IMMUNIZATION OF DOGS AGAINST RABIES WITH CULTURE VIRUS

THE literature on rabies contains conflicting evidence as to the immunizing potency of rabies vaccines. Positive results were obtained by Fermi in rats, by Stuart and Krikorian in rabbits, by Remlinger, Kelser and Shortt in rabbits and monkeys. In all cases, however, large amounts of vaccine in multiple doses had to be given, one per cent. or more of the body weight. A critical review of the literature was published by Webster.[1]

A primary problem in antirabic control is the immunization of dogs. For practical reasons single massive doses of various types of vaccines have been used, generally with unsatisfactory results. Recent examinations of the potency of various commercial vaccines, made by Webster,[2] showed that only two chloroform vaccines were efficacious in immunizing mice when doses used were relatively five times as large as those given to dogs.

In our work on formolized culture vaccines in mice[3] we found that a large proportion of mice could be immunized with 5 doses of 0.25 cc given intraperitoneally, if cultures having a mouse brain titre of 10,000 were used for making the vaccine. This dosage, however, is also disproportionately large. However, we noted that mice receiving one dose of culture vaccine (0.5 cc) i.p., were immune to a subsequent infective dose of mouse brain virus given intraperitoneally.

This procedure has been tested in dogs with most promising results. Dogs two to four months old were given 10 cc of a formolized tissue culture of the virus or a formolized suspension of infected mouse brain; 7 to 9 days later the dogs received an injection of 5 or 10 cc active culture, also intraperitoneally. About two weeks after the injection of the live culture the dogs were injected into the neck muscles 1.0 cc of 1:200 dilution of mouse brain infected with street virus; the dogs were observed 35 to 52 days. Thus far three different experiments have been completed. Of 9 control dogs 7 (78 per cent.) died, 5 in 14 to 23 and two after 32 and 37 days, respectively. Of 13 immunized dogs all remained perfectly well, ate well and grew normally during the period of observation; no symptoms of any kind were noted. In one experiment the dogs were under observation 57 and in the other two 76 days after the injection of the active culture; none of the dogs showed any untoward symptoms after the injection of live culture. (See Table I).

TABLE I

Virus dilution	Sera from immunized dogs				Serum of control unimmunized dogs
	1	2	3	4	
1:200	3/3	3/3	3/3	3/3	3/3
1:2000	3/1	3/1	3/1	3/0	3/3
1:20.000	3/1	3/0	3/0	3/0	3/2
1:200.000	...	...	...	...	3/1

Neutralization tests demonstrated that the sera of the treated dogs contained neutralizing antibodies. At the end of the observation period in Experiment I, blood was taken from the immunized dogs as well as from the surviving control dog. The neutralization tests were made with different dilutions of mouse brain virus; equal quantities of whole serum and the virus dilutions were mixed, kept in room temperature (24° C) 2 hours and in the ice-box overnight. The following morning three mice were inoculated with each serum virus mixture. The results given in Table I show that the sera of the immunized animals were able to neutralize 100 mouse cerebral lethal doses of the virus.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

APPARATUS FOR INFLICTING SUBCORTICAL LESIONS IN THE RAT BRAIN

ALTHOUGH the Horsley-Clark instrument was originally designed for mammals of medium size there has recently appeared in this journal an article describing certain modifications adapting this instrument for use on the rat (Clark, 1939). Because there are many investigators who do not possess a Horsley-Clark instrument and therefore can not profit from the suggestions advanced in the earlier report, we wish to describe a substitute instrument which has been in use in our laboratory for several years. Our apparatus, which is a modification of an instrument used by Ghiselli (1937), can be constructed by any competent machinist, and has proved a satisfactory substitute for the Horsley-Clark apparatus.

After the rat has been anesthetized with nembutal the scalp incision is made and the skull is trephined. The animal is placed upon the operating table (A). While the rat's head is held level in the lateral plane the ear plugs (B and B′) are firmly seated in the external meatus; and the set screws (C and C′) are tightened to hold the plugs in place. The triangular mouth bit (D) is slipped under the curving incisors,

[1] L. T. Webster, *Am. Jour. Hyg.*, 30: 113, 1939.
[2] L. T. Webster, *Jour. Exp. Med.*, 70: 87, 1939.
[3] I. J. Kligler and H. Bernkopf, *Brit. Jour. Exp. Path.*, 29: 378, 1938.