of dogs and were allowed to remain from 30 to 108 days. In addition, tantalum plates were buried in the abdominal wall of dogs. For comparative purposes a limited number of screws and plates of iron, galvanized tin and brass were used. At the termination of the experiment the animals were X-rayed and the metallic implants removed and weighed. The condition of the surrounding tissues was carefully noted and specimens removed for microscopic study.

X-ray Observations. The density of the bone adjacent to the tantalum plates was increased. There was also an increase in density along the shafts of the screws. In the animals with iron and brass screws there was a decrease in density and rarefaction as early as 30 to 32 days.

Weight Changes. These changes are shown in the accompanying table.

Gross Appearance of the Tissue. Surrounding the tantalum there was a thin film of shiny fibrous tissue. In no instancce was discoloration present. Beyond the film the tissues appeared normal. The periosteum of the bone adjacent to the tantalum plates was thickened. Around the iron there was a definite, but slight brownish discoloration. Brass produced a greenish discoloration and marked edema.

Microscopic Appearance of Tissue. The thin film of shiny tissue surrounding the tantalum plates was seen to be composed of fibroblasts. No edema and no inflammatory changes were noted. Sections of the decalcified bone showed no change in the bone substance and a small fibrous capsule adjacent to the periosteum. Sections of tissue exposed to other metals exhibit varying degrees of tissue reaction to the foreign body. Galvanized tin showed a large deposit on the plate, but only small amount of increase in the fibrous tissue about the plate. Brass showed an extreme tissue reaction with edema, fibrin deposit and cellular infiltration. Sections of tissue surrounding brass and iron show large amounts of metallic deposits.

Conclusions. Tantalum implanted in soft tissues is encapsulated by a thin layer of fibrous tissue. On the surface of the bone a similar reaction is observed, and in addition, X-ray reveals an increased density in the underlying bone. The metal is apparently well tolerated by tissue.

13869

A Microtome Sectioning Technique for Electron Microscopy Illustrated with Sections of Striated Muscle.

A. GLENN RICHARDS, JR., THOMAS F. ANDERSON* AND ROBERT T. HANCE. (Introduced by S. Mudd.)

From the Zoological Laboratory, University of Pennsylvania, Philadelphia; RCA Laboratories, Camden, N. J., and Biological Laboratories, Duquesne University, Pittsburgh, Penn.

The preparation of histological and cytological sections for study with the electron microscope presents several difficulties. These are due chiefly to the necessity of drying and to the low penetrating power of electrons.[1] The distortion commonly attendant on air-drying can be obviated by vacuum-drying in the frozen state. The low penetrating power of electrons is more serious since for high resolution electron micrographs it has been found that sections must be less than 0.25 μ and preferably less than 0.1 μ thick.[2] von Ardenne[3] has described a method, but, judging from the one micrograph published, his sections were not sufficiently thin for good resolution or adequate penetration. The present paper describes a technique by means

* RCA Fellow of the National Research Council. Present address: Johnson Foundation, University of Pennsylvania.

1 See any recent article on electron microscopy.

2 Richards, A. G., Jr., and Anderson, T. F., *J. Morph.*, 1942, **71**, in press.

3 *Z. wiss. Mikr. u. mikr. Tech.*, 1940, **57**, 291.

of which relatively uniform 0.1 μ sections of striated muscle have been prepared and examined with 60 kV electrons.

Some types of material can be cut free-hand[2] but much histological material requires a microtome. No commercial microtomes cutting such thin sections are now available but standard commercial models can be adjusted by a number of different ways to cut thinner sections. Our machinists have suggested three methods for adjusting microtomes to cut at fractions of a micron: (1) For Minot and similar sliding microtomes the cam knob that hits the setting device to control the movement of the ratchet wheel may be machined so that it has 4 quadrants each with a different radius. The radii can be adjusted so that with the machine set to cut at one micron the micrometer screw moves only approximately one-quarter of a micron (one-quarter of a tooth-spacing on the ratchet wheel) at a stroke, the cam knob being turned by hand between strokes. (2) The single micrometer screw used to move the specimen may be removed and replaced by a differential screw. (3) For indirect drive rotary microtomes, the same result may be obtained by decreasing the angle of the inclined plane. The first method is simplest but it has the least accuracy and does not give an automatic feed. In this preliminary work a Bausch and Lomb 1903 model of the Minot Automatic Precision Sliding Microtome was converted by the first method, but for further work this machine is being changed over to the more accurate differential screw drive with a small, strongly-braced specimen vise.

Muscle was fixed for 10 hours in a freshly prepared solution containing 50% ethyl alcohol, 10% formalin (=4% formaldehyde) and 5% glacial acetic acid. After washing in distilled water,† the tissue was transferred to "Carbo-Wax 4000"‡ in distilled water, the wax concentrated by evaporation of the water under a lamp, then the tissue transferred to pure molten wax (approximately 50° C) for a short while and finally blocked in a paper box.§ An infiltration time of 2 hours was used for a piece of tissue approximately 2 mm on an edge. The block was trimmed so that the surface of the specimen was at a slight angle to the surface of the wax, then placed on the specimen block of the microtome and sections cut perpendicular to the knife edge at settings on approximately 0.25 μ. Since the specimen was inclined at a slight angle, a tapering wedge of muscle was obtained in each uniformly thin slice of the wax.‖ Sections were accumulated in a dry dish. The somewhat hygroscopic wax was first softened by 15-30 minutes exposure to a saturated atmosphere, and then completely removed in distilled water.¶ Suitable sections were chosen under the light microscope and gently mounted on the wire disc[2] which serves for holding specimens in the electron microscope.** The specimens were then rapidly frozen by plunging the disc into

† Saline probably cannot be substituted here because of difficulties encountered in infiltrating with the wax. With *precipitated* protoplasm the distilled water is probably not harmful.

‡ "Carbo-wax 4000" is the trademark for a family of higher glycols prepared by The Carbide and Carbon Chemical Corporation, 30 East 42nd Street, New York City.

§ Since this manuscript was submitted, we have found that infiltration may be performed at room temperatures. Tissues are placed in a fairly deep dish of wax dissolved in water, and the water then removed in a desiccator. This necessitates 10-15 days to obtain a hard wax, but this can be accomplished without crystallization. Both heat and the shrinkage attendant on cooling the molten wax are thus eliminated.

‖ One might expect a good tapering wedge of material in any slice of an inclined specimen but in actual practice the best results are obtained from the thinnest wedges.

¶ Dropping sections directly into distilled water sets up such violent surface currents that this was discontinued in favor of a gentler method of dissolving the wax. The wax, of course, must be removed for it fills the interstices of the specimen (thereby decreasing contrast) and because it is a uniform slice containing a tapering wedge of muscle. This is one of the crucial points in the technic and the outstanding advantage of this particular wax is its ready removal with water.

** No membrane was used for support in these studies. The specimens were placed directly on clean wire screens. Specimens should lie flat on the screen and adhere to it.

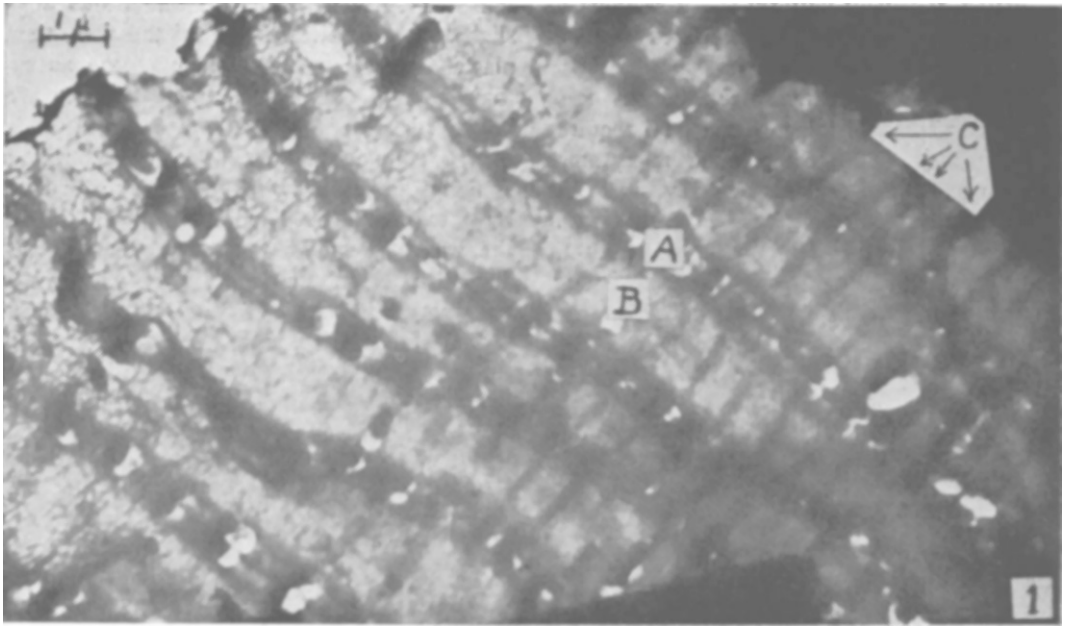

FIG. 1.
Electron micrograph of striated muscle of American coackroach. "A" is dark cross-band composed of 3 lines; "B" is faintly double cross-band in light band; "C" points to longitudinal bands. Direction of sectioning is from lower right corner to upper left corner. Original micrograph, 2200 ×, photographically enlarged to 6500 ×.

petroleum ether at the temperature of a dry-ice (solid CO_2) and alcohol mixture (—72° C). The screen was then transferred to a cold pillar (aluminum rod standing in mercury in a glass cylinder) which fitted into a vacuum system connected to a Cenco Megavac pump, and the specimens dried while frozen. Using a liquid-air trap between specimen and pump, drying was completed in 5-10 minutes. Finally, the specimens were warmed to room temperature, then exposed to air and introduced into the electron microscope.

Stereoscopic and other electron micrographs were taken of sections of striated muscle from the prothorax of the American cockroach *(Periplaneta americana)*. The sections were mostly around 0.2 μ thick but a considerable part of one section was not over 0.1 μ thick and was especially favorable for micrographing with 60 kV electrons (Fig. 1-2). The state of contraction of this muscle is not known. For cross (= transverse) striations the micrographs show dark (= denser) regions ("A") approximately 0.7 μ broad and 1.5 μ apart. Subsequent inspection of this section with the light microscope shows that these denser regions correspond to the dark cross bands seen with the light microscope. Each of these dark regions ("A" is made up of 3 equally spaced dark bands approximately 0.15 μ broad and separated by approximately the same distance. Between the composite dark bands is a lighter (= less dense) region approximately 1.5 μ broad. In the center of this light region is what appears to be a faintly double band ("B"); each of the faintly darker units of this is approximately 0.15 μ broad, 0.15 μ apart and in density intermediate between the light matrix and the dark bands mentioned first. A single muscle disc, then, shows 4 or 5 continuous cross bands; 3 dense ones closely spaced ("A") and one or 2 lighter ones ("B"). In the thinnest section and less distinctly in some of the thicker sections there are also longitudinal bands ("C") approximately 0.15 μ broad, 0.7 μ apart, and having approximately the same density as the 3 dark transverse

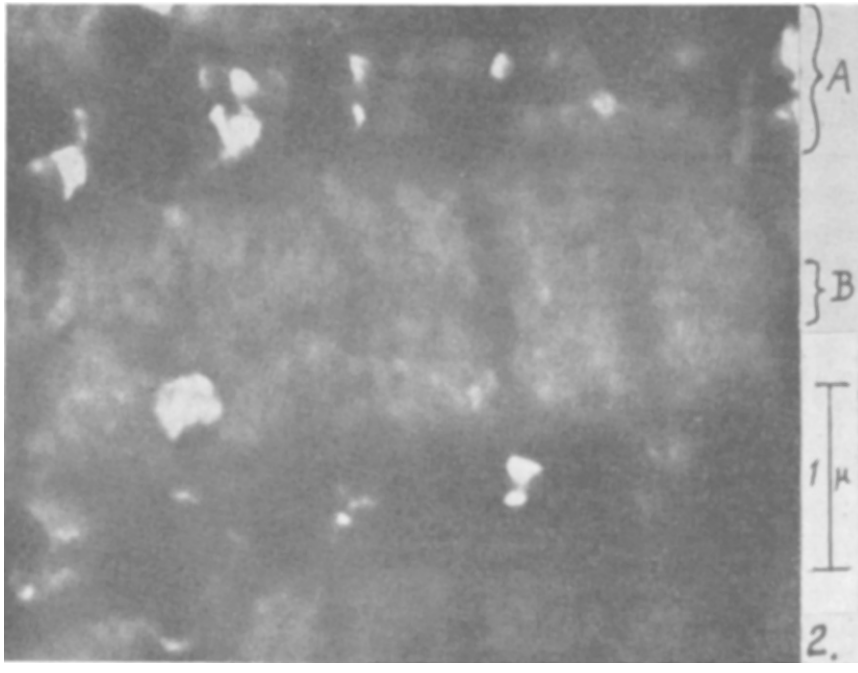

FIG. 2.
Photographic enlargement of a portion of Fig. 1. 23,000 ×.

bands. Stereoscopic pictures show that these longitudinal bands are really regions of greater density rather than being due to any waviness of the section (*i.e.* cutting artifacts). Their periodicity also suggests that they are true muscle components. These longitudinal bands appear to run continuously across the 5 cross bands. Note that a small hole is usually present on the right side of the longitudinal band where this band crosses the composite transverse band (the direction of knife movement was from right to left in Figures 1 and 2); this may mean that these are regions of greater hardness, at least in wax imbedded specimens. These longitudinal bands ("C") may be the myofibrillae but this cannot be stated for certain at present. Filling the interstices between all these bands and grading into them is what appears to be a gel-like material. It seems noteworthy that all the cross bands and the longitudinal bands are continuous and are of the same magnitude—approximately 0.15 μ—although not all of the same density. No further interpretation of these pictures will be attempted at this time since the object of the above description is to illustrate the results that have been obtained with this technique.

As in the usual types of histological technique slight changes in procedure will be necessary for different tissues and will have to be worked out as needed.

The slight irregularities in sharp microtome knife edges cause no appreciable difficulties in the usual histological studies (sections 5-20 μ thick) but cause relatively large linear variations in sections 0.1 μ to 0.2 μ thick. However, since only small areas are used from any one micrograph this is circumvented by selecting uniform areas for micrographing.

Other hard waxes (not soluble in water) can be used but, aside from necessitating the use of fat solvents such as alcohol and xylol, require a long hydration series with an extremely delicate section. Satisfactory results were not obtained by fixing a paraffin section onto a collodion membrane on a screen because the transferral through xylol and alcohol weakened the collodion membrane.

"Carbo-wax 4000" is satisfactory for cutting at small fractions of a micron (inclined specimens within the wax being cut as thinner wedges) and requires only water as solvent.

Full evaluation of the normality of these tissues cannot be given at present. Since fixed material is itself abnormal, more reliable results might be obtained by freeze-drying fresh tissue, sectioning and placing directly in the electron microscope. However, comparison of these micrographs with current ideas of muscle structure suggest that the results with fixed tissues will prove satisfactory.

13870 P

Exploratory Study of Early Effects of Urinary Gonadotropin in Prepuberal Male Rats.*

ELIZABETH Z. BURKHART. (Introduced by Carl R. Moore.)
Clarksville, Arkansas.

Earlier experiments[1, 2] have shown that the effect of a synthetic androgen can be readily detected in the accessory reproductive organs of castrated young adult rats within about 36 hours after its administration. Since testosterone propionate is a fat soluble substance which acts directly upon the accessory reproductive organs, it is of interest to determine the effectiveness of a gonadotropic substance which acts indirectly *i.e.* through its action upon the testis.

Twenty rats at an age of 20 days (29.2 ± 1.07 g) were employed, ten of which were treated on one day only with a pregnancy urine gonadotropin (Antuitrin-S) which is considered by some as entirely luteinizing.[3] Approximately 6 hours before autopsy the rats were weighed and injected with colchicine (0.1 mg per 100 g weight of rat); the gonadotropin and the colchicine were administered subcutaneously. Mitotic activity (see table) in seminal vesicles (s.v.) and in prostate (v. pr.) is recorded as the average number per field of colchicine-arrested cells in 25 fields at a magnification of 430 diameters. The gonadotropin was administered in a single dose or in 2 doses at 8-hr intervals, each injected animal receiving a total dose of 20 rat units (25 I.U.). At selected hours after the first introduction of the hormone a treated and control animal were sacrificed in pairs; both gonadotropin treated and control animals had received the same dose of colchicine.

Results. The ventral prostate and seminal vesicles of all control rats contained mitotic cells ranging in number from 2.32 to 11.88 and 2.12 to 8.28 per field respectively. Pale light areas indicative of secretory activity are already present in the glandular epithelium of the ventral prostate;[4] mitotic cells in the seminal vesicle are fewer per field than in the prostate and the secretory cells are less differentiated. Differences in the rate of development of these two glands have been described earlier.[5]

In both groups of treated animals the

* These investigations were carried on at the University of Arkansas. Grateful acknowledgments are made to the following individuals for invaluable aid: Professors S. C. Dellinger and D. V. Holcomb, Department of Zoology, University of Arkansas, for laboratory space; Dr. S. C. Ericksen, Department of Psychology, University of Arkansas, for rats of the proper age; Miss Grace I. Zimmerman, Dept. of Anat. and Hist., University of Maryland, and Prof. T. L. Smith, Dept. of Biology, College of the Ozarks, for needed technical equipment.

1 Burkhart, E. Z., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 137.

2 Burkhart, E. Z., *J. Exp. Zool.*, 1942, **89**, 135.

3 Gustavson, R. G., and D'amour, F. E., *J. A. M. A.*, 1941, **117**, 188.

4 Moore, C. R., Price, D., and Gallagher, T. F., *Am. J. Anat.*, 1930, **45**, 71.

5 Price, D., *Am. J. Anat.*, 1936, **60**, 79.