tration of virus in other studies(3). Also receptor-destroying enzyme has been used by Wagner(4) to show the temperature dependence of the penetration phase.

With influenza in minced chorioallantoic membrane a similar early suppression may be demonstrated by using higher concentrations of NH_4^+; but the increased NH_4^+, even when added after 4 hours, reduces HA yields to about 25% of the control. This is attributed to a toxic effect during the subsequent 24-hour incubation period.

Discussion. Jensen, Force and Unger(5) describe with influenza virus in another species of cell results essentially similar to ours(2). Using a stable line of epithelial cells originating from dog kidney, they were able to demonstrate protection by ammonium ions against the cytopathic effect of the PR8 strain. Similarly glutamine from which ammonia is formed will in the presence of bicarbonate protect Krebs 2 cells against the oncolytic action of influenza virus(2,6). These observations suggest that NH_4^+ at appropriate concentration may have a chemoprophylactic effect in certain cell-virus systems maintained *in vitro*. The concentration of NH_4^+ of 0.005% to 0.01% found to be non-toxic to cells in tissue culture but inhibitory for virus(5) is, however, greatly in excess of tolerated blood levels in the intact animal.

The ammonia effect seems to be most easily demonstrable under conditions favoring formation of large amounts of incomplete virus, although reduction of infectivity titers has been reported(5). Preliminary results with cultures of chorioallantoic membrane and influenza virus suggest that suppression of viral growth by NH_4^+ or glutamine may also occur in cells which produce complete infectious virus.

The process affected by NH_4^+ seems to require cellular energy production, but when oxidative metabolism is inhibited the requisite energy appears to be furnished by glycolysis. Because of the short duration of the period of NH_4^+ sensitivity after adsorption of virus, consideration can be given only to a mechanism which involves initial integration of the viral inoculum with cytoplasmic or nuclear components of the host cell. Ammonia may modify a biosynthetic process essential to viral invasion.

Summary. Following adsorption of influenza virus by Krebs 2 ascites cells there is a brief period of sensitivity to inhibition of viral replication by ammonia. After an hour at 35°C addition of ammonium phosphate causes no inhibition. The period of ammonium sensitivity is prolonged by low temperature, cyanide, or 2-4 dinitrophenol, but the presence of glucose in the medium prevents this effect of CN and DNP. The results suggest that the ammonium-sensitive phase of infection is an energy-requiring process which occurs soon after adsorption and penetration, and comprises initial integration of the viral inoculum with cell components.

1. Eaton, M. D., Jewell, M., Scala, A. R., *Virology*, 1960, v10, 112.
2. ———, Scala, A. R., *ibid.*, 1961, v13, 300.
3. Levine, A. S., Bond, P., Scala, A. R., Eaton, M. D., *J. Immunol.*, 1956, v76, 386.
4. Wagner, R. R., PROC. SOC. EXP. BIOL. & MED., 1955, v89, 103.
5. Jensen, E. M., Force, E. E., Unger, J. B., *ibid.*, 1961, v107, 447.
6. Eaton, M. D., Scala, A. R., Rouse, H. C., *ibid.*, 1960, v103, 832.

Received September 11, 1961 P.S.E.B.M., 1961, v108

Transfer of Allergic Encephalomyelitis Using Splenectomized Albino Rats.** (27061)

PHILIP Y. PATERSON* AND NORMAN C. DIDAKOW (Introduced by Lewis Thomas)
Department of Medicine, New York University, School of Medicine, New York City

Allergic encephalomyelitis (AE), induced in rats and other animals by injection of spinal cord plus Freund's adjuvant, has been under study in our laboratory as a model for

** This work supported by U. S. Public Health Service Grant.

* Recipient of Investigatorship of Health Research Council of City of New York.

defining immune mechanisms implicated in autoimmune disease(1). Although AE is believed on good grounds to have an immunological mechanism, the nature of the immune agent directly responsible for the characteristic encephalomyelopathy has remained unknown. The transfer of AE from spinal cord-sensitized donors to normal animals with cells or serum provides one means of clarifying whether the responsible immune agent is cellular or humoral in nature. Chase(2) has reviewed the past unsuccessful efforts of various investigations to achieve transfer of AE with serum or cells and using intact, randomly bred animals. Our attempts to accomplish transfer in this fashion have given similarly negative results(3).

Lipton and Freund(4) reported that AE may be transferred in rats by means of parabiosis. Paterson(5) subsequently described successful transfers of AE in randomly bred albino rats using lymph node cells (LNC) of sensitized donors and recipients which had been rendered immunologically tolerant by pretreatment with normal rat spleen cells. Best results were noted in recipients that received normal spleen cells of splenectomized prospective LNC donors. Our success was attributed, in part, to the use of tolerant recipients in which the donor LNC, by not being rejected by the recipient, could survive and function for a longer period of time and, thus have a better opportunity to react with the nervous tissue of the recipient.

Continued search for other methods of AE transfer in this laboratory have included: injection of donor LNC either into the carotid artery or into the brain of recipients, prolonged cross-circulation of donor-recipient pairs, use of heavily X-irradiated recipients and highly inbred intact rats. These methods of transfer gave only an occasional convincing positive transfer.

Our previous success in transferring AE with LNC from splenectomized donors(5) suggested to us that the spleen might well play a role in AE and its transfer. Initial experiments indicated that, indeed, splenectomy of sensitized donors permitted LNC transfer of AE to intact albino recipients. While our work was in progress, Koprowski et al(6) stated that splenectomy facilitated LNC transfer of AE within a highly inbred strain of rat. Their report did not make clear whether splenectomy of both donor and recipient gave better results than splenectomy of only the donor or only the recipient.

The present report is an extension of our earlier study(5) and describes the transfer of AE using LNC and splenectomized, commercially available albino rats. Our data agree with those obtained by Koprowski et al(6) in an inbred strain of rat and, in addition, indicate that splenectomy of only the donor is sufficient to facilitate transfer of AE to intact recipients.

Materials and Methods. The animals employed as donors and recipients were CFN ("specific pathogen free") Wistar rats† weighing between 125-175 g each. All techniques concerning splenectomy, intracutaneous sensitization of donors with guinea pig spinal cord plus adjuvant, preparation of donor LNC suspensions and processing of recipient brains and spinal cords for microscopic examination were carried out as previously described(5). Recipients routinely received 1 to 2 mg of heparin†† intravenously a few minutes prior to intravenous injection of donor LNC suspensions to prevent thromboplastin death. LNC preparations were periodically stained with methylene blue dye to assess their viability, and approximately 80% of the cells in a given preparation appeared viable as judged by their failure to take up this dye. Ratios of donor to recipients were usually 1:1; in one transfer experiment this ratio was 1:2.

Results. The results of 3 transfer experiments are shown in Table 1. Transfer of LNC from 11 splenectomized donors sensitized to spinal cord plus adjuvant to 12 splenectomized recipients gave positive results in 5 of these recipients. Each of these 5 recipients was found to have a few to numerous focal areas of vascular and perivascular inflammation characteristic of AE readily demonstrable with the brain or/and spinal cord. Transfer of LNC from 6 splenectomized donors to 6 intact (non-splenectomized) recipients also gave

† CFN albino rats were obtained from Carworth Farms, New City, N. Y.

†† Heparin Sodium, 1000 units (app. 10 mg) per ml, Connaught Medical Research Laboratories, Toronto, Canada.

TABLE I. Transfer of Allergic Encephalomyelitis (AE) in Splenectomized Rats using Lymph Node Cells.

Donor Rats*		Recipient Rats†		
No. of Rats	Splenectomized	No. of Rats	Splenectomized	AE Lesions
11	+	12	+	5/12‡
6	+	6	0	2/6
6	0	6	+	0/6
5	0	5	0	0/5

* Donor rats splenectomized 7-14 days before sensitized to guinea pig spinal cord plus Freund's adjuvant; lymph node cells obtained 6 or 7 days after sensitization.

† Recipient rats splenectomized 7-12 days before injection of donor lymph node cells; sacrificed for histological examination 10 or 11 days after single intravenous injection of from 200 to 500 x 10^6 donor lymph node cells.

‡ Numerator—No. of recipients with AE lesions; denominator—No. of recipients used.

clearcut positive results in 2 of these 6 recipients. In contrast, transfer of comparable numbers of LNC from 6 intact donors to 6 splenectomized recipients gave negative results, none of the 6 recipients having demonstrable microscopic abnormalities within their central nervous system. Transfer of comparable LNC from 5 intact donors to 5 intact recipients also gave negative results in agreement with our past attempts to transfer AE in intact albino rats. The data (Table 1) concerning transfer of AE from splenectomized donors to intact recipients have been fully confirmed, viz., of 6 additional intact recipients which received LNC of 6 splenectomized and sensitized donors, respectively, 2 of the 6 recipients were found to have characteristic lesions of AE.

None of the recipients exhibited clinical neurological signs of AE following LNC transfer, including the 7 animals which had typical AE lesions. This observation was consistent with the uncommon appearance of paralysis noted previously in tolerant albino rats(5). It may also be noteworthy that the success of transfer in these experiments (Table 1) as judged by the proportion of recipients having demonstrable lesions of AE, was less than that previously noted using pretreated and presumably tolerant albino recipient rats.

Control LNC transfers using splenectomized donors sensitized to guinea pig kidney plus adjuvant or adjuvant only and transfer of killed LNC (heated at 56°C × 30 min) obtained from splenectomized donors sensitized to spinal cord plus adjuvant have given negative results.

Discussion. The data (Table 1) indicate that AE may be transferred in splenectomized albino rats using LNC of donors sensitized to spinal cord plus adjuvant. The disease may be transferred with LNC of splenectomized donors to either splenectomized or intact (non-splenectomized) recipients. In contrast, LNC of sensitized intact donors do not appear capable of transferring AE to splenectomized or intact recipients. These results suggest that splenectomy of donors plays a more important role in facilitating AE transfer than does splenectomy of recipients.

Preliminary work using an inbred strain of rat (Fisher 44)††† confirms in full the observation of Koprowski et al(6) that splenectomy facilitates AE transfer in inbred rats. Our work to date with the Fisher 44 strain suggests that it is the splenectomy of the donor that facilitates transfer, since splenectomy of the recipient alone exerts little, if any, influence on the outcome of LNC transfer. In contrast to our transfers employing albino rats, we have noted appearance of hindleg paralysis and particularly intense disseminated lesions of AE in Fisher 44 rats following injection of LNC from splenectomized Fisher 44 donors. This observation provides further support for the thesis previously stated(5) concerning the importance of histocompatibility between donor and recipient with respect to success of AE transfer with LNC.

The role of the spleen in AE and its transfer is under study. It is conceivable that the spleen acts as a "trap" for circulating LNC and that by removing the spleen a greater number of LNC potentially capable of transferring AE are permitted to circulate and return to lymph nodes from which they originated. If such were the case, lymph nodes of splenectomized and spinal cord-sensitized donors might well contain a larger population of "sensitized" cells capable of transferring AE than found in lymph nodes of non-splenectomized donors. It is also possible that the spleen may produce the factor (presumably antibrain antibody) present in immune sera of rats recovered from

††† Fisher 44 strain of rat, known to be highly inbred, provided by Nat. Inst. Allergy and Infect. Dis., N.I.H., Bethesda, Md.

AE which Paterson et al(7) found effective in suppressing or preventing AE when passively administered to actively sensitized rats. Splenectomy might result in less production of this circulating factor and thereby permit LNC of a sensitized donor to be more efficacious in transfer.

It should be reemphasized here that the present, as well as our earlier(5), transfer studies in rats do not settle the question as to whether "sensitized" cells (delayed or tuberculin-type hypersensitivity) or conventional antibodies are directly responsible for initiating the damage recognized as AE. For it cannot be said as yet whether the lesions in recipients result from antibodies elaborated and shed by donor lymph node cells after their transfer or by interaction of the donor cells directly with antigen in the recipient's own nervous system. The simplified method of transferring AE in commercially available rats, described here, may help in answering this question.

Summary. Previously described methods of transferring allergic encephalomyelitis in commercially available randomly bred, albino rats sensitized to nervous tissue plus adjuvant, entailed use of parabiosis or pretreatment of recipients with normal spleen cells of prospective sensitized lymph node cell donors. Further study indicates that the disease may be transferred using lymph node cells of splenectomized donors sensitized to nervous tissue and either intact or splenectomized recipients. Splenectomy of only the recipient does not appear to facilitate transfer of the disease.

1. Paterson, P. Y., in *Cellular and Humoral Aspects of the Hypersensitive States*, edit. by H. S. Lawrence, P. B. Hoeber, New York, N. Y., 1959, p. 469.

2. Chase, M. W., in *Allergic Encephalomyelitis*, edit. by M. W. Kies, E. C. Alvord, Charles C. Thomas, Springfield, Ill., 1959, p. 348.

3. Paterson, P. Y., Harwin, S. M., Didakow, N. C., unpublished observation.

4. Lipton, M. M., Freund, J., *J. Immunol.*, 1953, v71, 380.

5. Paterson, P. Y., *J. Exp. Med.*, 1960, v111, 119.

6. Koprowski, H., Jervis, G. A., Schoeniger, D., *Fed. Proc.*, 1961, v20, 38 (Abst.).

7. Paterson, P. Y., Harwin, S. M., Didakow, N. C., *J. Clin. Invest.*, 1961, v40, 1069 (Abst.).

Received September 14, 1961 P.S.E.B.M., 1961, v108

Mann-Williamson Rat. (27062)

YASUO KUROYANAGI AND H. NECHELES*

Department of Gastrointestinal Research, Medical Research Institute of Michael Reese Hospital, Chicago, Ill.

The Mann-Williamson (M.W.) operation in the dog has been widely used in the study of experimental ulcer(1). We have succeeded in producing ulcer in the rat by a modified M.W. operation; the method and subsequent observations are reported here. We have used rats because they are cheaper than dogs and large numbers can be kept in relatively small space; also, the fact that anatomy and physiology of the stomach of the rat differ from that of the dog made the study desirable.

Methods. Fig. 1. Albino rats of Sprague-Dawley strain (both sexes) with initial weights between 172 g and 512 g (average 284 g) are fasted for 24 hours, water being given *ad lib.* The animal is etherized, and under clean but not aseptic precautions the abdomen is opened by a small incision in the upper midline; the pylorus is ligated and cut just proximal to the ligature. A 5-0 black silk thread is passed through the upper edge of the gastric stoma and secured with a small hemostat. The duodenum is pulled up; the bile duct with many pancreatic ducts entering it is seen coursing through the mesentery; it opens into the duodenum 2 to 3 cm below the pylorus. The jejunum is transected about 1

* Supported by U.S.P.H. grant A-2166.