Encephalomyelitis in the Rat Following Intracutaneous Injection of Central Nervous System Tissue with Adjuvant. (19840)

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By often repeated injections of CNS tissue over a period of several months, Rivers and his associates produced in the rhesus monkey sterile disseminated meningo-encephalomyelitis(1,2). With the aid of adjuvants (paraffin oil containing killed mycobacteria or killed Nocardia asteroides) the rapid production of this experimental malady became possible. Either single or a few injections of CNS tissue with this adjuvant induced encephalitis in the monkey, guinea pig, rabbit, mouse, and dog (3-9).

It is of interest to ascertain whether or not the albino rat is susceptible to the production of this experimental disease since the rat has a low capacity for antibody formation and active anaphylactic sensitization. Neither the phenomenon of Arthus nor skin sensitization to tuberculin has been produced in the rat. However, specific systemic reaction to tuberculin has been demonstrated in this species (10). Since the mouse with similar low capacity for immune response proved to be susceptible to allergic encephalitis, it seemed likely that the rat would not be refractory, although Lumsden(11) failed to induce the phenomenon in rats by subcutaneous injection of homologous brain with adjuvant. In the experiment to be described, homologous spinal cord with adjuvant was injected into the skin.

Procedure. Five male rats (Sherman strain) weighing from 145 to 195 g were used. They were fed Rockland Mouse Diet. The material to be injected was prepared in the following way: Rat spinal cord freed from the meninges was suspended in distilled water containing .25% phenol using a TenBroeck grinder. The suspension contained 33% tissue (wet weight). It was emulsified with an equal volume of a mixture of Arlacel A (1.5 parts) and Bayol F (paraffin oil) (8.5 parts) containing 4 mg (dry weight) of heat-killed and dried tubercle bacilli (Jamaica 22) per milliliter of the mixture. Emulsification was done with a syringe. The rats received 7 simultaneous intracutaneous injections of 0.1 ml of the emulsion, *i.e.*, 16.5 mg of spinal cord, 0.0335 ml phenolized water, 0.0075 ml of Arlacel A, 0.0425 ml of Bayol F and 0.2 mg tubercle bacilli per injection. Three injections were given on the left and 3 on the right side of the back, and one on the anterior surface of the neck. A group of 8 rats served as control. Each rat was given 2 kinds of emulsion: A) water-in-oil emulsion containing tubercle bacilli; B) water-in-oil emulsion containing spinal cord. Seven intracutaneous injections were made, 0.1 ml per site of preparation A on the left and seven 0.1 ml per site of preparation B on the right side of the rats. The amount of spinal cord and tubercle bacilli were the same as in the previous group.

Results. All of the 5 rats of the first group developed flaccid paralysis of the hind legs 11 to 15 days after the injection. Some of the rats had urinary incontinence. The rats were killed when *in extremis*. In the brain and spinal cord there was perivascular cuffing about capillaries and venules; occasional glial nodules were seen. The cells about the vessels were mononuclear cells. No polymorphonuclear leucocytes were found. In the meninges there was slight focal thickening with the accumulation of mononuclear cells; no polymorphonuclear leucocytes were found. The site of the injection was similar to that found in guinea pigs receiving CNS tissue in water-in-oil emulsion containing tubercle bacilli. There were vacuoles of oil underneath the epidermis surrounded by epithelioid cells, fibroblasts, Langhans giant cells, and a few lymphocytes. There was intense phagocytosis of tubercle bacilli and paraffin oil. In some areas there was necrobiosis of the inflammatory cells attracting polymorphonuclear leucocytes.

None of the control rats developed paralysis or other possible signs of encephalitis. Histo-

logical sections of the CNS revealed no pathological changes.

In a subsequent experiment meningoencephalomyelitis was produced in rats substituting spinal cord of guinea pigs for homologous spinal cord.

Summary. The introduction of homologous spinal cord in a water-in-paraffin oil emulsion containing killed tubercle bacilli into the skin of the albino rat produces mononuclear meningo-encephalomyelitis. The symptoms and pathological changes in the CNS and at the site of injection are similar to those found in the guinea pig.

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Recovery of Type I (Brunhilde) Poliomyelitis Virus from Mouse Brain and Spinal Cord.* (19841)

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Dick reported that type 1 (Brunhilde) poliomyelitis virus in the form of monkey central nervous system tissue inoculated into a variety of rodents could be recovered from their brains at least for 10 days from the time of cerebral inoculation(1). No adaptation of this virus to rodents occurred despite his employment of the method of alternating passage between rhesus monkeys and rodents and passage from rodent to rodent. There was neither clinical nor histological evidence of disease in the rodent hosts studied. It was stated "this is highly suggestive that no multiplication had occurred in the brains of these animals."

This interesting phenomenon was explored as part of a study in this laboratory on poliomyelitis virus-host adaptation. The purposes of this report are to confirm Dick's findings in murine brain and also a) to indicate recovery of virus at a site *distant* to the site of inoculation namely the spinal cord, b) to identify the virus recovered as type 1 virus which had not undergone detectable immunological alteration, and c) to indicate that the biological state of the virus was not such as to interfere with the propagation of a mouse adapted type 2 (Lansing) poliomyelitis virus in such mice.

Materials and methods. Type 1 (Brunhilde) poliomyelitis virus was utilized in the form of a 20% suspension of spinal cord enlargements of monkeys sacrificed at the height of paralysis following thalamic inoculation. This virus pool yielded a titer of $10^{-5.4}$ in monkeys. The mouse adapted type 2 (Lansing) poliomyelitis virus was an aliquot of a pool of infected mouse brains. The number of previous mouse passages approximates 250; average titers of $10^{-3.6}$ are observed with intracerebral inoculation of 0.03 ml in young adult mice. Swiss mice of Harlan stock were utilized in all the observations herein reported. Roller-tube tissue-cultures were utilized for the isolation and identification of poliomyelitis virus. Monkey testicle(2) was used as tissue

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