## 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.

and the arms to which the bit is attached are pulled away from the animal's head, stretching it tightly and pulling against the ear plugs. At the same time the height of the bit is adjusted to hold the head level in the longitudinal plane. Tightening the set screws (F and F′) makes this adjustment permanent.

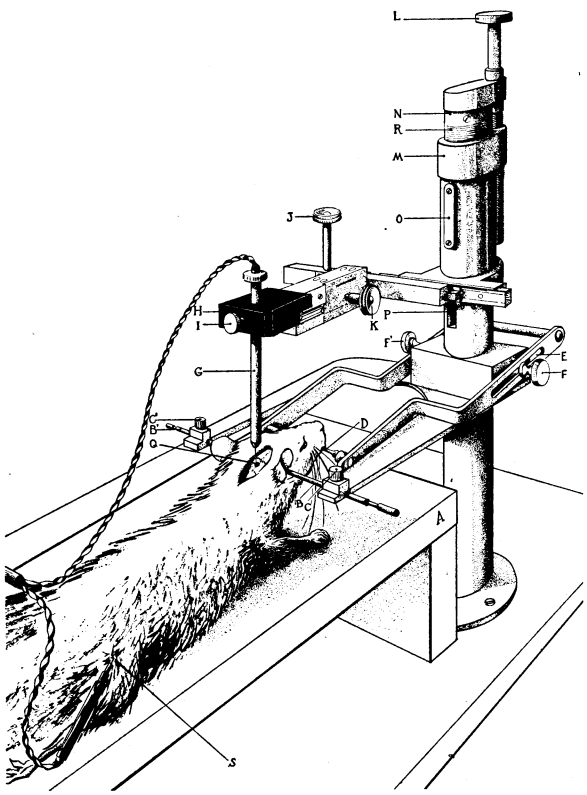


FIG. 1

The electrode holder (G) is adjusted coarsely by sliding it downward through the insulated block (H), and is fixed in position by tightening the set screw (I). The insulated block (H) is attached to a mechanical stage which, in turn, is fastened to a sliding collar (M) that moves vertically on a fixed shaft (N). The mechanical stage, taken from a microscope, is fixed to the collar by two small screws and the insulated block is similarly attached without in any way spoiling the stage for later use on a microscope. The mechanical stage hand screws (K and J) facilitate delicate lateral and longitudinal adjustment of the electrode.

After the horizontal position of the electrode has been adjusted, the entire unit (stage, insulated block and electrode holder) is lowered by turning the large screw (L) which controls movement of the collar (M) on the vertical shaft (N). The depth to which the needle is inserted may be read in millimeters on the scale provided (R). As the collar is moved vertically on the shaft, sidewise movement is prevented by the key (O) on the collar, which fits snugly in the slot (P) in the shaft.

A steel needle inserted in the hind leg of the animal serves as the indifferent electrode. The electrode (Q) in the brain is composed of fine insulated wire. The point of the wire is sharpened and the insulation is removed for a few millimeters above the point. When a direct current is passed through the electrode an electrolytically produced lesion is inflicted about the uninsulated point of the wire.

Cortical destruction is limited to the puncture made when the fine electrode is inserted. The extent of the subcortical lesion is a function of the length of uninsulated electrode, the strength of the current and the duration of its application. We have found it advisable to make the current at a very low voltage (less than 1 volt) and then step it up rapidly to the desired maximum. After the exposure the current is brought down to the low voltage before the circuit is broken. Make and break shocks are thus eliminated. Destruction of relatively large nuclei (*e.g.*, the lateral geniculate) may be achieved with an exposed electrode area of approximately $\frac{3}{4}$ mm if a current of 8 volts is applied for 30 to 45 seconds. Nearly all the corpus striatum is destroyed if 1.5 mm of the electrode is exposed and a current of 9 volts is applied for 90 seconds.

Absence of cortical landmarks and variability in the size of the rats used makes it difficult to place the electrode accurately. A few preliminary operations serve to determine the correct point of insertion necessary to achieve destruction of a particular region. Attacking the corpus striatum we have found the skull sutures to be the most practicable landmarks. If the electrode is inserted directly through the coronal suture at a point 3 mm laterally from the sagittal suture and lowered to a depth of 4 mm below the surface of the brain the uninsulated electrode lies directly in the center of the corpus striatum.

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