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The Role of the Adrenal in Relapses of Experimental Allergic Encephalomyelitis[1] (38951)

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The injection of myelinated central nervous system tissue or basic protein in adjuvant, engenders an inflammatory autoimmune process called experimental allergic encephalomyelitis (EAE). It causes paralysis which, in the rat, is usually followed by spontaneous recovery. Relapses are occasionally observed (1–4). The mechanism that leads to relapse in some animals has not been elucidated. McFarlin *et al.* (4) and Paterson *et al.* (3) considered the possibility that relapses were due to a second immune response of different type, or directed to a different determinant, than that which triggered the initial attack.

We, on the other hand, are impressed by the long persistence of antigenic inocula in water-in-oil emulsions (1). Indeed, it has been shown that local injection of pertussis vaccine can induce relapses after primary attacks of EAE, possibly by mobilizing residual antigen from oily depots (5). Therefore, we propose the following explanation for spontaneous relapses. The attack of EAE, especially paralysis, causes nonspecific stress with increased secretion of adrenocortical hormones. The corticosteroids exert anti-inflammatory and immunosuppressive effects which promote recovery. But with recovery, the stress is ameliorated and the adrenal hyperactivity is "switched off." The animal returns to its normal endocrine and immunological status. At this time, there may be sufficient residual or partially processed antigenic inoculum to be utilized as the source of another attack of EAE, that is, a relapse.

This hypothesis is supported by reports of increased plasma corticosteroids in EAE (6) and of the therapeutic efficacy of endogenous (7, 8) or exogenous (8–12) steroids or ACTH. The present work shows that, as required by our hypothesis, premature "switching off" of the adrenal hyperactivity by adrenalectomy soon after an attack of EAE caused a marked increase in the relapse rate. This finding is analogous to the enhancement of first attacks by adrenalectomy before sensitization or before passive transfer, reported previously (13, 14). However, adrenalectomy did not increase the relapse rate when EAE was produced by methods that did not create an oily depot of antigenic inoculum. These data do not disprove the immunologic speculations about relapses (3, 4) but our hypothesis has the virtue of being based on known homeostatic functions of the adrenal gland.

Methods. Female 2- to 3-mo-old Lewis rats (Microbiological Associates, Inc. or bred in our laboratory) were maintained on Purina laboratory chow and tap water in groups of five in metal cages with mesh bottoms. Guinea pig spinal cord was used in four different ways to produce EAE. 1—40% Cord homogenate was emulsified in an equal volume of Freund's complete adjuvant (13), and a dose of 0.05 ml was injected into the right hind footpad. 2—Same emulsion but dose of approximately 0.005 ml was injected into one or two cervical lymph nodes (15). 3—10% Cord homogenate in saline mixed with an equal volume of 10% suspension of carbonyl iron SF (a particulate adjuvant that is effective in aqueous media); the dose of 2 ml was injected ip (16). 4—Passive transfer of washed, living cells from the lymph nodes draining the sites of encephalitogenic inoculation (cord, Freund's complete adjuvant, pertussis vaccine); the iv dose of 1 or 2 ml represented all the cells from one or two isogeneic donors (17). See references for details.

Rats were checked for clinical signs of EAE, usually from day 8 (day 3 for passive

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transfer) to day 28, counting the day of immunization or cell transfer as day zero. The onset was characterized by weakness and flaccidity of the tail (1+), often with weakness of the hind limbs (2+). In one or several days, signs progressed to paralysis (3+) in most animals. In eight experiments, there were two rats that failed to develop clinical signs and they were discarded. At various times after onset, bilateral adrenalectomy was performed with ether anesthesia thru a middorsal skin incision. Muscle and skin were closed with silk and clips, respectively. Physiological saline was offered as sole fluid thereafter. Sham surgery involved visualization but no removal of the adrenals. Rats that died 1 or 2 days after surgery were deleted from the experiments as there was insufficient time for evaluation of remissions and relapses.

Results (Tables I, II). Relapses were rare or absent in intact or sham operated rats that had been immunized with spinal cord in Freund's adjuvant, even when 6-mo-old rats were used, as advised by McFarlin *et al.* (4).

When adrenalectomy was performed on the day of onset of early EAE signs (5 rats, day 10), or on the first day of paralysis (5 rats, day 11), the animals died within 2 days without remission of their disease. Therefore, these groups are not included in Table I. When adrenalectomy was postponed until days 13 or 14, a few rats already showed spontaneous improvement at the time of surgery, and most rats improved greatly or completely during the 2 days after surgery. However, from 3 to 10 days after adrenalectomy clinical signs recurred and usually progressed rapidly to paralysis and death. The incidence of relapses was 68%, including only those rats in which, following remission, signs advanced at least two levels (from normal to 2+ or 3+, or from 1+ to 3+). The only rats that did not have relapses were those that never had a remission, but rather progressed inexorably to death. All rats adrenalectomized on days 15 or 18 had remissions, but the incidence of relapses was lower, and no relapses were noted when adrenalectomy was done on day 25.

Immunization by direct injection into lymph nodes enabled us to produce EAE with very small volumes of oily inoculum. Nevertheless, adrenalectomy on days 12 or 13 produced as many relapses as in the groups inoculated in the foot.

Rats immunized with the aid of the carbonyl iron adjuvant had just as severe a

TABLE I. RELAPSES AFTER ADRENALECTOMY AT VARIOUS TIMES IN RATS WITH EAE PRODUCED BY SPINAL CORD AND FREUND'S ADJUVANT IN THE FOOT.

Day of onset[a]	Day of paralysis[b]	Day of adrenex	Day of relapse	Incidence, relapses[c]
10.4	11.8	None	—	0/37
10.7	12.7	None	—	0/10[d]
9.6	10.4	13, sham	23	1/10
9.3	10.5	12	16	2/4
10.2	11.7	13	16–23	9/14
10.5	12.4	14	17–21	6/8
10.6	11.8	15	18–19	2/5
11.0	13.5	18	22–23	2/5
10.8	13.0	25	—	0/5

[a] Group average. Signs at onset were weakness of tail (1+) and hind legs (2+). All rats had clinical signs of EAE.

[b] In some groups, one or a few rats did not become paralyzed until after adrenalectomy or never became paralyzed; therefore, they were not utilized in the calculation of average day of paralysis.

[c] Numerator = number of rats whose signs advanced at least two grades after adrenalectomy and after remission of initial attack. Denominator = total number of rats. Six experiments are summarized. Rats that died 1 or 2 days after surgery are excluded.

[d] Six-month old rats; all others were 2–3 mo old.

TABLE II. RELAPSES AFTER ADRENALECTOMY IN RATS WITH EAE PRODUCED IN VARIOUS WAYS.

Manner of immunization	Day of onset	Day of paralysis	Day of adrenex	Day of relapse	Incidence, relapses
Cord and oil, nodes	9.4	10.7	12, 13	15–18	6/8
Cord and iron, ip	9.0	9.7	None	—	0/10
Cord and iron, ip	8.6	10.2	11–14	—	0/25
Passive transfer, iv	4.8	6.0	6, 7	—	0/12

disease as those given Freund's adjuvant and the onset was even earlier. Adrenalectomy on days 11–14 was followed by a remission in almost all rats. Although two rats advanced from 2+ to 3+ after adrenalectomy, shortly before they died, there was no instance that fulfilled our strict criteria for a relapse (signs advance at least two levels).

Passive transfer of lymphoid cells from donors with EAE produced clinical signs of EAE in all the recipients and many of them became paralyzed. All recipients experienced a remission and only two died after adrenalectomy; nevertheless no relapses were noted.

Discussion. We were not able to confirm the high spontaneous relapse rate in intact 6-mo-old rats reported by McFarlin *et al.* (4), perhaps because we used a smaller volume of inoculum, less mycobacteria, and whole spinal cord rather than myelin basic protein as antigen. Our low rate of spontaneous relapses emphasized the dramatic effects of adrenalectomy. In fact, adrenalectomy on days 13 and 14 produced relapses in every rat immunized with oily inoculum except those in which the absence of a remission precluded detection of a relapse.

Could the "relapses" have been pseudoparalysis due to adrenal insufficiency? In defense of the designation "relapse," we note that these animals had histologic lesions of EAE at the time of relapse, that the clinical appearances were indistinguishable from primary attacks in intact rats, that relapses do occur occasionally in intact rats, and that adrenalectomy did *not* produce relapses when inappropriately timed or when performed on rats with EAE produced by nonoily inocula. The instances where adrenalectomy was ineffective also argue against a possible role for release of the CNS itself from direct effects of corticosteroids, as occurs in other systems (18).

The presence of intense nonspecific stress in rats with EAE was obvious on inspection and has been confirmed by elevated plasma steroid levels (6). It may be related to paralysis, inability to eat, the painful swollen foot, and the inflammation in draining lymph nodes and nervous system. Stress is probably the explanation for the 100% mortality rate when adrenalectomy was performed during early clinical signs; the 2-day survival corresponded to the time needed for dissipation of most residual circulating corticosteroids. When adrenalectomy was postponed to days 13 or 14, the several days of paralysis may have caused some adaptation to stress, and in addition there was evidence of beginning amelioration of EAE, both presumably due to adrenal cortical hyperactivity. Removal of the adrenals and their immunosuppressive steroids at this time seemed to enhance the disease process for some rats whose signs progressed inexorably until death. However, most of the adrenalectomized rats, like all the controls, continued to improve for a few days. The surgical procedure of removing adrenals is itself stressful and may increase plasma steroids transiently, but adrenalectomized animals did not improve any faster than sham operated or nonoperated controls. The variable time of occurrence of subsequent relapses may reflect the time needed after adrenalectomy for the elevated plasma steroids to decrease so that processing of the antigenic inoculum could be resumed. Also, the amount of residual inoculum and its availability (degree of encapsulation) may determine the time of relapse as well as its incidence after adrenalectomy at various times. The absence of clear-cut relapses after adrenalectomy among rats given carbonyl iron adjuvant, and the lack of even a suggestion of a relapse after cell transfer may be due to the aqueous, readily dissipated inoculum of the former, and the absence of antigenic inoculum in the latter. The importance of type (oil *vs* aqueous) rather than volume of inoculum is indicated by the high incidence of relapses after lymph node inoculation and adrenalectomy, where only minute amounts of oily inoculum were used.

According to our hypothesis, the mechanism of spontaneous or adrenalectomy-induced relapses is similar to that involved in relapses after cyclophosphamide (3), methylprednisolone (10), or tilorone (19) treatment of EAE. In all these situations, processing of antigen is interrupted by an immunosuppressive agent (endogenous steroids or exogenous drugs), the removal of

which permits processing to resume and thereby causes a relapse.

The course of EAE is probably determined by several factors, but these experiments were directly only to the adrenal homeostatic mechanisms. It is conceivable that adrenal mechanisms play a contributory role in the remissions and relapses of multiple sclerosis, the human disease for which EAE has been considered a model. However, our data indicate that relapses in EAE are related to a particular mode of immunization and are not an intrinsic part of the disease process. Therefore, the occurrence of relapses in some types of EAE does not strengthen its putative similarity to multiple sclerosis.

Summary. Adrenalectomy shortly after onset of clinical signs of experimental allergic encephalomyelitis (EAE) was uniformly lethal to rats within 2 days. If adrenalectomy was postponed until the third to fifth day of signs, most of the rats survived and experienced a complete or incomplete remission followed by a relapse. The success of adrenalectomy in inducing relapses suggests that spontaneous relapses in EAE are related to adrenal homeostatic mechanisms whereby paralysis, nonspecific stress, increased corticosteroids, immunosuppression, recovery from paralysis, decreased stress, decreased corticosteroids, and reimmunization follow each other in logical order. However, the failure of adrenalectomy to induce relapses in EAE produced with aqueous inoculum or by passive transfer with lymphoid cells suggests that relapses are related to the persistent antigenic depot from water-in-oil inocula, rather than being an inherent part of the immunopathologic process.

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