted peritoneal exudative and lymph node cells. *

<table>
<thead>
<tr>
<th>No. of donors</th>
<th>Strain&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of cells x 10&lt;sup&gt;9&lt;/sup&gt;</th>
<th>Signs observed Supernatant</th>
<th>Pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>A</td>
<td>0.345</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>A</td>
<td>6.75</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>13</td>
<td>A</td>
<td>6.71</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>15</td>
<td>A</td>
<td>7.15</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

* Peritoneal exudative and lymph node cells were disrupted in a Bronwill Biosonic at a setting of 90 kilocycles for 10-15 minutes and the supernatant fluid and the pellet injected into separate recipients.

<sup>a</sup> A, Amana strain.
Table 4. Passive transfer of Experimental Allergic Encephalomyelitis in guinea pigs with plasma from antilymphocyte serum treated donors.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Exp. no. Recipient letter</th>
<th>Day of transfer\textsuperscript{b}</th>
<th>Strain\textsuperscript{c} (No. of donors)</th>
<th>Volume of plasma injected</th>
<th>Signs observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a*</td>
<td>7</td>
<td>A (6)</td>
<td>25 ml</td>
<td>None</td>
</tr>
<tr>
<td>1b*</td>
<td></td>
<td></td>
<td>25 ml</td>
<td>None</td>
</tr>
<tr>
<td>2a*</td>
<td>7</td>
<td>H (3)</td>
<td>24 ml</td>
<td>None</td>
</tr>
<tr>
<td>3a*</td>
<td>7</td>
<td>H (4)</td>
<td>25 ml</td>
<td>None</td>
</tr>
<tr>
<td>4a**</td>
<td>9</td>
<td>R (5)</td>
<td>20 ml</td>
<td>None</td>
</tr>
<tr>
<td>4b**</td>
<td></td>
<td></td>
<td>25 ml</td>
<td>None</td>
</tr>
<tr>
<td>4c**</td>
<td></td>
<td></td>
<td>30 ml</td>
<td>None</td>
</tr>
<tr>
<td>5a**</td>
<td>9</td>
<td>R (5)</td>
<td>25 ml</td>
<td>None</td>
</tr>
<tr>
<td>5b**</td>
<td></td>
<td></td>
<td>25 ml</td>
<td>None</td>
</tr>
<tr>
<td>6a**</td>
<td>5</td>
<td>R (5)</td>
<td>20 ml</td>
<td>None</td>
</tr>
<tr>
<td>6b**</td>
<td></td>
<td></td>
<td>25 ml</td>
<td>Dead on day 2</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Antilymphocyte serum (12 ml/kilogram body weight) was injected four hours before collection of plasma and the plasma was then injected into separate recipients.

\textsuperscript{b} Indicates day of transfer of plasma after donor sensitization.

\textsuperscript{c} H, Hartley strain; A, Amana strain; R, Rockefeller strain.

\textsuperscript{*} Antilymphocyte serum used was unfractionated.

\textsuperscript{**} Antilymphocyte globulin was used.
DISCUSSION

Successful passive transfer experiments using peritoneal exudative and lymph node cell populations demonstrate that transfer of EAE can be accomplished between genetically variant guinea pigs. Since Chase (10) suggested in 1959 that the use of inbred animals would prolong the life of the transferred cells in the recipient, all attempts at transfer have been done using either pretreated tolerant animals or inbred strains. Early attempts at transfer of the disease (9) were unsuccessful, but Hill used a 1:1 donor recipient ratio for his experiments and, in other cases, attempts were made too late, after symptomatology was pronounced (16-22 days). Stone (13) later showed in 1961 that the disease could be transferred only from day 5 to day 13 after donor sensitization in the guinea pig. In addition to Hill's study, three experiments were reported by Chase (10) in 1959 in which he attempted to transfer passively the disease in outbred guinea pigs. Although he used a large donor to recipient ratio, his attempts were also made late in the course of the disease (13-15 days). Vulpe (in 33) reported in 1959 that he used 64 outbred guinea pig donors and collected cells from a portion of these on successive days after sensitization (7, 8, 9, 11, 13, 14). Cells from each day were injected into six recipients and none showed clinical disease. Only one had histological lesions.
In our experiments, 4 out of 7, or 57%, of the transfers with living cells were successful. This percentage is comparable with both Paterson's data (12) and with Stone's data (13). Paterson had a 63% success rate while Stone had 56% of his transfer experiments result in success when only the severe signs are tabulated. When minor signs of EAE, such as loss of weight, are incorporated into his data he achieved an 81% success rate. These comparisons indicate that inbred strains are not necessary for the successful passive transfer of EAE and that there is a certain percentage of unsuccessful transfers that result from unknown biological factors, such as donor sensitivity, insufficient numbers of cells, recipient insusceptibility and, possibly, the mechanisms of the transfer of information and the site of the disease itself.

It is possible that active sensitization of the recipient could be held responsible for the successful transfer of the disease, if antigen is inadvertently introduced along with the cellular preparations. However, in all unsuccessful cell transfers, the recipient animals failed to develop any clinical signs of the disease over a three-week period of observation. In addition, other investigators have shown that active sensitization is highly unlikely (17, 34). First, there is a difference in the time course. Actively induced EAE requires from 10-12 days to develop, while passively induced EAE develops in 5-8 days. Other evidence against active sensitization was demonstrated by Falk, Kies, and Alvord (34). They employed serial dilutions of spinal cord and Mycobacterium tuberculosis injected intraperitoneally
into normal guinea pigs. The lowest dose that could actively induce
the disease was 0.8 mg spinal cord (dry weight) per animal. The dose
used to sensitize donors in this study was approximately 2 mg wet
weight per animal. This is equal to approximately 0.028 mg dry weight.
Since the greatest number of donors used that resulted in transfer was
9, this gives approximately 0.252 mg total. This amount is well below
the 0.8 mg used in Falk's study. Falk et al. (34) also heated the cells
used for transfer to 60 C for 30 minutes as an added control for their
passive transfer experiments. This treatment resulted in a loss of
transfer. Since basic protein is not heat inactivated at this tempera­
ture, they concluded that if active sensitization was occurring in
their experiments, the transfer of the disease should occur regardless
of the heat treatment. Additional evidence against active sensitiza­
tion is the demonstration of the rapid passive transfer of EAE in the
brain by Levine and Wenk (17). They succeeded in producing the lesions
of EAE in as little as six hours. This suggests that sensitized lympho­
cytes are capable of producing the histological signs of EAE, presumably
without stimulation by introduced antigen.

The mechanism that is responsible for the transfer of EAE has
not been elucidated in this study. We did not find evidence of a
transfer factor in any of the leukocytic extracts. However, it is pos­
sible that there is a transfer factor associated with the disease. The
evidence for this belief includes the fact that transfer with cells
does not occur if the cells are inactivated by heating at 60 C for 30
minutes. Transfer factor in some other systems is inactivated by heat­
ing. In addition, Werdelin and McCluskey (35) showed in a series of
tritium labeled nucleoside experiments using either labeled donor cells or labeled recipient cells that X-irradiation of the recipient one day after transfer of tritium labeled donor cells did not affect the development of the disease. The authors suggest that sensitized donor lymphocytes are not required to be continually present in the lesion and in some way change the area so the disease process can ensue. This change could be explained by a transfer factor that initiates the response. Finally, Flad et al. (18) showed that in vitro incubation of sensitized lymphocytes with brain antigen caused a decrease in successful transfers. This decrease could be explained by the existence of a transfer factor that is liberated in the presence of antigen.

In view of this suggestive evidence, why do the leukocytic extracts fail to transfer the disease? The key may be the existence of the blood brain barrier. The barrier is defined by most investigators as consisting of the cerebral capillary endothelium, the basement membrane and the astrocytic end feet (36, 37). The precise anatomical location is under debate, but most agree that peculiarities in the cerebral capillaries and their relative position to neuroglial cells account for the barrier (38). The barrier at one time was thought to be absolute to cationic substances and to certain dyes (36), but it was later discovered that in many cases the control of the passage of a particular substance was a concentration, simple diffusion, or active transport problem (39).

With this background information, a consideration of the possible activities of injected donor cells follows. The sensitized
peritoneal exudative and lymph node cells are injected intraperitoneally. Then they enter the lymphoid system and the blood stream. Information is transferred such that the recipient's lymphocytes are sensitized. These sensitized cells enter the blood stream and pass through the cerebral capillaries. Normally, these lymphocytes would not leave the cerebral capillaries (38). So, how is the blood brain barrier altered to account for the passage of cells that are found in histological sections in the later part of the disease? Perhaps toxic substances affecting the permeability of the barrier are released with subsequent entry of the lymphoid cells themselves. Only when these cells and their products are allowed free entry into the white matter can the degradation of myelin and the ensuing clinical signs develop.

With this hypothesis in mind, the leukocytic extracts in our experiments may indeed contain a transfer factor which transfers skin sensitivity as do other transfer factors in other systems. Little is known about how transfer factor in other systems does this. It may be that the transfer factor in this system cannot cause the necessary products for initial damage of the barrier to be elaborated by the recipient's cells. Hence, the disease did not develop with any of our leukocytic extracts because there was no initial damage to the barrier. If this analysis of the pathogenesis is correct, it is not incredible that the intracerebral injection of lymphocytes (16) accomplish transfer between strains of rats because the barrier is affected even by the injection procedure. The six-hour development of EAE as demonstrated by Levine and Wenk (17) also allows for initial breakdown of the
barrier. In addition, Barlow (40) demonstrated increased permeability of the brain in EAE using trypan blue perfusion. Tourtellotte and Parker (41) also showed in the human correlate of EAE, Multiple Sclerosis, that an increased permeability in post mortum examination of white matter of certain anions and of endogenous serum proteins was in evidence. Thus, it becomes plausible that the existence and intricacies of the blood brain barrier could account for the difficulties encountered in the transfer of EAE.

Another plausible explanation for the failure of leukocytic extracts to transfer the disease is the recent information (42) that delayed skin sensitivity to basic protein may not correlate entirely with disease production. If this is so, then the transfer factor postulated in this system may not be sufficient to elaborate the full clinical response and thus may be incapable of transferring the disease regardless of the mechanisms of entry into the brain.
LITERATURE CITED


