susceptible mice did not result in transmission of the disease. This is apparent by the absence of paralysis in the uninoculated mice and by the lack of development of neutralizing antibodies. In the course of work with these agents we have seen numerous examples in which uninoculated newborn mice have been kept with their inoculated littermates and only the latter developed disease.

Discussion. It is evident from the results obtained that young mice are susceptible to C viruses orally administered, whereas adult mice neither develop signs of disease nor produce antibodies following such exposure to virus. Von Magnus(5) has recently observed that newborn mice are also much more susceptible than adult mice to oral administration of the TO strain of mouse encephalomyelitis virus.

These results indicate that although one must consider the possibility of accidental contamination of newborn mice in the laboratory (in view of the finding of virus in the intestinal contents of infected mice), the concentrations of virus required to infect by the oral route are much higher than by other routes. This lessens the probability of accidental infections through this cause. Placing groups of uninoculated mice next to or even in with groups of infected mice has not resulted in infection of the uninoculated group.

The fact that some mice surviving oral infection possessed neutralizing antibodies in their blood and some did not deserves comment. The possibility exists that the latter mice were more resistant to the administered virus and that a variation in susceptibility exists among individuals of the same litter of newborn mice. On the other hand, the apparent resistances may be due merely to the technic employed and these animals may not have actually swallowed the virus. It appears more likely that with the more concentrated virus (10^{-1}), mice develop the apparent disease or are not at all infected.

Summary. (1) Newborn mice may be infected by oral administration of at least 5 different strains, representing 4 immunologically distinct Coxsackie, or C, viruses. Adult mice were resistant. (2) Newborn mice surviving oral administration may produce neutralizing antibodies. (3) Adult female mice fed C virus did not transmit neutralizing antibodies to their offspring, in contrast to females inoculated parenterally. (4) In a comparison of routes of inoculation of newborn mice it was found that higher titers may be reached by subcutaneous than by oral administration. The subcutaneous route proved to be about 10,000 times more sensitive than the oral route. (5) Direct contact between inoculated and uninoculated mice did not result in the transmission of infection.

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Implanted Electrodes for Stimulating or Recording from Deep-Lying Brain Structures.* (18476)

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Recent studies (1-5) of brain stem mechanisms and their influences upon the electrical activity of the cortex have suggested the desirability of recording cortical and dience-

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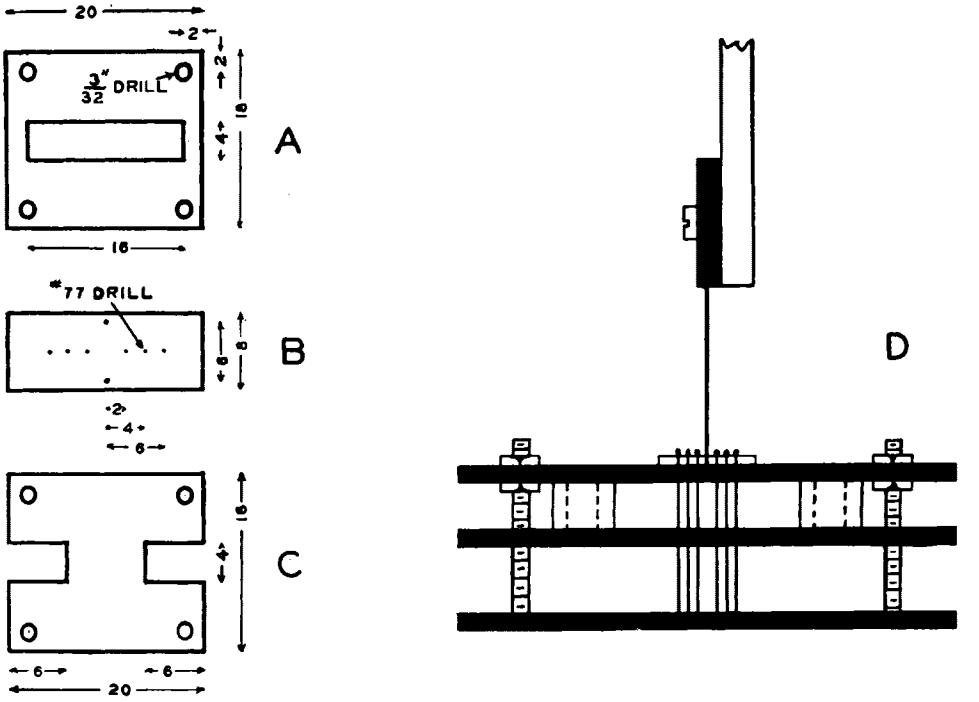

FIG. 1.

A. Standard base plate. *B*. Electrode carrier plate. *C*. Modified base plate used in reaching more lateral structures. *D*. Schematic representation of assembly jig and method of connecting electrode assembly to the Horsley-Clarke multiple electrode carrier. Dimensions are in millimeters.

phalic electrical activity in normal, unanesthetized cats during states of sleep and wakefulness. In order to record from or stimulate deep structures of the brain, a method of permanently implanting electrodes has been developed along the lines begun by Harris(6) and Hunter and Jasper(7). The possibility of wider application of this technic leads to detailed description here.

A permanently implanted electrode system for this purpose must meet three major requirements. It must be capable of *precise placement* or localization in order to explore systematically the matrix of small nuclei in the subcortical regions of the brain. It must be sufficiently *durable* to maintain electrical insulation in the presence of tissue fluids. It should not produce undue *tissue reactions* and irritability. A simple extension of the principles of stereotaxy permits accuracy of placement and satisfies the first criterion. Durability and non-irritability have been achieved by the proper selection of materials. Previous methods of implanting electrodes have used the Horsley-Clarke instrument for orienting the electrodes which are fixed into position by cementing them to sockets screwed into the calvarium. In the present method a new horizontal reference plane is established by attaching a flat base plate to the cranium parallel to the original Horsley-Clarke H (horizontal) reference plane. A second plate carrying the electrodes at predetermined H and L-R (left-right) positions is brought into place with the Horsley-Clarke instrument and cemented permanently to the base plate. In this manner several electrodes may be placed accurately to varying depths over a wide area.

Materials and procedures. The following sections describe in detail the construction of the various parts and sub-assemblies in this order. (1) The base plate. (2) The elec-

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trode carrier plate. (3) The electrodes. (4) The assembly jig. (5) The electrode carrier assembly. After these discussions, the actual implanting operation is outlined and sample recordings are presented.

(1) *The base plate.* A base plate large enough to minimize leveling errors and yet small enough to be tolerated under the scalp is desired. Satisfactory dimensions are 18 mm in the A-P (anterior-posterior) dimension by 20 mm in the L-R dimension. Clear Plexiglas sheet 1/16″ thick is used because of its rigidity, lightness, and non-irritability. A hole, 4 mm A-P by 16 mm L-R, is cut in the center of the base plate to allow for the passage of the electrodes at the time of implantation. Holes (3/32″) drilled in the corners 2 mm from the edges accommodate the 2/56, 1/4″, stainless steel filister head screws which attach the plate to the skull (Fig. 1-A). With such a base plate six or eight electrodes may be implanted to any depth within the region bounded approximately by the A 15, P 2, and L-R 6 planes. To reach more laterally to L-R 10 or 12 a modified design is used (Fig. 1-C).

(2) *The electrode carrier plate.* A piece of 1/16″ Plexiglas sheet, 8 mm A-P by 20 mm L-R is next cut to form the electrode carrier plate. Center lines are inscribed on this plate and six No. 77 holes for the electrodes are drilled 2 mm apart at the desired L-R distances (Fig. 1-B). With the diameter of electrodes used, a 2 mm separation is necessary to prevent undue damage to the brain. Two No. 75 holes are drilled 6 mm apart on the midline to hold the rods by which the electrode assembly is attached to the Horsley-Clarke multiple electrode carrier. These connecting rods are made by crimping a 24-gauge stainless steel tube inside a 20-gauge tube leaving 1 mm of the 24-gauge tubing free to be cemented into the proper holes in the carrier plate.

(3) *The electrodes.* Several 6″ lengths of 26-gauge Nichrome wire are first drawn straight. The larger pieces are cut into a number of 40 mm electrodes and insulated with 5 coats of Belden Insulating Varnish. Each coat is baked at 400°F for 30 minutes. After the electrodes have been properly insulated, 3″ lengths of Grass insulated EEG electrode wire are soldered on for lead-off wires. The soldered joint is insulated with two coats of Tygon primer and one coat of Tygon plastic paint. After the insulation is checked, the electrodes are cut to the proper length and the tips sharpened and polished with emery cloth. It has been determined empirically that the *top of the base plate* will, on the average, lie in the H plus 24 plane. Variation from this mean value is rarely more than plus or minus one millimeter, so that in probing for large structures the error is not significant. However, if extreme accuracy is required, it is necessary to construct several electrode assemblies with electrodes of different lengths; then the appropriate one may be selected when the exact level of the base plate has been determined at the time of operation.

(4) *The assembly jig.* To facilitate the building of the electrode assembly, a small jig was constructed (Fig. 1-D). This jig consists of a floor plate and two adjustable guide plates of 1/8″ brass sheet, 3″ square. Two rows of No. 60 holes spaced 2 mm apart were drilled in both guide plates. One row is for the odd L-R measurement, *i.e.*, L-R 1, 3, 5, etc., and the other for the even distances. The guide plates are held together by 3 pins, while spacer sleeves maintain a separation of 10 mm between the plates. Three 6/32, 2″ bolts attach the floor plate to the guide plates and allow for adjusting the jig to electrodes of various lengths.

(5) *The electrode carrier assembly.* After the jig has been set to the proper H value, the electrode carrier plate is aligned with the proper holes in the guide plates and the electrodes are inserted and cemented in place with Testor's Household Cement. The ends of the connecting rods are dipped into cement and inserted into their holes in the carrier plate. The whole assembly is left in the jig for twenty-four hours until the cement has set; then the electrode carrier assembly is removed and the spacing and insulation of the electrodes is checked. Slight adjustments in alignment may be made by slipping

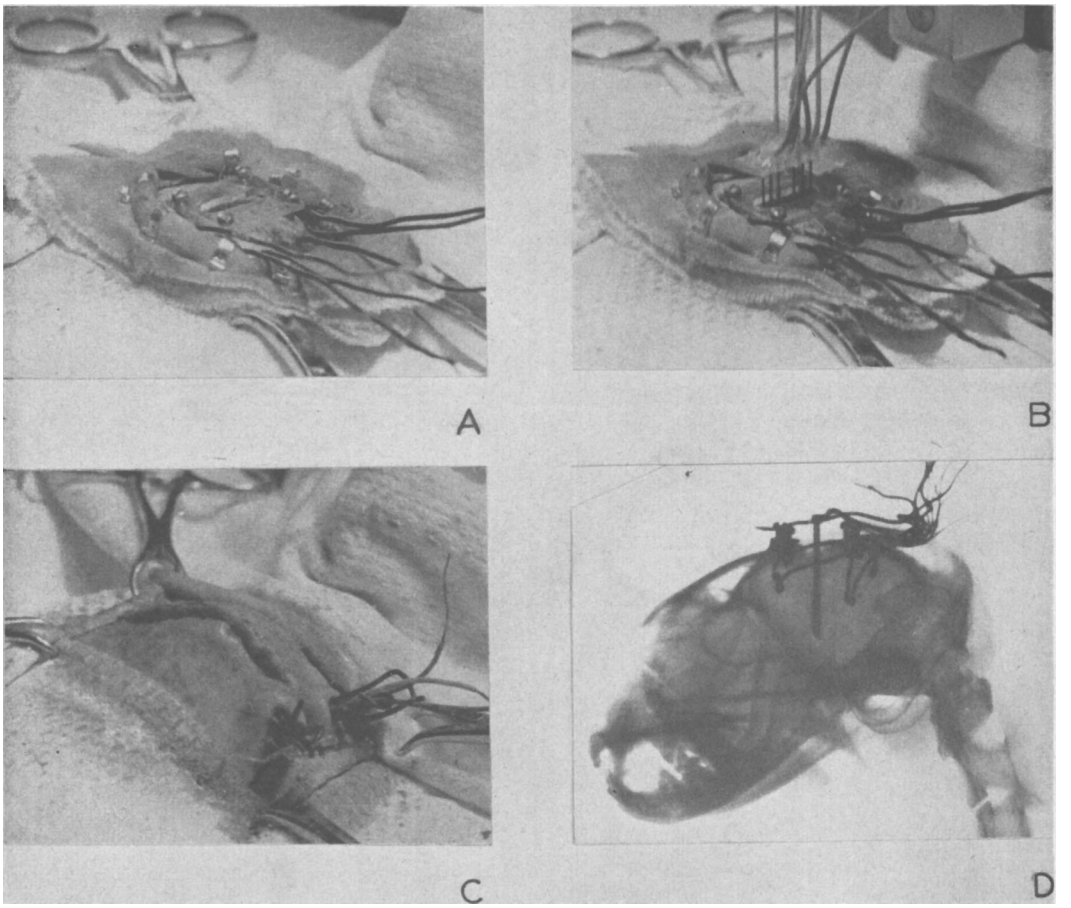

FIG. 2.

Progressive stages of the implanting operation. *A*. Placement of base plate. *B*. Insertion of electrodes. At this point ethylene dichloride is applied around the hole in the base plate and electrode assembly is lowered into place. *C*. Closure of incision over entire assembly with only lead-off wires protruding through stab wounds in skin at back of head. *D*. Lateral X-ray two weeks post-operative, showing six subcortical electrodes (straight vertical lines) reaching deep into the brain and six cortical electrodes (dark "bulbs" and wavy lines) resting on the dura. In all figures the animal is facing upper left.

a 20-gauge tube over the electrodes and bending them back into position. Tygon primer and plastic paint are now applied to the underside of the carrier plate around the electrodes to seal off the holes.

Implanting the electrodes. The electrodes are implanted under aseptic conditions. Zephiran Chloride, 1 1,000 aqueous solution, is used to sterilize the parts of the assembly. Two electrode carriers are required. A straight measuring probe is mounted in one of the carriers and calibrated in the usual manner. The second multiple electrode carrier holds the electrode assembly by means of the two connecting rods, and is calibrated for the A-P and L-R zero points. After the animal has been anesthetized (Nembutal-30 mg/K), shaved, mounted in the Horsley-Clarke instrument and draped, a midline incision is made along the top of the head, and the skin, muscles and periosteum are reflected. A 4 mm A-P by 20 mm L-R craniotomy is marked off over the proper A-P level with the aid of the measuring probe. After the bone has been trephined and rongeured away, the L-R calibration is checked and corrected by reference to the mid-sagittal sinus. Next, the 4 holes for the screws are laid out with

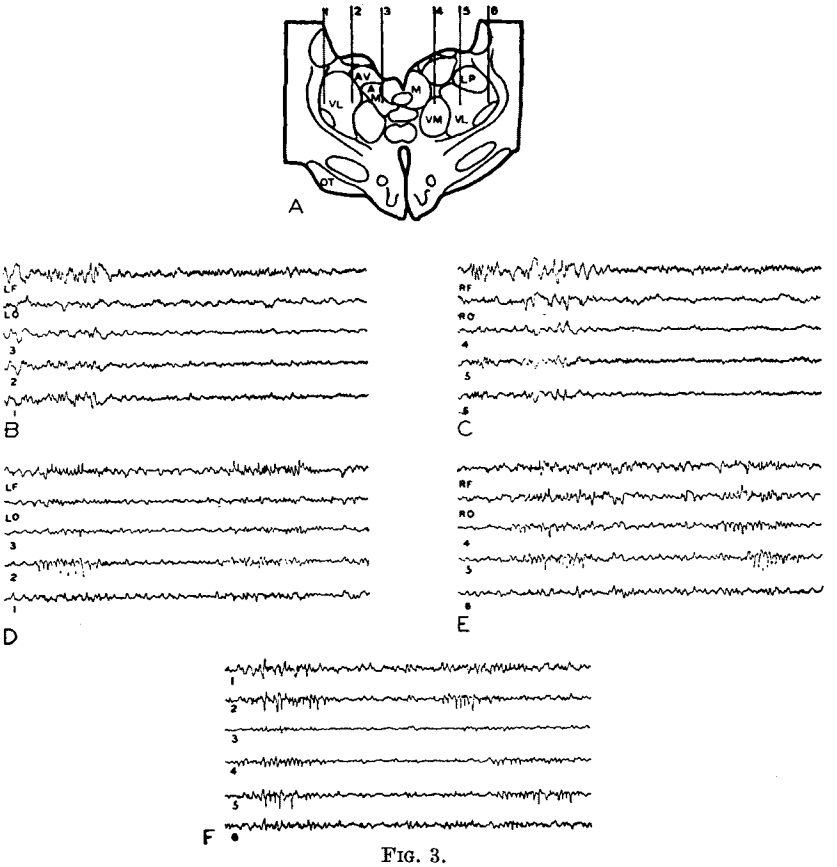

FIG. 3.

Section through rostral thalamus showing, *A*, placement of subcortical electrodes, and cortical and thalamic EEG's on the seventh post-operative day under conditions of normal spontaneous sleep and wakefulness, *B* and *C*; and under light barbiturate anesthesia (nembutal, 10 mg/k), *D*, *E*, and *F*. In records *B* and *C*, taken from the left and right sides of the brain, respectively, the animal is initially asleep and then wakens spontaneously. All tracings are monopolar to an "average" indifferent ground. The gain for the subcortical leads is 1.5 times that of the cortical records. The abbreviations are as follows: AV, anteroventral nuc.; AM, anteromedial nuc.; M, medial nuc.; VM, ventromedial nuc.; VL, ventralis lateralis nuc.; LP, lateralis posterior nuc.; OT, optic tract; LF, left frontal lead; LO, left occipital lead; RF, right frontal lead; RO, right occipital lead.

the measuring probe, drilled and tapped. Then a small amount of bone is whittled away from both sides of the craniotomy near the midline to create a broader foundation for the base plate, which is then attached to the skull and leveled with four stainless steel screws (Fig. 2-A). Again the measuring probe is used to check the leveling and to determine the final base plane. At times it is necessary to support one edge of the base plate with dental cement when the curvature of the skull is greater than usual. After the base plate is in place, the dura is slit and the electrode assembly is racked down until the carrier plate is about ¼" above the base plate (Fig. 2-B). A small amount of ethylene dichloride, the solvent for Plexiglas, is applied to the top of the base plate around the center hole and the electrode assembly is lowered into place. Three to 5 minutes are allowed for the junction to set and the connecting rods are removed. The lead-off wires are laid back along the skull and taken out through two stab wounds in the skin at the back of the head (Fig. 2-C). Closure is made in layers, making sure that the muscles are approxi-

mated as closely as possible around the lead-off wires. A clean dressing is applied and the animal is allowed to recover. Healthy animals generally do not require special post-operative care and rarely do they attempt to scratch at the wires. The animals remain docile and tractable and no difficulty has been experienced in soldering the lead-off wires to the EEG apparatus. When the animals have just been fed and are confined in a small, dark, warm, sound-proofed box, they drop off to sleep readily and continuous recordings can be taken over a period of hours. Examination of the brain of an animal with electrodes implanted for five months showed only negligible gliosis immediately around the electrodes and a slight herniation of the cortex through the slit in the dura. Accordingly, it is believed that by this method electrodes may be implanted in a normal brain and remain permanently serviceable.

Sample observations. The records shown in Fig. 3 were obtained from a cat on the seventh post-operative day. The animal was prepared as described above, and in addition 4 insulated, ball-tipped silver wires were inserted into small trephine holes to form the 4 cortical electrodes. These records are presented to demonstrate the feasibility of the method; a full account of studies on subcortical activity in normal wakefulness and sleep will be published at a later date.

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Temporary Accumulation of Eosinophilic Leucocytes in Spleen of Mice Following Administration of Cortisone. (18477)

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Allen(1) reported the occurrence of conspicuous numbers of eosinophilic leucocytes in the spleen in cases of sudden death caused by trauma and coronary occlusion. It was also noted that in the spleens of individuals who survived several hours the number of eosinophiles were progressively less as the period of survival increased. The explanation of this phenomenon was not known at that time. It was speculated, however, that it might be due to either histamine or acetylcholine release. Thorn(2) later described a test to ascertain the efficiency of the adrenal cortex by the disappearance of eosinophilic leucocytes from the circulating blood upon adrenal cortical stimulation by ACTH or adrenaline, or by injection of cortisone. It now appears most likely that the phenomenon described by Allen can be explained best by adrenal cortical stimulation during periods of stress and it would appear, from the observations in this report, that the eosinophiles upon disappearing from the circulating blood are stored temporarily within the spleen. In order to test this hypothesis the following investigation was undertaken:

Materials and methods. 24 male Swiss albino mice weighing 25 g each were divided into 2 groups of 12. One group was injected subcutaneously with 2.5 mg of cortisone acetate (Cortone acetate, Merck) and the other group served as controls. Eight hours post injection the animals were sacrificed by sharp blows on the back of the necks. This resulted in instantaneous death. All animals were necropsied; the spleen and thymus glands were fixed in formalin and in Zenker's solutions, were prepared for histologic examina-

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