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Acute Disseminated Encephalomyelitis Produced in Albino Mice.

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The production in monkeys of acute disseminated encephalomyelitis accompanied by demyelination following injection of brain tissue, first achieved by Rivers, Sprunt and Berry,[1] was not only confirmed by others, but similar lesions were later obtained with various preparations in rabbits and guinea pigs. The experimental disease has not hitherto been induced in mice. It would appear that if mice so convenient for experimental work could be shown to be susceptible, a step forward might be made. As the following results will show, it is believed that this has been accomplished.

Of 40 mice W-Swiss strain,[2] in 2 series of experiments, all were injected intramuscularly with 0.3 ml of a milky suspension containing 20 mg killed tubercle bacilli, 50 ml heavy liquid petrolatum and 10 g normal mouse brain in 50 ml saline solution. The tubercle bacilli were human type, pathogenic strain H37Rv,† acetone-dried and autoclaved for 15 minutes at 15 lb pressure. The normal mouse brain was obtained from a healthy stock of Rockefeller Institute or W-Swiss strains of albino mice. The materials were homogenized in a small-sized Waring blendor and mice were given 3 to 6 injections of the mixture at weekly or longer intervals.

Localized nodules were induced which were absorbed with difficulty and persisted for several weeks. The nodules contained grumous material, chiefly polymorphonuclear leucocytes and epithelioid cells but no visible microorganisms.

From 16 to 105 days after the first injection, 36 mice have thus far shown definite signs of involvement of the central nervous system. They exhibited all or some of the following patterns of behavior: ruffled fur; dyspnea; paresis; paralysis, generally of the hind limbs; generalized, coarse tremors; excitation alternating with somnolence; ataxia; tip-toe gait and hunched back. Subsidence of signs and later relapse were characteristic; quiescent periods endured from 2 to 15 or more days.

The chief histological changes in the brain and cord related to the marked mural and perivascular infiltration, observed more often in the white matter. The collections of cells were made up mostly of lymphocytes and microglial cells, some polymorphonuclear leucocytes and plasma cells, monocytes and compound granular corpuscles. The infiltrations tended to spread out perivascularly into the surrounding parenchyma. Vascular leucocytic or hyaline thrombi also occurred. The meninges were free from lesions or showed a spotty similar infiltration, especially about blood vessels. Neuronal degeneration was present in scattered areas, mostly in the cord, hind brain, basal ganglia and sometimes in the Purkinje cells. Disseminated, localized and diffuse glial infiltration, and small hemorrhages were visible. Demyelination was present, but not in all animals and generally in the parenchyma but this was not a prominent lesion in these early stages of illness.

Stock mice have not shown any of these signs, and repeated examinations of their central nervous tissues failed to show any apparent lesions. In addition, 20 W-Swiss mice were injected intramuscularly with 0.3 ml of acetone-ether, lipid extract of brain obtained from apparently normal mice. The concentration of lipid was 20 mg/ml and 2 to 8 injections of an emulsion of equal parts lipid and heavy liquid petrolatum were given at intervals of not less than one week. Of these 14 were killed from 14 to 113 days after the first injection, and their brains and spinal cords revealed no visible changes. Two ad-

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1 Rivers, T. M., Sprunt, D. H., and Berry, G. P., *J. Exp. Med.*, 1933, **58**, 39.

2 Webster, L. T., *J. Exp. Med.*, 1939, **70**, 87.

† The writers are indebted to Dr. G. Middlebrook for this strain.

ditional series of control tests were carried out: 1) 3 mice at the height of reaction to the induced encephalomyelitis were killed and suspensions of the brain and cord of each, in dilution of 10^{-1}, were injected intracerebrally into 10 mice. None of the 30 W-Swiss mice exhibited neurological signs. 2) The material used for inoculation was tested for the presence of an infective element transmissible to mice; none was found. It would thus appear that the induced encephalomyelitis is not caused by a transmissible agent present in the inocula or in the stock mice.

To conclude, disseminated encephalomyelitis was readily induced in W-Swiss mice by means of intramuscular injections of brain tissue combined with liquid petrolatum and killed tubercle bacilli—the latter being the constitutents of a modified "adjuvant" technic of Freund and McDermott.[3]

[3] Freund, J., and McDermott, K., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 548.

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Tests for Chemical Mutagens in *Drosophila* Using the Vaginal Douche Technic.*†

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In a previous note[1] data were presented demonstrating that the rate of mutation is increased in spermatozoa when the latter are exposed to methyl bis (β-chloroethyl) amine hydrochloride introduced into females of *Drosophila melanogaster* by vaginal douche. In addition, there seemed to be an increase in the number of mutations occurring in the egg chromosomes of females that were treated with the N mustard in this manner. It is the purpose of this paper to offer additional data which confirms these observations and to present data on the mutagenic activity of copper sulfate, formaldehyde, 2,4-dinitrophenol, hydrazine hydrate and trypsin, employing this technic. These results will be discussed together with those of other workers testing these chemicals in *Drosophila*.

Material and method. The Oregon-R wild type and the Muller-5 (sc^{81} B In-S w^a sc^8) stocks of *Drosophila melanogaster* were used in these experiments. Virgin females from either one of these stocks were aged for 2 to 5 days, injected with a chemical substance, and then individually placed in vials with several males from the other stock. The males had been isolated soon after hatching. Injections were made as described previously,[1] but in the later experiments a set-up of ring-stand clamps, arranged to steady the hands during injection, was substituted for the micromanipulator. Females were observed for copulation for 2 hours after the injection. Only a single mating was permitted, after which the males were killed and the females placed individually in "creamers" or vials containing culture medium. Transfers to fresh creamers or vials were made every 2 to 3 days until no further offspring were produced. As in the earlier experiments, all the first generation females except those lost through accident were tested for lethal mutations involving the X chromosome from the Ore-R stock. Except for a period in the N mustard experiments reported here, in which the food was not enriched with brewer's yeast, all the first generation females were also tested for lethals involving the X chromosome from the Muller-5 stock. The plan of the crosses made for

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[1] Herskowitz, I. H., *Evolution*, 1947, **1**, 111.