Metrazol Threshold in Experimental Allergic Encephalomyelitis.* (28525)

SEYMOUR LEVINE, HUSHANG PAYAN AND RALPH STREBEL
Departments of Pathology, New York Medical College-Metropolitan Hospital Medical Center, New York City, and St. Francis Hospital, Jersey City, N. J.

Experimental allergic encephalomyelitis (EAE) is an inflammatory "auto-immune" disease produced by injection of central nervous system tissue with adjuvants. Electroencephalograms of monkeys(1) and guinea pigs(2,3) with EAE have revealed convulsive activity or diminished amplitude. The spinal cords of guinea pigs with EAE have reduced threshold to electrical stimulation(4). In the present experiments, the increased excitability of the nervous system in EAE has been demonstrated by reduced threshold to pentamethylentetrazol (Metrazol)-induced convulsions.

Methods. Female, Hemlock Hollow Wistar rats, 155-185 (average 170) g, were fed Purina Laboratory Chow and tap water *ad libitum*. Metrazol threshold was determined as the number of intraperitoneal injections of 1% Metrazol, 15 mg/kg, repeated at exactly 15 minute intervals, required to produce generalized convulsions. EAE was produced by a single 0.05 ml injection of encephalitogenic emulsion in the right hind foot pad of rats under light ether anesthesia. The emulsion was prepared by cycling equal parts of 40% w/v rat spinal cord homogenate and Freund's complete adjuvant between 2 syringes connected by a double-hubbed needle. The adjuvant consisted of killed dried tubercle bacilli 4 mg/ml suspended in 8.5 parts Bayol F mineral oil and 1.5 parts Arlacel A emulsifying agent. Signs of EAE appeared after 9 days or more, and were graded in 4 degrees of severity(5): 1—paresis of 1 hind limb, 2—paresis of both hind limbs, 3—paralysis of both hind limbs, 4—preceding plus urinary incontinence. For purposes of comparison, brain lesions of known epileptogenic quality were produced by implanting pellets of 325 mesh cobalt powder into and beneath the right frontal cortex with the aid of a stereotaxic apparatus(6,7). As indicated in Table I, a single batch of 69 rats was divided into 4 groups: 1—untreated controls, 2—EAE, 3—cobalt, 4—EAE plus cobalt. On day 1, Metrazol thresholds of all rats were determined as a base line. On day 4, the third and fourth groups received cobalt implants. On day 6, the second and fourth groups received encephalitogenic emulsion. Metrazol thresholds were determined again on days 21 and 35. All rats were sacrificed on day 36; brains and cords were taken for histological study.

Results and discussion. Convulsions occurred in control rats usually after 3 or 4 injections of Metrazol (Table I). Rats with EAE, despite weakness or paralysis, convulsed after fewer injections, *i.e.*, the Metrazol threshold was decreased. Frequently, the paralyzed hind-quarters of rats with EAE did not participate in the convulsions. The Metrazol threshold returned partially toward normal on day 35 when EAE symptoms had largely disappeared. Rats with cobalt implants had a much lower threshold. This difference may be due to the disseminated and largely subcortical distribution of EAE lesions, contrasted to the focal and mainly cortical location of cobalt lesions. The low threshold of cobalt-implanted rats was not influenced by co-existence of EAE (last group, TableI).

Clinical signs of EAE appeared in all but 1 of 15 rats challenged with encephalitogenic emulsion; symptoms were severe (average

TABLE I. Metrazol Thresholds in EAE and in Cobalt Epilepsy.

Procedure	No. of rats	Metrazol thresholds* Day 1	Day 21	Day 35
None	10	3.4	3.7	3.4
EAE, day 6	15	3.8	2.8	3.1
Cobalt, day 4	15	3.4	1.7	1.7
EAE, day 6 / Cobalt, day 4	29	4.0	1.8	1.9

* Avg number of Metrazol injections required to produce convulsions.

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grade 2.9) and there was transitory loss of weight. In contrast, the group that received both encephalitogenic emulsion and cobalt implant had no loss of weight, signs of EAE were milder (average severity only 1.6) and 7 rats had no signs at all. The difference in EAE was confirmed histologically. This partial suppression of EAE was probably due to a non-specific stress effect of the cobalt implant(5). An effect on the hypothalamus of the large necrotic focus around the frontal lobe implant, with consequent pituitary-adrenal stimulation, cannot be ruled out.

Summary. Rats with experimental allergic encephalomyelitis (EAE) had reduced threshold to Metrazol-induced convulsions. The threshold of rats with cobalt implants was even lower. Rats with both EAE and cobalt implants had the same threshold as rats with cobalt implants only. The severity of EAE was reduced in this group, probably due to non-specific stress effects of the cobalt implants.

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Effect of *Pseudomonas pseudomallei* Endotoxin on Response to Cholinergic Stimuli in the Rabbit.* (28526)

G. J. HILDEBRAND, M. REDFEARN AND Y. SEYS (Introduced by A. P. Krueger)
(With the technical assistance of James Ng)
Naval Biological Laboratory, School of Public Health, University of California, Berkeley

Increased peristalsis, sustained rise in right ventricular pressure and fall in arterial pressure to shock level were among the effects produced by intravenous injection of *Pseudomonas pseudomallei* endotoxin in rabbits. These responses were similar to those produced in rabbits and other animals by various bacterial endotoxins(2,3,4). Since it appeared that some of these effects might result from hyperactivity of cholinergic mechanisms that became sensitized to cholinergic stimuli by endotoxin, we investigated the possibility of an enhanced response to cholinergic stimuli in rabbits injected with *P. pseudomallei* endotoxin.

Materials and methods. Female New Zealand white rabbits, weighing from 3.1 to 5.9 kg were used. The animals were anesthetized by intravenous (i.v.) injection of sodium phenobarbital (100 mg/kg) and sodium pentobarbital (15 mg/kg).

Endotoxin was isolated from cultures of *P. pseudomallei* strain 118-6 (rough), that were grown in a chemically defined medium(6) containing 0.1 M gluconolactone, 0.1 M KH_2PO_4, 0.0045 M $MgSO_4 \cdot 7H_2O$, 0.000023 M $FeSO_4 \cdot H_2O$. The medium was adjusted to pH 6.4 with NH_4OH. The organisms were killed with formalin at a final concentration of 1% and extracted with phenol by the method of Westphal(5). The phenol extract containing endotoxin was fractionated by precipitation with acetone and differential centrifugation. The endotoxin isolated contained less than 2% nitrogen and was devoid of both protein and nucleotide residues. The

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