oral fluid intakes were restricted to 20-percent ethanol for 30 days (*6*). By the fourth day of infusion 14 percent was preferred and after cessation of infusion, preference for high concentrations persisted. Moreover, neither the offering of water for a 5-day period nor the drug injections altered the oral thresholds observed during final testing. In the latter case, both drugs exhibited identical effects; they may have ameliorated slightly the noxious taste of 15-percent ethanol.

In this study, preference for ethanol (*10*) was positively related to the amount of ethanol injected intracranially over a period of time. Although rats of the strain we used normally reject even weak solutions of ethanol (*7*), the infusion of this fluid into brain tissue transformed the predisposition toward ethanol avoidance into preferences for much higher concentrations than have been reported in the literature. In addition, the persistence of the preference for over 2 weeks after cessation of infusion tends to support the hypothesis that the alteration to the central nervous sysem (*11*) may be permanent.

Although it is presently difficult to delimit the general biochemical and neural mechanisms related to fluid selection in this study, several important factors stand out in relation to the preference shifts. From autoradiographic and other evidence (*8, 12*), the improbability of systemic dispersion of ethanol infused intracranially may rule out the etiological involvement in alcoholism of the liver (*13*), endocrine glands (*14*), or bodily nutritional balance (*15*). Corroboration of this concept may rest in the comparison between rats infused intracranially and rats of the same strain orally acclimated to ethanol (*6*). The preference threshold for rats orally acclimated was 6 percent after 600 ml of 20-percent ethanol was consumed over 30 days. This is in sharp contrast to the rapid and almost linear rise in ethanol preference following the brief span of infusion (Fig. 2) of a fraction of the oral amount. For example, after infusing during a 12-hour period a total of 0.1 ml of 10-percent ethanol, equivalent to 0.000083 of the oral intake, a 7-percent solution was selected; after 48 hours or 0.00033 of the oral amount, an 11 percent solution was preferred. Essentially, the presence of ethanol in the central nervous system may have elicited greater perference for the fluid than oral acclimation, because orally ingested quantities of ethanol undergo systemic dilution and partial metabolism prior to passage to the brain.

It is also possible that intracranial infusion acts as a nonspecific stressor, causing the rats to drink ethanol to relieve the stress. However, it has been demonstrated (*7*) that an organism requires time to learn ethanol's placating qualities before preferring this fluid. The rats in this study preferred to drink the fluid without any prior experience.

Finally, the role of acetaldehyde and other metabolic intermediaries in chronic alcoholism is presently unclear. Since evidence on the degradation of alcohol by brain tissue is seemingly not incontrovertible (*13, 16*), it is possible that some of the effects of infusion are due to acetaldehyde or another derivative. In any event the results clearly indicate that chronic and direct alteration of the brain's biochemical "environment" can produce significant changes in later behavior. In this instance, a new biochemical theory of alcoholism may have to be evolved with its primary focus on a metabolic aberration of the central nervous system (*17*).

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4. The Degroot coordinates used for cannula placement were: anterior, 5.8; lateral, 1.5; horizontal, + 3.0.
5. Sterile ethanol solutions for intracranial injections were volumetrically prepared with U.S.P. 95 percent ethyl alcohol and distilled H_2O. Oral solutions were prepared similarly except that tap water constituted the solvent.
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9. Dosages of 80 mg/kg of both drugs were prepared in a gum acacia suspension and injected intraperitoneally 30 minutes prior to each test session. Half of the group received one drug before one test session and the other drug before the other session. The remaining animals were given the two drugs in reverse order.
10. A table indicating mean grams of ethanol ingested at each level of oral concentration is available from me upon request.
11. Under light microscopy, analyses of coronal brain sections stained with thionin failed to reveal any evidence of morphologic changes or lesions other than those of the ventricular cannula tracts.
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17. Supported by National Science Foundation grants G16187 and G19843 and by grants from the Wallace Laboratories and the Colgate Research Council. I am indebted to Professor Raymond Myers for examining the brains histologically and to L. Beideman, R. Carey, B. Holman, P. Kinkaide, L. Pellegrino and W. Rogers for helping with the intracranial infusions.

7 August 1963

Ultramicrotome: A Simple, Easily Constructed Instrument

Abstract. *A practical microtome may be built with nominal skill, hand tools, and a drill press. Thermal specimen advance and cutting action by rod flexion eliminate pivots and critical moving parts. The cost of materials is negligible. Resultant sections are adequate for routine work in electron microscopy.*

The ultramicrotome described consists of a seasoned hardwood base which supports a flexible rod of metal. A specimen-holding chuck is fastened to the free end of the rod, which is mounted horizontally. There is a movable stage designed to hold a glass knife. A vertical guidepost set in the wooden base completes the assembly. The exploded diagram (Fig. 1) illustrates the separate parts, specifications for which are given in the legend. Substitute materials with similar physical characteristics should also be adequate. However, attention should be given to a few critical points. The grain of the wooden base should run parallel to the long axis of the instrument. This is because its coefficient of expansion is very low compared to that of cross-grain construction or other materials [increase in length per unit length per degree Celsius $\times 10^{-6}$: oak parallel to fiber, 4.92; oak across fiber 54.4; commercial aluminum, 24 to 28.7, and so forth (*1*)]. The brass rod must be very securely fastened to the back plate, preferably by soldering. It should be mounted so that it presses gently against the Teflon sleeve of the guide post throughout the entire vertical traverse of the downward cutting movement. When the rod is in its resting position the cut surface of the specimen in the chuck should be about ½

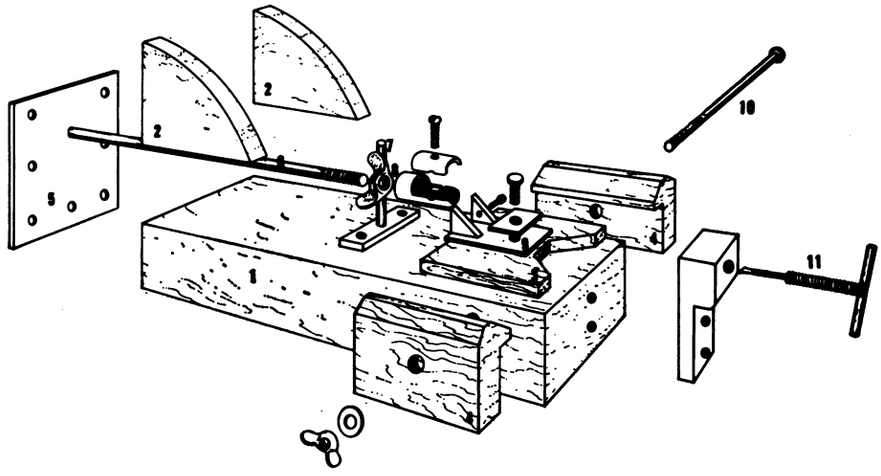

Fig. 1. Exploded lateral view of ultramicrotome. *1*, base; *2*, back plate supports; *3*, knife stage base; *4*, knife stage base clamps; *5*, back plate (brass or steel at least 0.3 cm thick); *6*, specimen holder rod (soldered or bolted to back plate; free end threaded with 8 × 32 die); *7*, guide post (0.6-cm diameter brass rod; surface against which specimen holder rod moves fitted with Teflon sleeve, or highly polished); *8*, specimen holder and locking nut (vice-type chuck drilled to accommodate a 0.6-cm diameter plastic embedment; upper segment receives a 2 × 52 screw which inserts into the tapped lower segment; base of embedment rests against shaft of screw which is tightened for firm support of the specimen by the vise); *9*, knife stage (made of metal to support triangular glass knife; lower hole through which vertical screw passes is drilled oversize to permit positioning of the knife; this screw engages a nut countersunk in the bottom of the knife stage base); *10*, carriage bolt with washer and wing nut for adjusting tension on knife stage base clamps; *11*, knife stage advance mechanism (a 6 × 32 screw threads into a 12 × 24 screw; screwdriver tip engages screwhead mounted in knife base). The instrument is 33 cm long, 13 cm wide, and 14 cm high.

cm below the knife edge. The sliding surfaces of the knife stage should be waxed. All permanent screws should be fastened as tightly as possible.

Plastic tissue embedments and glass knives are prepared and mounted in the usual fashion (*2*). The knife should be laterally positioned so that the part of the knife edge that will do the cutting is directly above the tip of the trimmed plastic embedment. The rod is flexed up and down against the guide post while the knife is advanced slowly by turning the double screw. When the surface of the plastic pyramid is cut by the dry knife edge to yield sections of suitable dimensions, the stage is firmly secured by the lateral screw. The knife trough is then filled with fluid (10-percent acetone in distilled water) and sectioning may begin. On the upstroke the specimen is made to by-pass the knife edge by pressing to the left and upward with the finger tips. The rod is then permitted to flex into position against the guide post, with the specimen being about 1 cm above the knife edge. The rod is released, and a section is cut as the specimen passes the knife edge on its downward way to the resting position. Another cutting cycle may then begin.

Thermal specimen advance is obtained by placing a naked 25-watt electric bulb about 5 to 7 cm from the center of the rod. The resultant rate of advance is suitable for a cutting speed of about 1 cycle per second. If the knife edge is not by-passed on the upstroke there is danger of picking up previously cut sections. It is expedient to flatten the sections with chloroform vapor while still on the fluid. They are then dipped onto a grid in the usual manner (*2*). These operations should be observed under a magnification of about ×10, for which an ordinary jeweler's loupe is satisfactory.

The instrument (Fig. 1) routinely produces usable sections of thicknesses comparable to those generally used in electron microscopy. There is a notable lack of cutting compression in many of them (Fig. 2) as evidenced by round nuclei and so forth. Large fields for low-power work are not difficult to obtain (Fig. 3). In addition to being practical, this type of instrument is valuable for the way it impresses the special problems of ultramicrotomy on the builder. It is a simple construction that can be recommended to anyone wishing first-hand experience with the principles and requirements of thin sectioning for electron microscopy (*3*).

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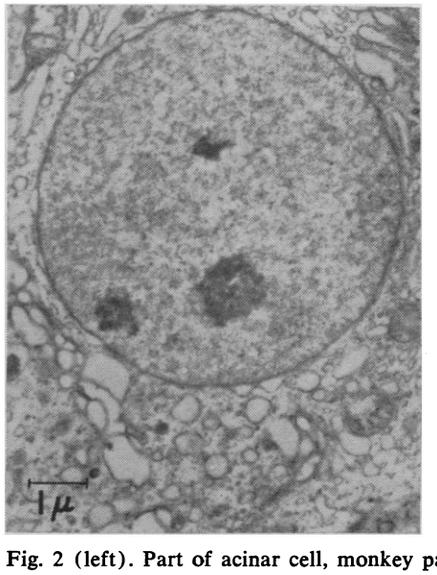

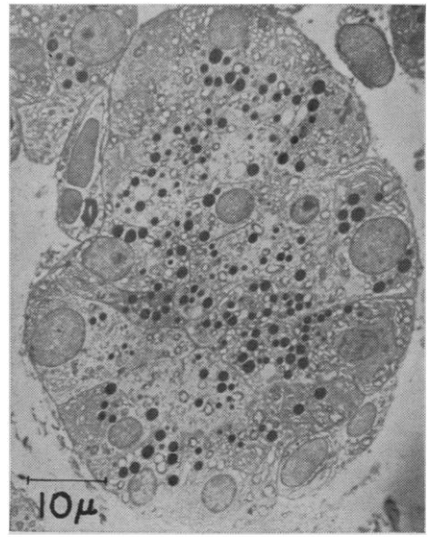

Fig. 2 (left). Part of acinar cell, monkey pancreas. Fig. 3 (right). Acinus, rat pancreas.

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23 September 1963