and related substances. Our results indicate that DCI elevation of plasma FFA is dependent on its *in vivo* administration. *In vitro* studies suggest that DCI does not have an intrinsic lipolysis promoting property unlike epinephrine which stimulates FFA release *in vivo*(10,14) and *in vitro*(15,16). The evidence of Muhlbachova *et al.*(11) that DCI is mimetic to epinephrine, arterenol and isoproterenol does not preclude either the *in vivo* modification of DCI to a compound with greater lipolytic activity or release of FFA mobilizing factors within the organism. Another possible explanation is that DCI may interfere with utilization rather than the elaboration of plasma FFA. However, Armstrong *et al.*(17) have recently reported that DCI not only elevates plasma FFA levels, but also found that an increased rate of turnover is associated with elevation of plasma FFA levels.

Summary. 3,4,Dichloroisopropylarterenol (DCI) elevates the FFA level in anesthetized dogs. However, *in vitro* it does not cause FFA release from adipose tissue. Isoproterenol induces elevations of plasma FFA and glucose which are reduced by DCI (4 mg/kg). Glucagon hyperglycemia is also antagonized by DCI. DCI effects *in vivo* can be overridden by increasing the dose of isoproterenol or glucagon.

1. Powell, C. E., Slater, I. H., *J. Pharm.* 1958, v122, 480.
2. Moran, N. C., Perkins, M. E., *J. Pharm. and Exp. Ther.*, 1958, v124, 223.
3. Mayer, S. E., Moran, N. C., *ibid.*, 1960, v129, 271.
4. Fleming, W. W., Hawkins, D. F., *ibid.*, 1960, v129, 1.
5. Root, M. A., *Fed. Proc.*, 1961, v20, 168.
6. Murad, F., Rall, T. W., Sutherland, E. W., *ibid.*, 1960, v19, 296.
7. Mayer, S. E., Moran, N. C., Fain, J., *J. Pharm. and Exp. Ther.*, 1961, v134, 18.
8. Love, W. C., Ashmore, J., *Fed. Proc.*, 1961, v20, 166.
9. Hoffman, W. S., *J. Biol. Chem.*, 1937, v120, 51.
10. Dole, V. P., *J. Clin. Invest.*, 1956, v35, 150.
11. Muhlbachova, E., Wenke, M., Hynie, S., *Physiol. Bohemoslovenica*, 1961, v10, 181.
12. White, J. E., Engel, F. L., *J. Clin. Invest.*, 1958, v37, 1556.
13. Sutherland, E. W., Rall, T. W., *Pharm. Rev.*, 1960, v12, 265.
14. Gordon, R. S., Jr., Cherkes, A., *J. Clin. Invest.*, 1956, v35, 206.
15. ———, PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 150.
16. White, J. E., Engel, F. L., *ibid.*, 1958, v99, 375.
17. Armstrong, D. T., Steele, R., Altszuler, N., Dunn, A., Bishop, J. S., DeBodo, R. C., *Am. J. Physiol.*, 1961, v201, 9.

Received September 5, 1961. P.S.E.B.M., 1962, v109.

Suppression of Experimental Allergic Encephalomyelitis by Stress.* (27183)

SEYMOUR LEVINE, RALPH STREBEL, EUGENE J. WENK AND PINCKNEY J. HARMAN
Department of Pathology, St. Francis Hospital, and Department of Anatomy, Seton Hall College of Medicine and Dentistry, Jersey City, N. J.

Stress under certain conditions increases the non-specific resistance of the organism against injuries caused by various noxious agents(1-3). Stress influences certain immunologic phenomena as well, causing inhibition of active and passive anaphylaxis(4,5) and skin homograft rejection(6). The present report demonstrates that stress can suppress experimental allergic encephalomyelitis (EAE), a manifestation of delayed type hypersensitivity to nervous tissue.

It also occurred to us that the low incidence of EAE in certain species following administration of encephalitogenic emulsion by intraperitoneal route might be due to stressful effects of the inflammatory reaction to the inoculum in the peritoneal cavity. Waksman

* Supported by research grants from Nat. Multiple Sclerosis Soc. and Nat. Inst. of Neurol. Dis. and Blindness, U.S.P.H.S.

found that the intraperitoneal route gave poor results in rabbits(7). We have confirmed this in 2 strains of albino rats. At necropsy, these rats exhibited granulomatous peritonitis, large adrenals and atrophied thymuses. These findings suggested a stress effect. The present experiments demonstrate that the ineffectiveness of intraperitoneal inoculation of encephalitogenic emulsion can be attributed at least partly to the stressful effect of granulomatous peritonitis.

Methods. Female 120-167 g Sprague-Dawley rats were fed Purina Laboratory Chow and tap water *ad libitum*. Encephalitogenic emulsion was prepared by cycling 33% w/v rat spinal cord homogenate and equal volume of Freund's complete adjuvant between 2 syringes connected by a double-hubbed needle. Adjuvant was prepared by suspending killed dried tubercle bacilli 4 mg/ml in 8.5 parts Bayol F and 1.5 parts Arlacel A. EAE was produced by administering encephalitogenic emulsion in the right foot pad as a single 0.05 ml dose or intradermally as 7 injections of 0.1 ml each in plucked skin of flanks and dorsal neck, under light ether anesthesia. Local inflammatory response to encephalitogenic emulsion was graded clinically from 1 to 4 based on size and ulceration of lesions at sites of inoculation. Clinical signs of EAE appeared usually from 10 to 18 days after inoculation and were graded in 4 degrees of severity: 1—paresis of 1 hind limb, 2—paresis of both hind limbs, 3—paralysis of both hind limbs with or without weakness of fore limbs, 4—the preceding plus urinary incontinence.

Restraint, noradrenaline or cold baths were used as stressors(1-3). Restraint was accomplished by taping all 4 paws of prone rats to a board. Their teeth were clipped and they were fed powdered chow. Duration and frequency of restraint are detailed under *Results*. Noradrenaline (Winthrop) was injected subcutaneously twice daily suspended in 0.2 ml corn oil, from 6 days before challenge until sacrifice. Doses started at 25 γ, increased at intervals by 25 γ until a maximum dose of 300 γ was attained. Cold baths in water at 0 to 4°C were given twice daily, increased from ½ to 2 minutes during the 6 days before challenge, and maintained at 2 minutes thereafter.

Some rats, challenged intradermally with encephalitogenic emulsion, were given simultaneous intraperitoneal injection of 0.7 ml of an emulsion of equal parts of adjuvant and saline. The intraperitoneal adjuvant-saline emulsion was given to produce a sterile granulomatous peritonitis. For control purposes other rats, challenged intradermally with encephalitogenic emulsion, were given adjuvant-saline emulsion intradermally as 7 injections of 0.1 ml each in ventral and dorsal midlines.

Rats were killed with chloroform 28 days after challenge. Spinal cord and hindbrain were fixed in 10% formalin or Bouin's solution, embedded in entirety in paraffin, sectioned, stained by hematoxylin-eosin and occasionally by Luxol fast blue-periodic acid-Schiff. 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. For each experimental group, incidence of EAE and average severity of both clinical and histological signs are recorded in the Tables.

Results. Suppression of EAE by stress. (Table I). Among 28 control rats challenged

TABLE I. Suppressive Effect of Stressful Procedures on EAE Induced by Intradermal Flank-Neck Route.

Stressful procedure	No. of rats	Clinical EAE	Histologic EAE
		%	%
None	28	57 (1.2)†	61 (1.5)
Restraint D−6* to D+28	42	7 (.2)	21 (.4)
" D−6 to D+14	7	14 (.1)	29 (.4)
" D+9 to D+28	9	78 (1.6)	67 (1.3)
" D+6 only	10	70 (2.0)	70 (2.4)
Noradrenaline	7	29 (.9)	57 (.8)
Cold baths	9	22 (.7)	44 (.9)
Intraper. adjuvant	18	0 (.0)	11 (.1)
Intradermal adjuvant	11	64 (.9)	36 (.7)

* Reckoned from day of challenge as day zero. D = Day.

† Figures in parentheses in Tables I and II represent avg severity, graded individually from 0 to 4 as described in text.

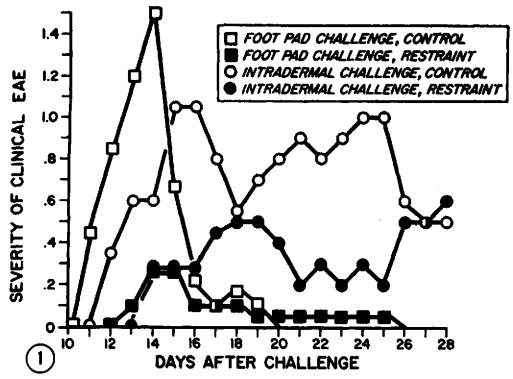
with encephalitogenic emulsion by intradermal flank-neck route, 16 (57%) developed clinical signs of EAE and 17 (61%) had histologic lesions. Among 42 rats challenged simultaneously and in identical fashion but subjected to restraint 3 hours daily or 7 hours every other day from 6 days before challenge until sacrifice, only 3 (7%) developed clinical signs of EAE and 9 (21%) had histologic lesions. Severity of clinical signs and histologic lesions paralleled the data on incidence. Restraint applied to 7 rats 3 hours daily, from 6 days before challenge until only 14 days after challenge, also suppressed clinical and histologic evidence of EAE. On the other hand, no protection was obtained from restraint 3 hours daily, starting 9 days after challenge and continuing until sacrifice, or from 17 hours of restraint applied once only 6 days after challenge. Twice daily injections of noradrenaline or twice daily cold baths from 6 days before challenge until sacrifice gave only slight reduction of clinical and histologic signs of EAE.

Suppression of EAE by granulomatous peritonitis. (Table I). Eighteen rats, challenged with encephalitogenic emulsion by intradermal flank-neck route and given adjuvant-saline emulsion intraperitoneally, exhibited almost complete suppression of EAE. None had clinical signs and only 2 (11%) had histologic lesions which were of minimal severity. Controls that received the same quantity of adjuvant-saline emulsion in the skin had only slight decrease of EAE compared to controls that received no extra treatment.

Effect of stress after foot pad challenge. (Table II). Foot pad inoculations of encephalitogenic emulsion are known to be more effective in eliciting EAE than intradermal inoculations in flank and neck (8,9). In the present experiments the foot pad route produced higher incidence of clinical EAE (70%), more severe signs, earlier onset and earlier peak, but more rapid and complete resolution (Fig. 1, control groups, open symbols). A single 17-hour period of restraint 6 days after foot pad challenge gave no protection, but restraint applied 7 hours every other day throughout the experiment reduced the incidence of clinical signs of EAE to 20% with corresponding reduction in severity (Table II and Fig. 1, closed squares). How-

TABLE II. Effect of Stressful Procedures on EAE Induced by Foot Pad Route.

Stressful procedure	No. of rats	Clinical EAE	Histologic EAE
		%	%
None	10	70 (1.9)	100 (3.2)
Restraint D−6 to D+28	20	20 (.4)	100 (2.6)
" D+6 only	10	100 (2.3)	100 (2.4)



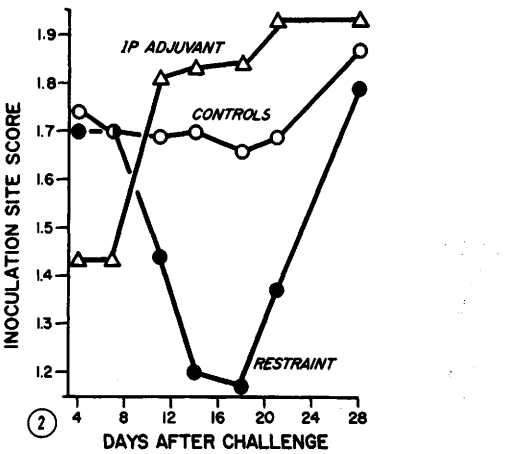


FIG. 1. Avg severity of clinical signs of EAE in one experiment on 4 groups of rats challenged with encephalitogenic emulsion by intradermal flank-neck route (circles) or foot pad (squares). Rats subjected to restraint 7 hr every other day (closed symbols) had much less clinical evidence of EAE than controls that received no additional treatment (open symbols).

FIG. 2. Avg scores for intradermal sites of inoculation of encephalitogenic emulsion in groups of rats subjected to restraint 3 hr daily (●), to intraperitoneal inj. of adjuvant (△), or to no additional treatment (○).

ever, histological examination showed no difference in incidence or severity of EAE lesions between control and restrained groups, unlike results after intradermal challenge. Similar discrepancy between clinical and histologic findings was noted also in an experiment in which suppression of EAE after foot pad challenge was sought by intraperitoneal adjuvant injection.

Other observations. Normal increase of body weight was retarded by repeated restraint and an occasional rat lost a few grams, but return toward normal started after about 2 weeks. Noradrenaline and cold baths did not interfere with normal weight gain. After intraperitoneal adjuvant, rats appeared unwell for a few days, exhibited swollen abdomens and excessive gain in weight, but appeared healthy thereafter. At necropsy, they had sterile granulomatous peritonitis, usually with ascites (up to 16 ml).

Ulceration of inoculation sites in some control rats diminished slightly between 11 and 18 days after inoculation followed by enlargement till sacrifice (Fig. 2). Rats subjected to repeated restraint showed a much greater decrease in size of inoculation sites between 11 and 18 days after inoculation, but by time of sacrifice they had almost regained the size of controls. Inoculation sites of rats treated with intraperitoneal adjuvant were smaller than controls during the first week but increased after that to become larger than controls, and exhibited no decrease between 11 and 18 days. Sites of inoculation of encephalitogenic emulsion in rats given extra adjuvant in the skin did not differ in size from those of untreated controls. Lymph nodes draining sites of inoculation of encephalitogenic emulsion contained epithelioid cell granulomas and foci of suppuration surrounding oil droplets derived from the inoculum. These lesions were slightly decreased after repeated restraint but there was great individual variation.

Discussion. Suppression by early restraint and lack of suppression by late restraint indicate that stress influences sensitization rather than a later stage in development of EAE.† The suggestion that excessive secretion of anti-inflammatory adrenal corticosteroids is responsible for inhibitory effect of stress on anaphylaxis and skin homograft rejection(1,5,6) may apply as well to EAE. This hypothesis is now being explored by experiments on adrenalectomized rats. The hypothesis is compatible with our observation that inoculation sites during part of their evolution were smaller in stressed rats than in normal rats (Fig. 2), and the observation of Kabat *et al.*(10) that cortisone treatment prevented EAE by suppressing epithelioid cell response at inoculation sites.

Suppression of EAE by stressors might be subject to other interpretations. We investigated body temperature because restraint may be associated with slight hypothermia (11), but measurements of rectal temperature showed hypothermia only after cold baths and not after restraint or noradrenaline. Thus there was no correlation between body temperature and suppression of EAE. We have not excluded a possible role of nutrition because there was a correlation between ability of the stressor to suppress EAE and to interfere with normal weight gain. EAE can be prevented by specific nutritional deficiencies(12,13), but there are no experiments pertinent to restriction of body weight on a qualitatively adequate diet. Furthermore, such experiments would be difficult to interpret because food deprivation itself is considered to be a powerful stress(1).

The observations that repeated restraint caused more suppression of EAE after intradermal than after foot pad challenge, the lack of effect of single periods of restraint, and the slight effects of noradrenaline and cold baths suggest that suppression of EAE depends on a balance between strength of the encephalitogenic stimulus and severity of the

† Professor N. Allegretti of Zagreb has permitted us to quote the following experiment (to be published): suppression of EAE was obtained by intraperitoneal injection of adjuvant alone when this injection was given 1,2,3,4,5,6, or 8 days after intradermal challenge with encephalitogenic emulsion; no suppression was observed when the intraperitoneal adjuvant was given 10, 12 or 14 days after challenge. These results may be compared with our own inability to suppress EAE when daily restraint was not initiated until 9 days after challenge.

applied stress. Different degrees of inherent susceptibility to EAE(9) and to stress may influence results also.

Suppression of EAE by intraperitoneal adjuvant was probably due to stressful effects of granulomatous peritonitis. The fact that adjuvant in the peritoneal cavity caused far more suppression of EAE than adjuvant in the skin suggested a non-specific rather than immunologic mechanism. Suppression of EAE by granulomatous peritonitis may explain the low incidence of EAE in rats following intraperitoneal challenge with encephalitogenic emulsions. Stress may be involved also in the action of drugs or other treatments on EAE (for example, stressful effects of intraperitoneal salicylate(14) and infantile stimulation(15).

Summary. Stress, in the form of restraint repeated throughout the experiment or during its first half, suppressed development of clinical and histologic signs of experimental allergic encephalomyelitis (EAE) in rats after intradermal challenge with encephalitogenic emulsion. Clinical signs, but not histologic lesions, were suppressed by restraint when the highly effective foot pad route of challenge was employed. Intraperitoneal injections of adjuvant also suppressed EAE in rats simultaneously challenged with encephalitogenic emulsion in the skin. It is suggested that suppression of EAE by intraperitoneal adjuvant was due to the stressful effects of the consequent sterile granulomatous peritonitis. Thus, the occurrence of granulomatous peritonitis may explain the failure to produce EAE when rats are challenged by intraperitoneal route with encephalitogenic emulsions that contain adjuvant.

1. Selye, H., *Stress*, 1950, Acta, Inc., Montreal.
2. Bajusz, E., Selye, H., *Am. J. Physiol.*, 1960, v199, 453.
3. Selye, H., Bajusz, E., Strebel, R., *Can. J. Biochem. Physiol.*, 1961, v39, 519.
4. Karady, S., Selye, H., Browne, J. S. L., *J. Immunol.*, 1938, v35, 335.
5. Treadwell, P. E., Rasmussen, A. F., Jr., *ibid.*, 1961, v87, 492.
6. Wistar, R., Jr., Hildemann, W. H., *Science*, 1960, v131, 159.
7. Waksman, B. H., Morrison, L. R., *J. Immunol.*, 1951, v66, 421.
8. Waksman, B. H., Pearson, C. M., Sharp, J. T., *ibid.*, 1960, v85, 403.
9. Levine, S., Wenk, E. J., *Am. J. Path.*, 1961, v39, 419.
10. Kabat, E. A., Wolf, A., Bezer, A. E., *J. Immunol.*, 1952, v68, 265.
11. Bartlett, R. G., Jr., Bohr, V. C., Inman, W. I., *Can. J. Biochem. Physiol.*, 1955, v33, 654.
12. Schneider, H. A., Lee, J. M., Olitsky, P. K., *J. Exp. Med.*, 1957, v105, 319.
13. Mueller, P. S., Kies, M. W., Alvord, E. C., Jr., Shaw, C.-M., *J. Exp. Med.*, 1962, v115, 329.
14. Field, E. J., Miller, H., *Postgrad. Med. J.*, 1960, v36, 236.
15. Zeman, W., in "Allergic" Encephalomyelitis, Proceedings of a Symposium: Experimental "Allergic" Encephalomyelitis and its Relation to Other Disease of Man and Animals, M. W., Kies and E. C. Alvord, Jr., eds., Charles C Thomas, Springfield, p462.

Received September 5, 1961. P.S.E.B.M., 1962, v109.

Heparin-Like Substances in Cement Lines of Vascular Endothelium of Guinea Pigs.* (27184)

GOROKU OHTA, HIROYA SASAKI, FUJITSUGU MATSUBARA, KIYOO TANISHIMA AND SIRO WATANABE (Introduced by Hans Popper)
Department of Medical Zoology and Department of Pathology, School of Medicine, University of Kanazawa, Kanazawa, Japan

Recently, Samuels and Webster demonstrated vascular endothelial cement lines by treating large blood vessels in stretch preparations with heparin followed by toluidin blue staining(1). This suggests an affinity

* Supported in part by a grant-in-aid for Fundamental Scientific Research of the Ministry of Education, Japan.