to serve as the only respiratory muscle. Lack of proper central connections prevents spontaneous use of this power in the animals tested.

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A Simple Electrode Carrier for the Exploration of Subcortical Structures.* (19508)

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Electrode carriers for use in the stimulation of or recording from subcortical areas have been developed by Hess(1), and his school (2), Harris(3), Hunter and Jasper(4), and recently by Knowles(5). These technics for implanting brain electrodes, particularly that developed by Knowles, are elaborate and laborious, requiring careful construction of various parts and sub-assemblies, such as base plates, electrode carrier plates, assembly jigs, and electrode carrier assemblies. In addition, the curvature of the skull necessitates, in the Knowles method, the formation of a broad foundation to receive the electrode carrier or base plate; and finally the electrode carrier must be fixed securely and aligned with great exactness in the plane of the stereotaxic apparatus, a process demanding repeated checking and leveling of the electrode carrier. All this is avoided in the method described here, yet the electrodes are accurately placed, the procedure is simple and quick, and no assemblies or sub-assemblies are used.

Procedures. Under general anesthesia a piece of 3/8 inch plexiglas, 15 mm x 20 mm (a, Fig. 1) is fastened over a trephine opening in the skull as securely as possible, disregarding its orientation to the stereotaxic apparatus in which the animal is fixed. A dental drill mounted in the electrode holder of the stereotaxic apparatus is then used to drill guide holes (b, Fig. 1) at the chosen coordinates and in the desired direction. Upon recovery from anesthesia, the electrodes (c, Fig. 1) can be inserted without discomfort to the animal to the desired depth. The depth may be varied throughout the experiment, or the electrode

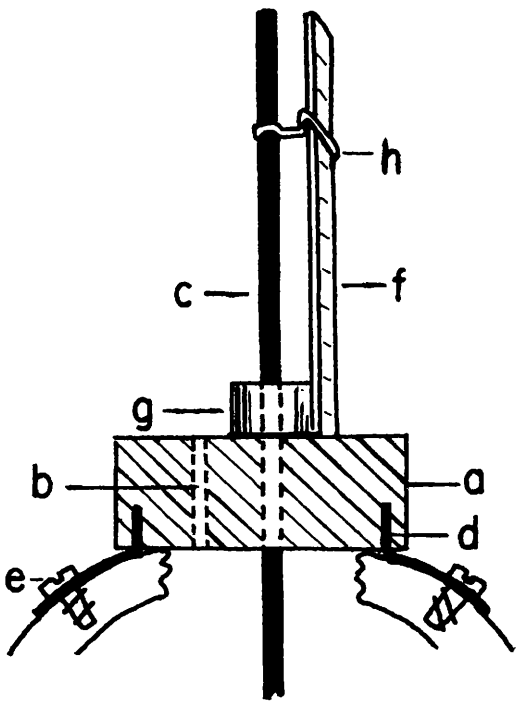

FIG. 1. Plexiglas plate with electrode in place. a—plexiglas plate; b—guide holes; c—electrode; d—solder lug; e—screw; f—scale; g—stop; h—scale indicator; i—spring.

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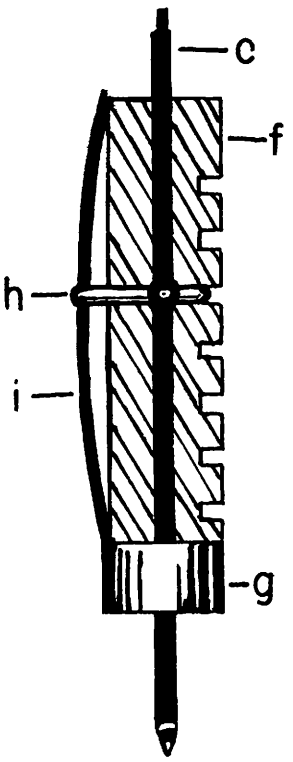

FIG. 2. Electrode with scale and stop.

may be cemented in place at any desired depth. Thus the electrodes are oriented with reference to the stereotaxic coordinates but the plate carrying them is not. This prevents errors which might be introduced by slight disorientation of the carrier plate such as occurs in the methods previously cited.

The plate itself is attached to the skull of the anesthetized animal by a pair of small, doughnut type solder lugs (d, Fig. 1) which are heat crimped, one on each side, into the bottom of the plexiglas. This is placed on the skull in a secure position, and the flexible lugs are bent to follow the curvature of the bone. The opening of each lug is marked on the bone and holes for the fastening screws (e, Fig. 1) are drilled with a dental burr. Self-tapping sheet metal screws fix the plate rigidly. The electrode holder carries a dental drill which is fitted with a bit of desired size whose shank had been adapted to fit the chuck of the drill. The diameter of the bit should insure a snug fit of the electrode in the guide hole. The transparent plexiglas permits drilling of the guide holes without injury to the brain, and the desired depths for the electrodes are easily reckoned since the distance between the cortex and the top of the plate can be seen and measured. The guide holes can be placed within a millimeter of each other without difficulty.

In addition to the plexiglas plate, the apparatus consists of one or more bipolar, concentric needle electrodes, insulated to the tip, and a scale (f, Fig. 1 and 2) notched at one or 2 mm intervals, and cemented at its base to a small cylindrical stop (g, Fig. 1 and 2). The stop is made of hard rubber, center drilled to allow free movement of the electrode, and has one side filed flat to accept the millimeter scale. The scale indicates the position of the electrode in the vertical plane. The scale indicator (h, Fig. 1 and 2) is a flat loop of wire fastened to the upper portion of the electrode. The indicator fits into a notch at the desired scale reading and is held at the chosen level by a spring (i, Fig. 2) which is a bow of piano wire cemented to the base of the scale. Upward movement of the stop-electrode combination is prevented by a small rubber band slipped under the solder lugs just prior to fastening the plate to the skull, and brought twisted over the top of the plexiglas where it can be set to hold the stop flush against the plate.

With the plate fastened to the skull of the anesthetized animal and the guide holes drilled, the incision is closed with a purse string suture. A strip of gauze is then laid around the plate and sealed over the wound with collodion. The animals seem in no way discomforted by the presence of the plexiglas plate.

This method has been used both for stimulating the midbrain, thalamus, and hypothalamus, and for recording potentials from these areas. Histological examination has established the accuracy of electrode placement.

Summary. A simple, inexpensive and quickly executed method for exploring subcortical areas is described.

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Intracellular Development of Vaccinia Virus.* (19509)

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The cytoplasmic inclusions formed in cells infected with vaccinia virus have been recognized for 60 years and extensively studied with the optical microscope. It is generally accepted that the inclusions consist of masses of elementary bodies, but the manner in which they are formed is still uncertain and several conflicting views are held by various authors. The literature on this subject has been reviewed by Van Rooyen and Rhodes(1).

Recent technics of making ultra-thin tissue sections(2-5) have been utilized by Bang(6) and Wyckoff(7), who have each published electron micrographs made from thin sections of infected chorioallantoic membrane. They clearly show the inclusions to be aggregates of elementary bodies. The development of the inclusion bodies has not been reported, however, and it is with this phase of the problem that the present paper is concerned.

Methods. Ten to 12-day embryonated eggs were inoculated on the chorioallantoic membrane with approximately 10^8 infectious doses of virus in order to have as many cells infected as possible. The suspensions of virus were prepared and the inoculations were carried out according to the method of Briody and Stannard(8). The virus was a commercial calf lymph strain of vaccinia (Lederle) passed once in rabbit skin and 3 times in eggs. Membranes were harvested 48 hours after inoculation and fixed for 24 hours in 4% formalin buffered with M/10 phosphate at pH 7.0. The tissue was dehydrated in graded alcohols, starting at 10%, and imbedded in 1:8 methyl:butyl methacrylate. Polymerization was carried out at 60°C overnight using 2-4 dichlorobenzoyl peroxide as catalyst(3). Sectioning was performed on a Spencer microtome modified with a 1:20 wedge and driven by a motor as described by Hillier and Gettner(4). A glass knife(5) was used for cutting. The sections were lifted from the water with collodion covered grids and the methacrylate was removed from the sections with toluene before examination with an RCA electron microscope, Model EMU.

Results. In the ectodermal layer of 48-hour membranes nearly every cell examined was infected with virus and in any one group of cells, inclusions ranging from a few particles to those occupying most of the cytoplasm could be observed. In almost every cell containing a small or medium size inclusion, the inclusion was located at the nuclear membrane and consisted of particles of varying sizes enmeshed in a dense matrix (Fig. 1-5). The matrix appeared less dense in the larger inclusions. Recognizable virus was never seen within the nucleus.

Occasionally, cells were seen to be ringed with virus particles which filled the intercellular spaces (Fig. 5). Cells in such areas contained little and, occasionally, no detectable virus. In other areas, cells appeared to have

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