A Rapid Bone Sectioning Technic.* (17518)

PAUL G. ROOFE, FRANK E. HOECKER, AND CARROLL D. VOORHEES (Introduced by William C. Young)

From the Departments of Anatomy and Physics, University of Kansas.

This paper describes a recently developed apparatus and technic for rapidly cutting undecalcified bone sections sufficiently thin for microscopic and autoradiographic study. This technic is particularly applicable to the sectioning of old, dried bone or bone from mature animals. Sections may be cut at the rate of 20 to 40 per hour and they may be cut serially with the loss of not more than 150 microns of material between sections.

I. *The Sectioning Apparatus.* A conventional brain microtome was adapted by substituting a rotary, motor driven saw blade for the stationary knife blade. The bone to be sectioned was clamped in the reciprocating carriage which fed the bone to the saw. The

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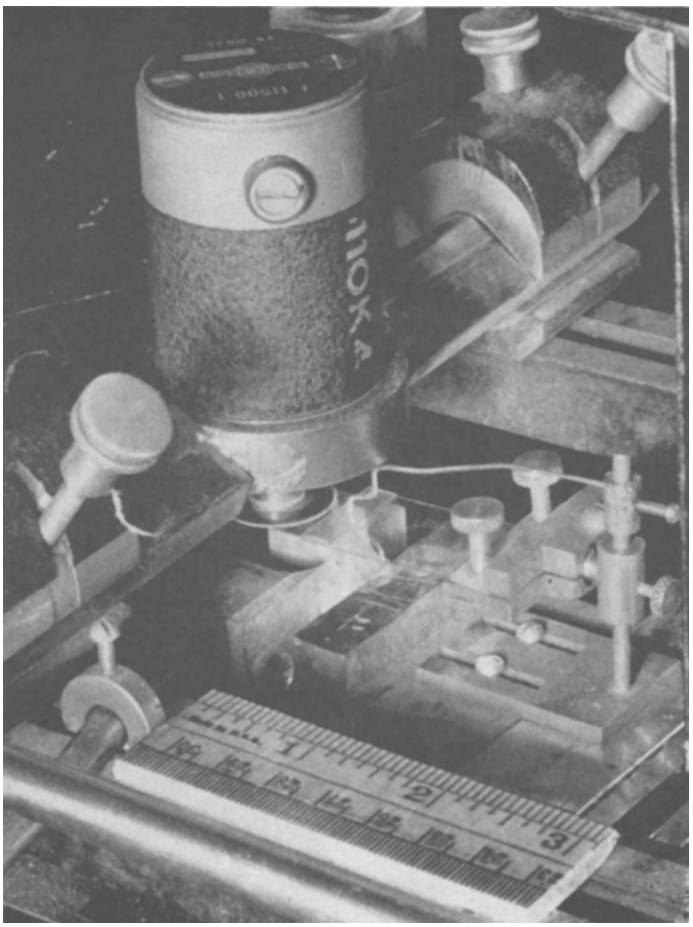

FIG. 1.
Bone-sectioning apparatus showing motor mounting and arrangement for clamping flexible plastic coverslip in contact with upper end of bone.

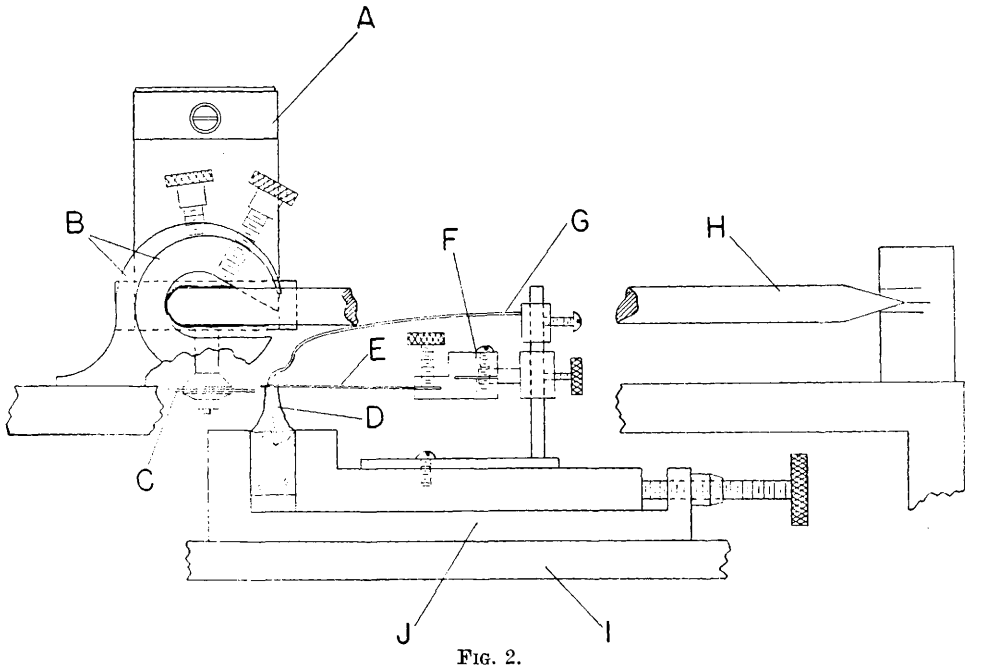

FIG. 2.
Details of bone-sectioning instrument. A. Motor. B. Motor mounting blocks which permit rotation of the motor about a horizontal axis perpendicular to the plane of the paper. C. Saw blade. D. Bone mounted in bioplastic. E. Flexible coverslip resting on upper surface of bone to retain cut section. F. Coverslip clamp. G. Wire for adjustment of pressure between coverslip and bone. H. Index arm for adjustment of attack angle of saw. I. Carriage way. J. Bone carriage.

motor used in this apparatus is a "war surplus" item† designed to drive a rotary switch in aircraft. Its small size (2½ inches x 1½ inches diameter) and the rigid ball bearing mounting of the armature are highly desirable features. However, other designs of motors would no doublt be usable provided the armature end-play was eliminated. The motor is mounted on a solid steel bar machined to fit the knife blade clamps on the chassis of the microtome. These clamps are adjustable so that rotation of the motor about an horizontal axis perpendicular to the path of the bone is possible. This permits a fine adjustment of the angle of attack of the saw blade, preventing it from cutting out of or into the bone as the section is cut. Figure 1 is a close-up photograph of the essential features of the apparatus. The circular saw blades‡ (commercially available for use in motor driven hand tools) are approximately ⅞ inch in diameter, have 88 teeth without set and are 150 microns thick. The blades are attached to the armature shaft, the end of which is machined to the diameter of the hole in the blade, by clamping it between thick, cone-shaped washers which serve to hold the blade rigid. The speed of the motor is controlled by a variable transformer.

Since these blades are manufactured in quantity by stamping, it is frequently found that the mounting hole is not exactly centered. This results in excessive vibration which interferes with the cutting of the very thin sections. This difficulty may be overcome by placing the blade in a mandrel which holds it over an accurately centered hole slightly larger than the original hole. The mounting hole is then ground to the slightly larger size of the centered hole. This, of course, necessitates the use of a machined motor shaft the same size as the enlarged hole.

II. *Preparation of Bones.* The cutting of very thin sections (20-30 microns) necessitates that the bone be surrounded with a shell

† Bendix Radio, E. 11500-1, 28 Volts D.C.

‡ Dremel Manufacturing Co., 2400 Eighteenth St., Racine, Wisc.

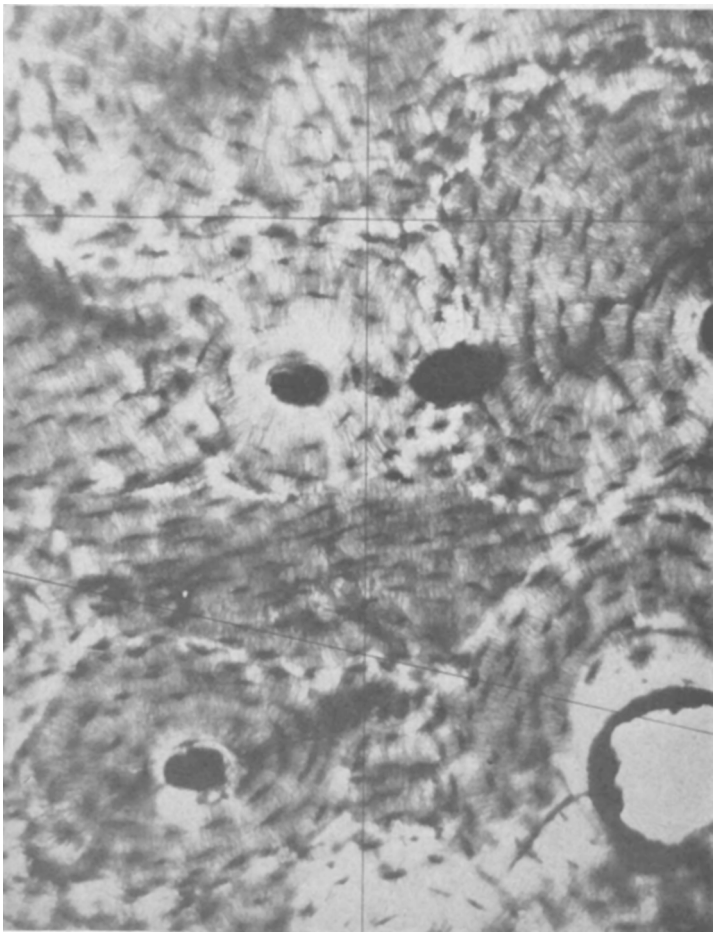

FIG. 3.
The cut section is approximately 30 microns thick and was cut from a piece of human bone from an individual 30 years of age at the time of death, which occurred 18 years ago.

of bioplastic which serves as a binder to prevent shattering of the section during cutting. The bone is prepared by embedding in Bioplastic§ which, after hardening, is trimmed to leave only a thin shell surrounding the bone. This is best accomplished by using a router bit in a motor driven hand tool. A rectangular block of the plastic is left attached to the end of the bone by means of which it may be clamped in the microtome carriage.

III. *Sectioning Procedure.* Before cutting each section, a flexible, plastic coverslip is placed over the free end of the bone. The coverslip is held in place by a holder mounted on a short metal rod, the base of which is attached to the carriage of the microtome.

A damper, consisting of a small piece of a microscope slide, is placed on top of that part of the coverslip immediately covering the free end of the bone. The damper, besides being attached to the coverslip holder by two strong threads, is held in place by a spring-wire keeper. The keeper is mounted in a position above the coverslip holder on the same rod. The end of the keeper is bent at right angles to the long axis and rests upon the damper. Fig. 2 is a schematic diagram of the arrangement of the various parts de-

§ Ward's Natural Science Establishment, Rochester, N.Y.

scribed. The heat generated by the saw blade softens the plastic coverslip just enough to secure the section after the bone and surrounding plastic are completely cut through. The coverslip also prevents the section from warping. The damper serves to reduce a slight vertical flutter which appears in the saw blades when traveling at high speeds and also to minimize other extraneous vibrations. The keeper facilitates a fine adjustment of the pressure exerted upon the coverslip. The operating speed of the motor, and hence the saw blade speed, depends upon the type of bone being cut (degrees of hardness, inherent structure, etc.); the cross sectional area of the bone; the speed at which the bone is fed to the saw blade; and the thinness of the desired sections. Due to the extreme variability of these factors, it is difficult to set down definite rules for governing the saw blade speed; hence an insight into the problem must be acquired largely through experience.

The cut should be made in one firm smooth motion as rapidly as possible so that the section will be in contact with the saw blade a minimum period of time. It has been found that the bone should be moved against the saw blade at a speed approximately 0.5 cm/sec. for best results. After cutting, the sections can be removed from the coverslip by means of a thin razor blade.

If sections are being cut from a bone having a relatively small cross sectional area, a section will sometimes be lost because not enough of the coverslip is softened to secure the section. This loss can be prevented by stopping the cut before the trailing edge of the plastic is completely severed. The cut can then be finished with a thin razor blade without loss of the section. Figure 3 is a photomicrograph of a section of human bone cut by this method. The cut section is approximately 30 microns thick and was cut from a piece of human bone from an individual 30 years of age at the time of death, which occurred eighteen years ago. The bones were kept in a sealed glass jar in the dry condition for the eighteen-year period. They were prepared for sectioning by embedding in Bioplastic as described above without any other treatment of any kind. Several thousand sections have been cut by this method.

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Effect of Pilocarpine on Epinephrine Reversal Produced by 2-(N-p'toly-N-(m'-hydroxyphenyl)-aminomethyl)-Imidazoline. HCl (C-7337)* (17519)

JAMES L. MORRISON, RICHARD J. LANGSTON, AND ARTHUR P. RICHARDSON

From the Department of Pharmacology, Emory University School of Medicine, Ga.

The recent introduction of a number of new drugs, which antagonize the pressor action of epinephrine, has created renewed interest in the well known phenomenon of epinephrine reversal. This problem is of considerable practical importance since many of these newer agents are being used in the treatment of human disease as a possible substitute for surgical sympathectomy. There have been a number of hypotheses suggested which attempt to explain the phenomenon of epinephrine reversal, none of which seems adequate. Substances which produce an epinephrine reversal are considered to block some part of the neuro-muscular apparatus which is concerned with the excitor actions of epinephrine, thus uncovering the inhibitory functions of epinephrine. The antagonism of epinephrine reversal by a wide variety of agents has been reported in recent months.(1-3)

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1. Chen, G., and Russell, D., *Fed. Proc.*, 1949, v8, 280.

2. King, T. O., and Koppanyi, T., *J. Am. Pharm. Assn.*, 1949, v37, 346.