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Effect of Injecting Crystalline Tetanal Toxin and Tetanal Antitoxin Into Mice.

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Pillemer and Wartman[1] have shown that the administration of crystalline tetanal toxin in single doses ranging from 0.25 to 500,000 M.L.D. produced no pathological lesions in the muscles or central nervous system of white Swiss mice. Similar results were obtained upon the injection of numerous sublethal doses of toxin. Experiments were subsequently undertaken to determine whether the administration of tetanal antitoxin would produce any pathological lesions in mice that had received various amounts of crystalline toxin. This seemed important because many of the patients with tetanus on whom autopsy was performed have received antitoxin.

Experiments. White Swiss inbred mice, which weighed between 15 and 20 g, were divided into 8 groups. Mice in Groups I to IV were given 1 M.L.D. of crystalline tetanal toxin followed by 25 units of tetanal antitoxin. In Group I the antitoxin was administered one day after the toxin; in Group II 2 days afterwards; in Group III 3 days afterwards; and in Group IV 4 days afterwards. Mice in Group V were given 0.5 M.L.D. of toxin followed 5 days later by 25 units of antitoxin. In Group VI the procedure was reversed and the mice were given first 25 units of antitoxin and then 1 M.L.D. of toxin every day for 6 successive days, receiving in all 6 M.L.D. of toxin. Animals in Group VII were given 25 units of antitoxin, and those in Group VIII 2500 units of antitoxin. None of the mice in Groups VII and VIII received tetanal toxin.

Crystalline tetanal toxin[2,3] and a highly purified antitoxin (Lederle) were used in all experiments. The toxin was dissolved in 0.3 M glycine and injected into the gluteal muscles at the base of the tail on the right side. The antitoxin was diluted in 0.9% saline and injected intraperitoneally. The mice were amply fed and allowed as much water as they would drink.

The mice were killed at the desired intervals with ether, except those in Groups III and IV which died. Autopsies were performed immediately after death, and tissues were fixed for 24 hours in 10% formalin and Zenker's fluid. Sections were stained with hemalum and aqueous eosin and, in addition, the brain, spinal cord, and peripheral nerves were stained specifically for Nissl substance and myelin. Sections were made of the following organs: the anterior, middle, and posterior thirds of the brain; the lumbar, thoracic, and cervical portions of the spinal cord; anterior and posterior nerve roots; sciatic nerve; the cerebral and spinal meninges; the skeletal muscles from both the injected and the opposite side of the tail, both gastrocnemius muscles, and the muscles along the vertebral column.

Results. When 25 units of tetanal antitoxin were given to white Swiss mice within 24 hours after the intramuscular injection of 1 M.L.D. of crystalline tetanus toxin, the animals were protected from the development of clinical tetanus. When the antitoxin was not given until later, the signs of classical tetanus developed and the mice died. No detectable pathological changes occurred in animals which survived for as long as 14 days. If only 0.5 M.L.D. of toxin was injected, the administration of 25 units of antitoxin was effective even if delayed for several days. When given as long as 5 days after the toxin, some of the mice developed

[1] Pillemer, L., and Wartman, W. B., *J. Immunol.*, 1947, **55**, 277.

[2] Pillemer, L., Wittler, R. G., and Grossberg, D. B., *Science*, 1946, **103**, 615.

[3] Pillemer, L., Wittler, R. G., Burrell, J. I., and Grossberg, D. B., *J. Exp. Med.*, 1948, **88**, 205.

tetanus, but the signs were only mild to moderate. In these mice, also, no significant pathological lesions were discovered.

The intraperitoneal injection of either 25 or 2500 units of tetanal antitoxin caused no recognizable clinical or pathological manifestations.

Summary. The administration of 25 units of tetanal antitoxin to white Swiss mice which had previously received 1 M.L.D. of crystalline tetanal toxin, produced no discoverable lesions in the central nervous system, peripheral nerves, or skeletal muscles, irrespective of whether the animals developed clinical tetanus, or of how long they survived. Likewise, the intraperitoneal injection of either 25 or 2500 units of tetanal antitoxin caused no changes which could be detected by ordinary histopathological technics.

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Effect of *Lithospermum ruderale* on the Gonadotropic Potency of the Pituitary Gland.*

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(Introduced by Raymond N. Bieter.)

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The crude plant, *Lithospermum ruderale,* has been shown to cause prolonged or persistent diestrous in mice with previously regular estrous cycles.[1,2] Since the administration of either follicle-stimulating hormone or estrogenic hormone induces estrus in animals under treatment with Lithosperm and since Lithosperm causes a decrease in the weights of pituitary glands the suggestion has been made that the drug acts on the pituitary gland to decrease the formation or secretion of gonadotropic hormone.[1] The present paper deals with the biological assay for gonadotropic potency of pituitary glands from treated and control animals.

Experimental. Female mice, 21 days old, were divided into 3 groups, distributing littermates between the groups as evenly as possible. Group I called *Lithosperm donors,* consisted of 9 mice, each of which was given an injection of two pituitary glands from donor mice which had been on a 20% ground Lithosperm diet for one week or more. In Group II, the control donors, each of 9 mice received an injection of 2 pituitary glands from donor mice on control diet, Purina fox chow. Group III served as uninjected controls, receiving no pituitary injections. Donor mice for Groups I and II were adult females and were sacrificed during diestrus only. To make the pituitary injections a method similar to that of Smith and Engle[3] was used. Donor mice were killed with ether, the skin on back of head calvarium and brain removed and pituitary gland lifted out. Recipient mice were lightly anesthetized with ether and the pituitary glands placed through a small incision into the thigh muscles. Mice were autopsied at 26 days of age, *i.e.* 5 days after injection. The ovaries, uterus plus vagina and adrenal glands were dissected out and weighed in the moist state.

The data obtained are presented in Table I. Evidence of gonadotropic stimulation was

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1 Cranston, E. M., *J. Pharm. and Exp. Therap.,* 1945, **83**, 130.

2 Drasher, M. L., and Zahl, P. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 66.

3 Smith, P. E., and Engle, E. T., *Am. J. Anat.,* 1927, **40**, 159.