

Flat single coil receivers which operate at a radio-frequency of 1 megacycle are made as follows: The coil is made by winding $17\frac{1}{2}$ turns of number 26 AWG enameled copper wire on a form $\frac{3}{4}$ in. diam and $\frac{3}{16}$ in. wide. The coil is painted with collodion to hold the turns together before removing from the form. Next a 2,500 mmfd resonating condenser, germanium crystal, and 3,000 mmfd by-pass condenser are mounted inside the coil. The electrode and ground leads are then attached to the circuit. The circuit is cast into a plastic disk of polyethylene. The ground plate is a tantalum or silver disk $\frac{3}{4}$ in. in diam and 0.02 in. thick with edges rounded and smooth.

The leads used in connecting the electrode and ground plate to the receiver are of the utmost importance. Flexible conducting leads have been made by overwinding a thread 0.015 in. in diam with two copper ribbons 0.0005 in. thick and 0.02 in. wide. The lead is insulated by covering with polyethylene tubing which has an 0.023-in. bore and an 0.014-in. wall thickness.

The electrode which wraps around the nerve is made from 0.002-in. silver foil $\frac{1}{4} \times \frac{3}{8}$ in. This is attached to the flexible lead by inserting the lead and a small wedge of silver foil into a small metal tube and then crushing the tube. The electrode is covered on the outside by 0.118 in.-diam polyethylene tubing $\frac{1}{2}$ in. long which has been cut lengthwise so that the nerve can be inserted. The side of the tubing opposite the cut is pierced in the center to permit passage of the conducting lead and is fused to the end of the tube covering the lead. The other end of the tubing is fused to the polyethylene disk covering the receiver.

The three-coil spherical receiver is made in the same way as the single-coil receiver except that the coils are not cast in solid polyethylene but two hemispherical shells are placed around them and fused together. This makes a lightweight covering which is moisture proof.

There are many advantages to the use of radiofrequencies for transmitting the signal to the receiver and then applying the rectified output of the receiver to the nerve. Relatively low power equipment can be used. The stimulating voltage applied to the nerve can be made to have practically any wave form by proper modulation of the r-f transmitter.

In most experiments we have found it desirable to apply rectangular pulses to the nerve. This is accomplished by modulating the transmitter with a square wave generator in which the width, repetition rate, and amplitude of the stimulating pulses can be controlled over a wide range.

Electrode polarization may be made negligible by the use of rectangular pulses. The pulse width is kept very short (a few milliseconds), and the repetition rate is kept well below what appears to be the fatigue level of the neuromuscular junction. Rectangular pulses lend themselves to detailed analysis in determining optimum conditions for efficient and effective stimulation of specific nerves.

Our method has been used to stimulate radial nerves in the forelegs of unrestrained dogs. Functional electrodes

have been maintained *in situ* for 11 months. Buried units have successfully stimulated the front paw via the radial nerve for over $8\frac{1}{2}$ months. Stimulation of the splanchnics and the vagi in the lower thorax has been carried out for similar periods. Studies have been made of the reaction of the nerves to the electrodes. Grossly, there is no scar tissue response to the encompassing electrode, although microscopic sections of the nerves have shown moderate perineural fibroblastic proliferation.

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Recovery of the Virus of Eastern Equine Encephalomyelitis from Mosquitoes (*Mansonia perturbans*) Collected in Georgia

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Although mosquitoes have long been considered the probable vector for the virus of eastern equine encephalomyelitis (E.E.E.), so far there has been no published account of the recovery of this strain from mosquitoes taken in nature. However, the virus has been isolated from the chicken mite (*Dermanyssus gallinae*), and from chicken lice (*Eomenacanthus stramineus*) as recently reported by Howitt *et al.* (4). Merrill, Lacaille, and TenBroeck (5) had demonstrated earlier that the eastern strain could be transmitted experimentally by several species of *Aedes* (*A. sollicitans*, *A. cantator*, and *A. taeniorhynchus*). They were unable to transmit the virus by *Culex pipiens* or *Anopheles quadrimaculatus*. Davis (1) found that five species of *Aedes* mosquitoes prevalent in Massachusetts, (*A. vexans*, *A. sollicitans*, *A. cantator*, *A. atropalpus*, and *A. triseriatus*) could transmit E.E.E. virus when tested in the laboratory, but no transmissions were obtained from other genera (*Culex*, *Mansonia*, or *Anopheles*). This note is to report on the

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recovery of the E.E.E. virus from the mosquito *Mansonia perturbans* Walker taken in nature.

During the summer of 1948, a few horses in different parts of Georgia were diagnosed as having encephalomyelitis. The brains from two animals were sent to the U. S. Public Health Service Virus Laboratory in Montgomery, Alabama, where the virus of eastern equine encephalomyelitis was recovered from them. During the same period, mosquitoes were collected from different areas in the state by members of the Epidemiology Division of the U. S. Public Health Service in collaboration with the Georgia State Department of Health. Forty-three *Mansonia perturbans* were taken from farms in Burke and Jenkins Counties, where sick horses previously had been reported. These mosquitoes were put into glass ampules, which were sealed in a flame and then quickly frozen with dry ice and alcohol. They were sent in this frozen state to the laboratory in Montgomery. There they were pooled, washed with buffered saline, suspended in 3 ml of buffered saline containing 30% normal rabbit serum, and spun in the angle centrifuge for 20 min at 13,000 rpm. The supernatant fluid was removed and 0.03 ml was inoculated intracerebrally and 0.1 ml intraperitoneally into nine 12-day-old white Swiss mice. After 2-4 days, three animals died and four became sick and were killed. The brains were removed but, because of a bacterial contamination, the suspension of brain tissue was passed through a Seitz filter before inoculation of a second group of mice. The latter animals either died or showed typical convulsions in 2-3 days. Cultures of the brains were negative for bacteria, so that a third passage was made to mice. All of these animals either died or showed symptoms of an acute encephalitis. In a further passage the titer was 10^{-8} in mice.

This virus was then identified as that of the eastern equine encephalomyelitis strain, both by means of the neutralization test in mice and by cross immunity inoculations into immune guinea pigs. The virus was neutralized by the E.E.E. antiserum but not by that of the W.E.E. or St. Louis strains. Guinea pigs, previously proven immune to the stock E.E.E. strain by intracerebral inoculation of 70,000 lethal mouse doses of virus, withstood a dose of the new strain that was fatal for the control animals and for those immunized to the western equine virus. Moreover, an antigen prepared from a mouse brain suspension of this virus gave a positive complement-fixation against E.E.E. antiserum but not against those of the W.E.E. or St. Louis strains. The antigen titer was 3+ in a dilution of 1:32 against the E.E.E. serum.

It should be stated here that the Venezuelan equine virus has been recovered by Gilyard (2, 3) from another species of the genus *Mansonia* (*Mansonia titillans*) taken in nature in Trinidad. Both *M. titillans* and *M. perturbans* are persistent feeders on warm-blooded animals, including horses and chickens. This makes these mosquitoes potentially dangerous vectors of equine encephalitis viruses if they are true vectors, rather than merely transient carriers. Further studies to establish the role of *M. perturbans* in the epidemiology of eastern equine encephalitis are planned for the coming season.

In summary, a filterable virus, proven to be antigenically and immunologically identical with that of the eastern equine encephalomyelitis virus, has been recovered from wild-caught specimens of *Mansonia perturbans* Walker. The infected pool of these mosquitoes was collected in Burke and Jenkins Counties, Georgia during the summer of 1948.

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A Convenient Microsyringe

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An accurate microsyringe is useful for dispensing minute quantities of fluids in laboratory experiments. This type of instrument was used for inoculating individual honeybee larvae in the honeycomb by adding spores of *Bacillus larvae* to the food in which the larvae float during the first two days of life (1, 2). Recently it has been improved to increase its accuracy and convenience, and has been used for feeding minute quantities of DDT suspended in 50% sugar syrup to individual adult worker bees. By this method as little as 1 μ g or less of DDT can be fed to each bee. The unit of discharge is 1 μ l.

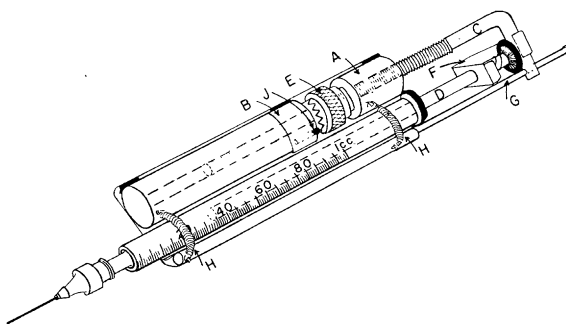


FIG. 1.

The complete instrument is diagramed in Fig. 1. The syringe is a 1-cc glass tuberculin type hypodermic syringe, the 1-cc calibration occupying a linear distance of 56.5 mm. The syringe holder is made of sheet brass formed to accommodate and hold the syringe in position. Two bushings, A and B, serve as bearings to guide the 3/16-in. feed screw C, which regulates the movement of the plunger D of the syringe. The screw is threaded 3/16-32. The feed device E is a knurled nut tapped to fit this screw, and on one end are cut notches or gear teeth. A hole is drilled