and weight. It seems valid to conclude, therefore, that the significantly greater gains made by pigs in lot 2 were due to the addition of vitamin B_{12} concentrate. However, since this preparation undoubtedly contains impurities, it cannot be stated definitely that the growth-promoting activity of the concentrate was due to vitamin B_{12} *per se,* alhough the latter hypothesis seems likely.

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A Technique for Chronic Remote Nerve Stimulation[1]

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Chronic remote nerve stimulation is the usual terminology applied to a technique whereby a nerve in an intact animal is stimulated for periods of weeks or months by an electrical current, the ultimate source of which is removed from and not connected to the animal. By this method the effect of stimulation of a specific nerve in unanesthetized, intact animals can be assayed, thus eliminating disturbing emotional factors due to restraint. Chronic remote nerve stimulation permits close imitation of normal and certain abnormal physiological states.

Various methods for attaining remote stimulation have been tried in the past. Loucks (*12*), and Chaffee and Light (*2*) reported techniques in which a secondary coil was buried beneath the integument, leads from this being connected to electrodes on given nerves. A primary coil positioned over or around the buried unit induced a current in the secondary coil. High currents are required in the primary circuit to induce adequate currents in the secondary. This proves cumbersome and inefficient. These disadvantages can be partially offset by restraining the animal and closely approximating the two coils as Harris (*9*) is doing. However, since restraint of the animal must be employed, such techniques eliminate many of the desirable features of chronic remote excitation. Its great advantage over systems of chronic stimulation by means of lead wires brought out through the skin, as utilized by Hess (*10*), Cannon (*1*), Manning and Hall (*13*), Cressman and Blalock (*3*), Kottke, Kubicek, and Visscher (*11*), is that there is no possibility of infection traveling down the leads. This method also restricts the wave form of the stimulating voltage developed across the secondary coil.

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Newman, Fender, and Saunders (*14*), and Greig and Ritchie (*8*) have reported success using radiofrequencies to provide an electromagnetic field for exciting the buried secondary coil. Fender utilized a frequency of 430-kc while Greig and Ritchie employed a 100-kc frequency. Along with other technical difficulties, polarization at the stimulating electrodes and broken lead wires due to intra-animal motion were the major factors in preventing the attainment of chronicity. Fender (*4–7*) did report one period of 5½ months of successful splanchnic stimulation but broken leads and polarization were always major handicaps in his experiments.

For the past year we have had in operation a system of remote stimulation utilizing radiofrequency for transmission of the signal to the receiver. Detailed studies of polarization with various wave forms have been made. Buried units have been devised and subjected to all the traumata possible in unanesthetized, unrestrained animals. Following is a description of the system which has met our minimal standards of chronicity and remoteness, i.e., stimulation over periods of 4 months in a cage 8 ft. in diam.

The receiver circuit consists of a flat pickup coil tuned to resonate at 1 megacycle by a condenser connected in parallel with it. The r-f voltage output from this is rectified by a germanium crystal and then applied to the nerve which is to undergo stimulation. A by-pass condenser is connected across the output terminals of the receiver to prevent r-f voltage from being applied to the nerve and to increase the receiver sensitivity.

An analysis of the receiver circuit shows that there are optimum values of the circuit elements for maximum sensitivity. The voltage output of the coil under optimum conditions is proportional to the 3/2 power of the coil diameter and to the 1/2 power of the radiofrequency. This would indicate that, within the limits of the experiment, optimal conditions may be obtained with a pickup coil as large in diameter as possible and of a high radiofrequency. The crystal rectifier should have as low a resistance as possible. The number of turns on the coil should be adjusted so that the product of its inductive reactance and Q is equal to the equivalent load resistance presented by the nerve and crystal rectifier. Sufficient capacitance should be added across the coil to produce resonance.

Two types of receivers were constructed: one was a flat disk with a single pickup coil and the other was a sphere with three coils mounted mutually at right angles. The flat receiver is used in experiments where the subject is held fixed for a time in such a position that the receiver coil is properly oriented with respect to the r-f magnetic field. The exciting field may be induced by a large primary coil which entirely surrounds the subject or by a small coil held in close proximity to the receiver. The spherical receiver is essentially three separate receivers with their output terminals connected in parallel. By mounting the pickup coils mutually at right angles the receiver will respond to an r-f magnetic field in any direction. With this receiver the subject may move around freely within a single primary coil 6–10 ft in diam and receive nearly uniform stimulation at all times.

Flat single coil receivers which operate at a radiofrequency of 1 megacycle are made as follows: The coil is made by winding 17½ turns of number 26 AWG enameled copper wire on a form ¾ in. diam and 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 ¾ 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 ¼ × ⅜ 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 ½ 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 8½ 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, Lacaillade, 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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