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Enhancement of Experimental Allergic Encephalomyelitis by Adrenalectomy.* (27799)

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Corticosteroids or stress suppress, and adrenalectomy enhances, certain immunologic phenomena(1). Experimental allergic encephalomyelitis (EAE) is thought to be due to delayed hypersensitivity to nervous tissue. Corticosteroids(2) or stress(3) are known to suppress EAE. The following experiments show that adrenalectomy enhances development of EAE.

Methods. Female 150-250 g CFN-derived or Sprague-Dawley rats were fed Purina Laboratory Chow and tap water *ad libitum*. Encephalitogenic emulsion was prepared by cycling 40% w/v rat spinal cord homogenate and equal volume of Freund's adjuvant between 2 syringes connected by a double-hubbed needle. Freund's adjuvant (complete) contained 4 mg/ml killed tubercle bacilli suspended in Bayol F-Arlacel A (8.5: 1.5). EAE was produced by administering encephalitogenic emulsion as 7 intradermal injections of 0.05 ml each in plucked skin of flanks and dorsal neck, under light ether anesthesia. Both rat strains were known to be of low susceptibility to EAE when challenged in this fashion(4). Total bilateral adrenalectomy was performed under pentobarbital anesthesia by making a dorsal midline skin incision and longitudinal incisions through muscle and peritoneum on each side of vertebral column; rats with adrenal remnants at necropsy were eliminated. Adrenalectomized rats were maintained on 1% NaCl as sole source of fluid. Adrenal enucleation was performed by snipping off a bit of each adrenal capsule and expressing the glands through the rents; capsules were left *in situ* and subsequently regenerated cortical but usually no medullary tissue. Sham operations were carried to the point of visualizing both adrenals. Except as indicated, each experimental group consisted of 10 rats.

Clinical signs of EAE consisted of paresis or paralysis of one or more limbs and, in severe cases, urinary incontinence. Dragging or limpness of the tail was a common but not completely reliable sign and was not scored. Surviving rats were exsanguinated under chloroform anesthesia 21 days after challenge. The entire spinal cord and hindbrain fixed in Bouin's solution, plus inoculation sites and draining lymph nodes fixed in formalin were embedded in paraffin, sectioned, and stained by hematoxylin-eosin. Histologic evidences of EAE were graded by number of lesions: 1—up to 4 or 5 lesions on the entire slide, 2—a few lesions in each of several different low-power fields, 3—lesions in many different fields, 4—many lesions in all or almost all fields. Slides were read without knowledge of the group from which tissues were derived.

Results. Total adrenalectomy 2 days prior to challenge produced a great increase in incidence and severity of clinical and histologic signs of EAE compared to unoperated controls (Table I). Thymus was much larger and spleen, inoculation sites and draining lymph nodes were slightly larger than controls. Inoculation sites and draining lymph nodes had slightly more epithelioid cell granu-

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TABLE I. Enhancement of EAE by Adrenalectomy.

Treatment*	No. of rats	Clinical EAE (incidence)	Histologic EAE (severity)	Thymus, %	Spleen, %
Adrenalectomy	17	71%	2.1†	.14‡	.21‡
" , 0.3 mg cortisone	8	63	2.9	.13	.20
" , 3.0 " "	10	0	1.1	.03	.19
None	10	6	.4	.08	.16

* Adrenalectomy 2 days before challenge; rats maintained on saline. Cortisone acetate IM once daily.

† Group avg, graded individually from zero to 4 according to severity.

‡ Moist weight after formalin fixation, as % of body weight, excluding rats that died before end of experiment.

lomatous reaction to encephalitogenic emulsion than controls. In contrast to past experience with intact rats, most severely paralyzed rats died, probably due to inability to respond to the stress of the disease. Daily intramuscular injections of 3.0 mg cortisone acetate, starting on day of challenge, reversed the effects of adrenalectomy. Clinical signs of EAE were completely absent, and histologic lesions were reduced, although not quite to the level of intact untreated controls. Thymus was very atrophic. Inoculation sites and draining lymph nodes were smaller and had far fewer granulomas than untreated adrenalectomized rats or intact controls. Cortisone acetate in 0.3 mg daily dose did not reverse the enhancing effect of adrenalectomy. In control experiments, there were no differences in EAE between unoperated and sham operated rats, or between sham operated rats maintained on 1% NaCl or on tap water.

Partial adrenalectomy. In 3 separate experiments, bilateral adrenal enucleation increased moderately the incidence and severity of clinical and histologic signs of EAE. Enucleation one day prior to challenge was more effective than enucleation 4 days before, probably because the shorter interval gave less time for regeneration. In one experiment, enucleation of one adrenal and total removal of the other gave more enhancement of EAE than bilateral enucleation.

EAE produced with incomplete adjuvant. EAE can be produced in the rat with emulsions of nervous tissue in incomplete adjuvant (*i.e.*, lacking tubercle bacilli)(5). To assess the effect of adrenalectomy on this system, antigen and route of inoculation were chosen so that controls had a low incidence of EAE. In 2 trials, guinea-pig cord antigen-incomplete adjuvant emulsion was inoculated by intradermal route. Total adrenalectomy 2 days prior to challenge enhanced EAE slightly, more notably in clinical signs than in histologic lesions. In one trial, 0.05 ml rat cord antigen-incomplete adjuvant emulsion was inoculated in the foot pad. Total adrenalectomy 2 days before challenge was without effect on EAE.

Discussion. The fact that enhancement of EAE by adrenalectomy was largely reversed by cortisone suggests that enhancement was due to removal of cortical rather than medullary tissue. In accord with this suggestion was our observation that enucleation of one adrenal and removal of the other was more effective than bilateral enucleation (the former procedure removed more cortical but the same amount of medullary tissue as the latter). However, neither argument is conclusive.

The mechanism of enhancement of EAE by adrenalectomy is uncertain. There was a general parallel between EAE and lesions in inoculation sites and draining lymph nodes, a finding in agreement with previous observations under a number of different circumstances(4). This parallel suggests an effect of adrenalectomy on sensitization. It should be noted, however, that enhancement of EAE by adrenalectomy was far more spectacular than its augmentation of inflammatory lesions in inoculation sites and lymph nodes, and, conversely, that suppression of histologic EAE lesions produced by cortisone in adrenalectomized rats was quantitatively less impressive than its suppressive effect on lesions in inoculation sites and lymph nodes. These

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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