quantitative discrepancies might be explained by employment of a non-physiological steroid, by administration of only one dose daily, by differing sensitivities of various target structures, or other factors. However, we have not excluded other explanations for the enhancing effect of adrenalectomy, including direct effects of abnormal steroid levels on the nervous system(6).

Summary. Adrenalectomy or adrenal enucleation increased the incidence and severity of experimental allergic encephalomyelitis in rats. This effect was reversed by cortisone. These data are related to the effects of adrenalectomy and steroids on inflammatory lesions in inoculation sites and draining lymph nodes, but other mechanisms are not excluded.

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Lymphatic Dissemination of Encephalitogenic Emulsion and Its Relation to Prevention of Allergic Encephalomyelitis by Lymphadenectomy.* (27800)

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Excision of the regional (popliteal) lymph node draining the site of inoculation of nervous tissue-adjuvant emulsion has prevented experimental allergic encephalomyelitis (EAE) in rabbits(1,2). However, removal of the popliteal lymph node either 5 days before or 7 days after foot pad inoculation of mycobacterial adjuvant has failed to prevent arthritis ("adjuvant disease") in rats(3). Nevertheless, there is evidence that both conditions are due to delayed hypersensitivity to constituents of nervous tissue and mycobacteria respectively(3). This apparent discrepancy can be reconciled by the following observations on dissemination of encephalitogenic emulsions in the lymphatic system of the rat and prevention of EAE by lymphadenectomy.

Methods. General procedures, preparation of encephalitogenic emulsion, clinical and histologic methods and evaluations have been described in the accompanying paper(4). Female Sprague-Dawley and CFN-derived rats, strains of relatively low susceptibility to EAE(5), were given pertussis vaccine intraperitoneally, 0.6 ml diluted to 3.0 ml with saline, 4 days before challenge, to increase reactivity(6). Female Hemlock Hollow Wistar rats, highly susceptible to EAE, were used without pretreatment. Lymphadenectomies were performed under pentobarbital (25 mg/kg) anesthesia supplemented with ether. Care was taken to remove all members of lymph node chains. Inguinal nodes were removed with surrounding fat and mammary tissue through a groin incision. Axillary and elbow nodes were removed through an incision just below the axillary fold. Sham operation consisted of a like incision and dissection sufficient to visualize the node without removing it. Wounds were closed with clips. Surgery was performed immediately before inoculation with encephalitogenic emulsion or 7 days later. Rats were sacrificed 21 days after challenge.

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TABLE 1. Prevention of EAE by Excision of Axillary, Inguinal and Elbow Lymph Nodes in Rats Challenged with Encephalitogenic Emulsion in Right Flank.

Surgical procedure		No. of rats	Clinical EAE (% incidence)	Histologic EAE (severity)*
None		30	63%	2.7
Excision	R, D7	11	9	.5
"	R, D0	11	0	.7
"	R-L, D7	22	0	.3
"	R-L, D0	10	0	.6
"	L, D7	9	67	2.9
"	L, D0	10	20	2.5
Sham	R-L, D7	21	81	2.6
"	R-L, D0	10	40	1.2
Hemigirdle	R, D0	10	10	1.0
"	L, D0	10	0	1.4

Abbreviations: R, right; L, left; D7, 7 days after challenge; D0, day of challenge.

* Group avg, graded individually from 0 to 4 according to severity.

Results. Foot pad inoculation. Encephalitogenic emulsion was widely disseminated following injection of 0.05 ml into right hind foot pad of 10 control rats. Many oil droplets, representing metastatic particles of injected emulsion, with granulomatous and suppurative lesions(5), were observed in right popliteal nodes of all 10 and in right inguinal nodes of 8 rats. Among more distant nodes, 8 right lumbar, 3 left lumbar, 3 right axillary and 3 left inguinal nodes had lesions, and a few of these were severe. The incidence and severity of EAE in this group were essentially the same as that in a group of 12 rats subjected to excision of right popliteal and inguinal nodes 7 days after challenge. At sacrifice, remaining lymph nodes of the operated rats had even more lesions than corresponding nodes in control rats. The presence of emulsion in these distant nodes explained the failure to prevent EAE by popliteal-inguinal lymphadenectomy.

Flank inoculation. Dissemination of encephalitogenic emulsion was less widespread after injection of 0.05 ml into each of 2 adjacent intradermal sites in the mid-axillary line of the right flank, cephalad of the inguinal lymph node level. Among 30 control rats, there were oil droplets and granulomatous and suppurative lesions in all right axillary and most right inguinal nodes. Minor lesions were present in only 6 right elbow, 1 left axillary and 1 left inguinal node. No lesions were found in left elbow, right or left popliteal, superficial cervical or lumbar lymph nodes. Removal of right (or right and left) axillary, inguinal and elbow nodes, on the day of challenge or 7 days later, prevented development of EAE or decreased its severity (Table I). Rats subjected to right axillary-inguinal-elbow lymphadenectomy had more lesions in the left axillary and inguinal nodes than did the controls. This observation implied that the absence of customary homolateral drainage channels favored slight redistribution of encephalitogenic emulsion to contralateral nodes, but this was not sufficient to permit normal development of EAE. Vicarious sites of drainage were not discovered after bilateral lymphadenectomy.

Control groups subjected to contralateral (left) axillary-inguinal-elbow lymphadenectomy on day of challenge or 7 days later, or to bilateral sham surgery 7 days after challenge, had a normal incidence of EAE. Unexpectedly, bilateral sham operations performed on day of challenge decreased the severity of EAE (Table I). Only this group among the controls had, also, a marked decrease of lesions in draining lymph nodes. In an attempt to explain this observation, additional groups were challenged by injection into the right flank immediately after interrupting lymphatic connections. This was accomplished by 2 hemigirdling incisions through skin and fascia of right side of body from dorsal to ventral midlines, parallel to each other and to the ribs. The inoculation site was separated from right axillary and elbow nodes by the upper incision and from right inguinal nodes by the lower; the nodes were neither visualized nor disturbed. Like the bilateral sham operation, this procedure reduced the severity of EAE (Table I) and drastically decreased lesions in draining lymph nodes. However, hemigirdling incisions on the contralateral (left) side also diminished the severity of EAE; lesions in draining (right) lymph nodes were slightly decreased.

Discussion. The wide dissemination of encephalitogenic emulsion after foot pad inocu-

lation is probably responsible for the efficacy of this route in producing EAE(3), and also for the failure to prevent EAE in rats by popliteal-inguinal lymphadenectomy. It is likely that failure to prevent adjuvant-induced arthritis by popliteal lymphadenectomy(3) can be explained in the same way. Flank inoculation gave more restricted propagation of emulsion so that excision of homolateral axillary, inguinal and elbow nodes prevented development of EAE. The absence of usual drainage pathways to homolateral nodes promoted redistribution (as others have found by lymphography(7)) to contralateral nodes, and this may explain the slightly greater protection afforded by bilateral lymphadenectomy.

Reduction of EAE after the bilateral sham and hemigirdling operations performed on day of challenge may have been due to interference with lymphatic drainage (inadvertent for the former, intentional for the latter) or to stress(8). The drastic decrease in lymph node lesions indicated that both types of surgery had inhibited dissemination of emulsion. Partial protection after contralateral hemigirdling suggested participation of a stress factor related to the long incisions and numerous wound clips. The lack of effect of bilateral sham surgery performed 7 days after challenge was compatible with either suggested mechanism. Probably both were operative. Sham surgery was, therefore, not a suitable control for lymphadenectomy experiments. Contralateral lymphadenectomy, where applicable, appeared to be an acceptable control.

Summary. Encephalitogenic emulsion was widely disseminated in superficial and retroperitoneal lymph nodes following inoculation into the foot pad of rats. Therefore, it was not possible to prevent experimental allergic encephalomyelitis (EAE) by removing only popliteal and inguinal nodes. In contrast, dissemination was more restricted following flank inoculation so that EAE was prevented by homolateral or bilateral excision of axillary, inguinal and elbow nodes, either on day of challenge or 7 days later. Interruption of lymphatic vessels during surgery near lymph nodes (as in "sham" operations), the stressful effects of surgery, and the redistribution of emulsion following elimination of customary drainage pathways were also found to affect experiments designed to influence EAE by lymphadenectomy.

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