and locus of the destroyed region is an important desideratum. The older methods of destruction by mechanical means, such as by the use of a scalpel, brain knife or especially devised trephine, do not allow for this precise control. They can not be used for destroying internal cell masses when the destruction of the overlying tissues is to be held at a minimum. Adequate control is also lacking in the method involving the injection of chemicals into the parts to be affected. Although the intervening tissue destroyed may be reduced to an exceedingly small amount, it is impossible to accurately control the locus and size of the regions infiltrated with the chemical.

The electro-cautery method offers a more accurate control over the amount of destruction and has proved extremely valuable for ablating external layers of tissue. It is not used for destroying internal masses. High frequency currents have likewise offered a means for attaining an accurate control over the amount and locus of the region affected. They have been used with marked success by surgeons in the removal of various types of neoplasms, and a rather refined technique of electro-surgery has resulted from this particular application. These currents have also been used for heating internal cells masses, even to lethal temperatures. No account has been found of high-frequency currents being conducted into the inner parts of a tissue by means of an insulated electrode, thus producing localized destruction of cells.

In endeavoring to study the functions of certain diencephalic centers in emotional responses of animals it became necessary to devise some technique for destroying these deeper nuclei of the brain without releasing them from the restraining influence of the higher cortical centers. This meant that the destruction of the intervening nervous tissue had to be held at a minimum. After considerable experimentation a technique has been devised utilizing high frequency electric currents, which accomplishes well-defined destruction in internal cell masses and which enables the operator to exercise a very precise control over the locus and the amount of the tissue affected.

The high frequency current is generated by a shielded thermionic oscillator[1] which has an output of 50 watts at a frequency of 3×10^6 cycles per second. Most of this energy is dissipated in a voltage stabilizing circuit. A relatively small amount of the current is applied to an electrode, which is insulated except for one or two millimeters at the tip. This electrode produces negligible mechanical injury when it is inserted into the tissue because of its small diameter, which is approximately 0.3 mm, including the insulation. By using the proper duration and density of current, a definite globular region of thermally coagulated tissue surrounding the electrode tip may be produced. A large indifferent electrode is used to complete the circuit. The amount of tissue destroyed varies with the amount of uninsulated electrode surface, as well as the current density. A thermionic voltmeter across the active and indifferent electrodes has proven more useful in controlling the current than has direct measurement of the current flow. Shreds of tissue adhere to the electrode tip if the voltage is too high, which is of course objectionable in many cases. This usually may be prevented if the proper voltage-duration ratio is used.

In operating upon the deeper nuclei of the brain the first step is that of trephining the cranium. Following this the electrode is inserted with a goniometer-manipulator, accurately oriented with respect to the animal's brain by various landmarks on the skull. The manipulator is designed so that the electrode can be placed in any position and inserted at any angle desired. The animal's head is immobilized by means of suitable clamping devices. The precise adjustments of the goniometer for accomplishing destruction in any desired area are previously worked out from the stained sections of the brain of another animal. The proper voltage-duration ratio for producing a given amount of destruction must also be worked out previous to the operation.

To date, the method has been used only for producing internal lesions in the encephalon. It should, however, prove serviceable wherever controlled lesions in the deeper structures of the body are required. Because of the accurate control over the amount of destruction, the method can be used for making extremely minute lesions which are difficult of accomplishment by mechanical means.

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[1] The writers wish to express their appreciation to the National Research Council for a grant-in-aid for constructing the high frequency unit.

A SIMPLE CALIBRATION METHOD

I HAVE devised a method for calibrating pipettes which is so simple that any technician who can perform a determination of uric acid in blood may use it. The method is believed to be original.

The example which follows is based on the uric acid determination, but other substances may be used.

The stock standard solution of Folin and Wu[1] is a 0.1 per cent. solution of uric acid, 0.020 cc of which contains 0.020 mg of the substance—the amount contained in 5 cc of the dilute standard.

To calibrate a capillary pipette to deliver, say, 20 cmm, it is necessary first to mark it approximately

[1] *Jour. Biol. Chem.*, 54: 153, 1922.

with a wax crayon. Then fill to the mark with the stock standard solution and discharge into a small volume of water in a test-tube. Draw up a little water and discharge this also into the test-tube. In another tube place 5 cc of the dilute standard solution. Make both tubes up to equal volumes with water and proceed with the determination in the usual way. With the standard set at 20 a reading at 20 would, of course, mean that the crayon mark is correct.

It is, of course, necessary that the 5 cc pipette be correct, as all capillaries will reproduce whatever error it may have.

The method may be employed to calibrate any size pipette by using solutions of appropriate concentration. The volume of a single drop also may be determined: a great convenience sometimes.

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SPECIAL ARTICLES

PREVENTION OF EXPERIMENTAL EQUINE ENCEPHALOMYELITIS IN GUINEA PIGS BY MEANS OF VIRUS ADSORBED ON ALUMINUM HYDROXIDE

RHOADS[1] reported the prevention of experimental poliomyelitis in monkeys by the use of virus adsorbed on aluminum hydroxide, Wilstaetter's Type C.[2]

In experiments with the virus of equine encephalomyelitis[3] an attempt was made to immunize guinea pigs against the experimental disease by means of the virus so adsorbed. It was found, however, that the adsorbent, when injected subcutaneously in amounts comparable to those previously employed, produced large, nodular masses which resolved with difficulty and often became inflammatory. Inasmuch as aluminum hydroxide prepared at pH 6.6 and sterilized by heat adsorbs as much as 99 per cent. of the virus from active material, the use of less than one twentieth of the amount of the colloid hitherto employed resulted in active immunization of the test animals. Moreover, the sites of injection showed only inconsiderable, localized indurations that disappeared within 5 or 6 weeks.

The source of virus was either the brain of guinea pigs which had succumbed to the experimental disease, or tissue cultures.[4] The latter were found to be more desirable, since less protein is present and hence less adsorbent is required. Another advantage is that in tissue-culture virus concomitant infectious agents can more easily be controlled.[5]

Guinea pigs received three subcutaneous injections of one cubic centimeter each, at 7-day intervals, of the adsorbed virus. The adsorption is of such a character that none of these animals revealed signs of infection during the period of immunization. On the tenth day after the third injection, they were shown to be resistant to an intracerebral inoculation of virulent guinea pig brain material in dilutions of 1:600 to 1:1600.[6] It should be emphasized that the virus was introduced directly into the brain in the test dose, which was lethal for control animals in dilutions of 10^{-5} or 10^{-6} when given subcutaneously or intracerebrally, respectively.

Thus far 40 guinea pigs have been inoculated with the aluminum hydroxide-virus material: 16 with the Western strain of tissue-culture virus, 10 with a mixture of Eastern and Western strains of similar material; 9 with the Eastern strain of guinea pig brain virus, and 5 with a mixture of Eastern and Western strains of the brain virus. None of these animals was affected after the intracerebral injection of the homologous strains as a test for resistance. On the other hand, all the 14 control, non-immunized guinea pigs died of experimental encephalomyelitis within from 72 to 96 hours after the test inoculation.

Investigations are now under way on the possible use of this method in preventing experimental encephalomyelitis of the monkey and the horse, the length of time the adsorbed virus retains its potency (thus far determined to be at least 10 weeks), and the duration of the resistance after the immunization.

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THE THERAPEUTIC BEHAVIOR OF LUCILIA SERICATA MEIG. LARVAE IN OSTEOMYELITIS WOUNDS

THE blowfly maggot (*Lucilia sericata* Meig.) removes by ingestion the acid-forming and bacterial-

[1] C. P. Rhoads, *Jour. Exp. Med.*, 53: 399, 1931.

[2] R. Wilstaetter and H. Kraut, *Ber. chem. Ges.*, 56: 149, 1923.

[3] We are indebted to Miss B. Howitt, of the University of California, for the Western, and to Dr. C. Ten-Broeck, of the Rockefeller Institute, for the Eastern strain of the virus.

[4] H. R. Cox, J. T. Syverton and P. K. Olitsky, *Proc. Soc. Exp. Biol. and Med.*, 30: 896, 1933; J. T. Syverton, H. R. Cox and P. K. Olitsky, SCIENCE, 78: 216, 1933.

[5] T. M. Rivers and S. M. Ward, *Jour. Exp. Med.*, 58: 635, 1933.

[6] All operations on animals were performed with the aid of ether anesthesia.