lar substance into the tissues or the gastric juice, as evidenced by the extracellular granules and cytoplasmic vacuolation previously described. This supports the point of view of Paff and Bloom (*8*) concerning the secretory life cycle of the mast cell.

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A New Brain Slicing Technique for Tissue Respiration Studies

Joseph G. Graca and William N. Makaroff

Department of Pharmacology,
University of Minnesota, Minneapolis

The problem of preparing brain slices for respiration studies quickly and accurately, with a minimum of damage to the tissues, has never been adequately solved. Brain tissue lacks firm consistency and homogeneity and is therefore subject to more maceration and damage when using free-hand methods of cutting than are some of the other tissues of the body. This problem is magnified in the small animal, such as the mouse or the rat, where the organ is not only gelatinous but is also small. Under these conditions Umbreit (*1*) has recognized that "the free hand method of slicing even by experts leaves much to be desired." In the course of work requiring serial sections of mouse brain tissue for respiration studies using the conventional Warburg respirometer, the senior writer has developed a new technique, rapid and consistent in results, which does not require any prolonged training in manipulation. Further, there is minimal damage to the cut surfaces of the tissue, since no pressure is exerted in cutting, and the tissue is not subjected to the wiping action of a wide blade. In general, the procedure consists of embedding the brain in agar and cutting it with a thin, flat nichrome wire knife, mounted on a microtome developed for this technique.[1]

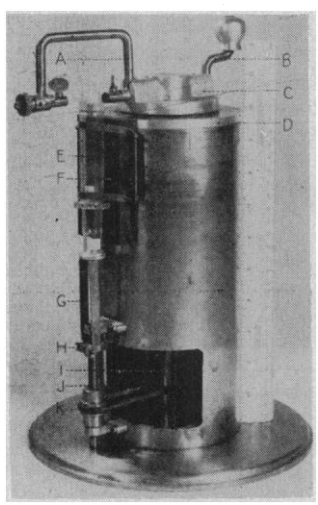

FIG. 1. Microtome with a partially cut embedded brain: *A*, blade frame; *B*, handle; *C*, head; *D*, cylinder cap; *E*, syringe; *F*, mounting block; *G*, brass frame; *H*, split block; *I*, drive shaft; *J*, helical gear; *K*, rubber drive belt.

The microtome (Fig. 1) is mounted on a steel cylinder frame 25.5 cm high having a diameter of 19 cm, which is permanently fixed to a flat, wide base for stability. A shaft 12 mm thick, fixed to the head holding the blade frame and handle, passes through the cylinder cap and ends in a centered well in the bottom plate. The blade rotates on a horizontal fixed plane passing through the embedded tissue, which moves upward a constant distance with each sweep of the knife. The tissue is held in a 10-cc syringe barrel cut off about 2 cm from the tip. The mechanism actuating the plunger of the syringe is a helical gear, connected to the center shaft by a rubber belt, which raises the tissue as the knife is rotated. The instrument described is fixed to cut the tissue 400 μ thick,[2] but any thickness of tissue may be cut by altering the diameter of the drive shaft or helical gear. This may be done with pulleys of various sizes or with a cone-type drive shaft. The syringe barrel holder is a 5 × 9 cm block of bakelite permanently attached to the cylinder wall and cut vertically in the center to accommodate the syringe barrel snugly when attached to the block with two rubber bands. A groove is cut into the block to accommodate the rim of the barrel. This prevents vertical displacement of the syringe and allows the knife to pass just over the surface of the barrel. The plunger rests on a brass frame riding on each side of the helical gear fixed at the top and bottom to the cylinder frame. A split block, forming the bottom of the brass frame, is grooved to fit the gear and rides upward as the gear is rotated. The plunger may be adjusted as may be required for the tissue, or to return the frame to the bottom after cutting, by opening the screw lock on the frame block, causing the two parts to spring apart slightly and drop. After setting it to the proper depth it may again be tightened.

The U-shaped blade frame (5 × 8 cm) is patterned after a jeweler's saw. Flat jaws for holding the blade are tightened by thumbscrews, and tension may be exerted on the blade by adjusting the screw passing through the outer part of the frame. The knife blade itself is made from a 0.005″ × 0.030″ nichrome heating element wire sharpened on the leading edge with a small stone and Turkish emery. It is not nec-

[1] The authors wish to acknowledge the capable technical assistance of William Marshall in the construction of the microtome.

[2] The measurements were made on the fixed preparations with an eyepiece micrometer.

essary to have as keen an edge as is required for paraffin sectioning. The photomicrograph (Fig. 2)

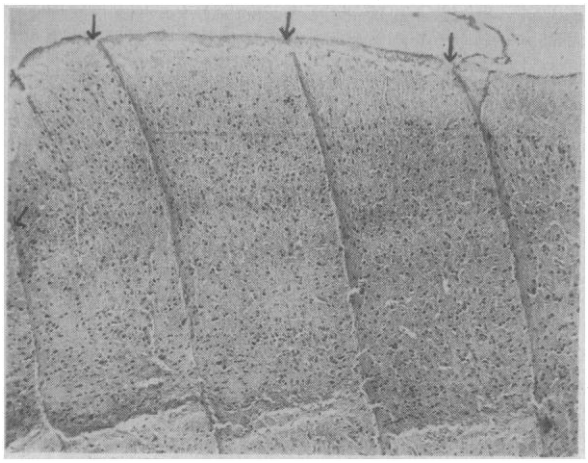

FIG. 2. Formalin-fixed block of sliced brain tissue, cut (7 μ) at right angles to slices (400 μ). Stained with hemotoxylin and eosin. Margins of slices are indicated by arrows.

shows a formalin-fixed preparation stained with hemotoxylin and eosin and cut at approximately right angles to the slices to show the edges of the sliced tissue. A study[3] of these sections showed that tearing and distortion of the tissue were minimal. The cut surfaces appear to be more a separation of the components of the brain on a plane than a sharp cut. The integrity of the cell structures along the line separating the slices appears to be preserved.

Preliminary preparations for embedding the brain consist in melting agar in a water bath, then transferring it to a constant temperature bath held at 39° C. For convenience in handling, a number of test tubes of agar may be prepared beforehand and kept stoppered until used. A large beaker of crushed ice for precooling a small, thin glass vial (2×8 cm) to receive the excised brain and the syringe and plunger is also prepared. A watch glass containing about 5 ml of the same solution to be used in the respirator flask is used to separate the slices transferred from the microtome in preparation for weighing and transferring the slices to the flasks. This is supported by a small beaker or shallow dish containing ice.

The actual procedure begins with the removal and transfer of the brain to the precooled vial in the ice bath, where it is left for about 1 min. The brain is then teased into the well formed by dropping the plunger about 2 cm from the top edge of the syringe barrel. This space is filled to the brim with the agar, and the brain oriented to the desired position, which will be maintained if the syringe is precooled and the agar held at 39°–40° C. The syringe, with a glass vial slipped over the tip to hold the agar in place, is then returned to the ice bath for about 30 sec, mounted on the microtome and the embedded brain cut by rotating the knife. It is important that the sections of cut tissue are not removed until the desired number of slices is obtained or the whole brain sectioned. This stacking of cut tissue helps keep the surrounding agar firmly around the tissues and prevents the slices from being peeled off and torn, as happens when the slices are removed one at a time as cut. Sagittal sections of a whole mouse brain may be cut in less than 10 sec. The entire stack is transferred to the precooled watch glass containing the tissue media. The slices are separated from each other and from the encircling rings of agar by gentle agitation of the solution and some teasing. The selected slices are then weighed and placed in the flasks in the usual manner.

The technique described here offers the advantage of rapidly preparing consecutive brain slices of constant thickness for tissue respiration studies. Further work to adapt this procedure to other tissues is now in progress and will be reported later.

[3] The authors are indebted to Fae Tichy, of the Neuropathology Department of this university, for her valuable assistance in evaluating this important phase of the technique.

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Production of a Solid Rhenide

Ernest Griswold, Jacob Kleinberg, and Justo B. Bravo[1]

Department of Chemistry, University of Kansas, Lawrence

The existence of uninegative rhenium in aqueous solution has been definitely established (*1–3*). Up to the present time the only method that has been successfully employed for the production of rhenide has involved the reduction of perrhenate in acid solution either by means of a Jones reductor or cathodically. A maximum concentration of approximately 0.001 *M* perrhenate has been quantitatively reduced to uninegative rhenium in acid solution (*4, 5*), and a rhenide concentration of 0.0026 *M* has been obtained in an acid solution containing pyridinium chloride (*5*).

We have successfully produced for the first time a solid rhenide material in admixture with potassium hydroxide by reduction of potassium perrhenate in ethylenediamine-water solutions by means of potassium metal. The reaction is carried out in a closed system under nitrogen atmosphere. The valence number of rhenium in the white solid was established in the following way: An aqueous solution of the solid was prepared, and separate aliquot parts were analyzed for rhenium content and for reducing power in terms of number of equivalents of standard oxidizing agent (potassium dichromate) required to oxidize the rhenium to the perrhenate state. Typical values of the valence number of rhenium in the solid established in this manner were: −0.97, −1.06, −1.00, −0.95, −1.16, −0.81.

[1] The authors wish to acknowledge the support of the Office of Naval Research in this investigation.