

to a delay in the rate of hormone absorption,⁶ it would appear that absorption rates are not similarly affected by these substances in different species.

Summary. Augmentation of the effect of pituitary gonadotropic extracts by the addition of hemin to the injection mixture has been demonstrated in immature male rats. Testes, seminal vesicles and prostates were

significantly heavier in rats receiving hemin with the pituitary extract than in rats receiving the same dosage of pituitary extract alone.

No augmentation was demonstrable in immature male pigeons or immature male chickens. Further increase in weight of the testes was lacking when hemin was added to the pituitary extract in the case of chickens or whole blood in the case of pigeons.

⁶ Maxwell, L. C., *Am. J. Physiol.*, 1934, **110**, 458.

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Ultra-Violet Photomicrography of Muscle.

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A simplified quartz microscope with the 2537 Å line of mercury as the light source has recently been described.¹ Since it is known that nucleic acid, nucleoprotein and proteins absorb in this region, photomicrographs of tissue taken with an ultra-violet light source can be expected to show selective absorption. Also, since these substances do not absorb with the same intensity, it is to be expected that the photomicrographs will show more detail than those obtained with visible light and with the usual staining methods. This technic, then, should be of particular advantage in the cytological study of muscle when it is desired to correlate closely certain morphological and physiological changes. In ordinary light the appearance of muscle is misleading, since the dark and light portions which are seen in unstained material are due to inhomogeneity of the material of the fibers and not to the presence of material with selective absorption.²

Microphotographs of muscle made with the electron microscope have been published recently and a new technic was described whereby sections of muscle can be obtained

in thicknesses of 0.2 to 0.5 μ .³ The present report shows that ultra-violet photomicrographs can be taken of sections of tissue embedded in the routine manner, and cut at thicknesses of 5 μ , and that they depict with great clarity structures similar to those revealed by the electron microscope. Moreover, sections of tissue prepared and studied in this way are free from the distortion which results when they are desiccated as in preparation for study with the electron microscope. No report has been found of an attempt to obtain ultra-violet photomicrographs of muscle since that given by Meigs.⁴ The photomicrograms which he obtained were made from sections of muscle taken from the frog and from the wings of insects; no studies of human muscle were described.

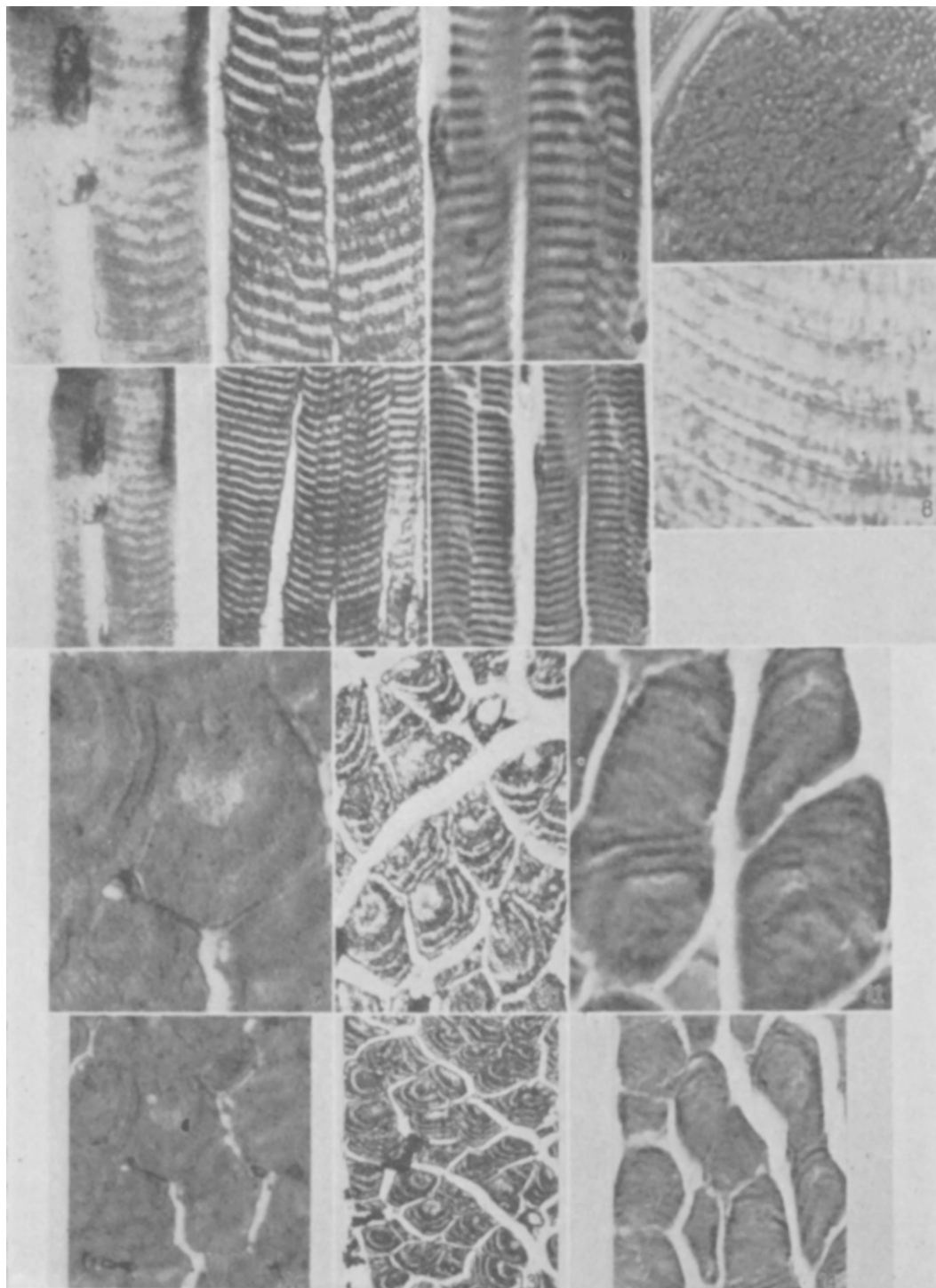
The apparatus used in this study was that described by Lavin and Pollister¹ except that the focusing screen was not attached to the under side of the ground glass. Instead, the screen was placed in a holder, the image brought in focus, and a photographic plate substituted for the screen in order to secure a permanent record. The screen was made

¹ Lavin, G. I., and Pollister, A. W., *Biol. Bull.*, 1942, **83**, 299.

² Bernal, J. D., in *Perspectives in Biochemistry*, Cambridge University Press, 1937, pp. 45-65.

³ Richards, A. G., Jr., Anderson, T. F., and Hance, R. T., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51**, 148.

⁴ Meigs, E. B., *Z. f. allg. Physiol.*, 1908, **8**, 81.



Photomicrographs of stained and unstained sections of human voluntary muscle made in visible and in ultra-violet light, in glycerin and when desiccated as in preparation for study with the electron microscope.

by removing the emulsion from a photographic plate and coating one surface with willemite.*

Human pectoral muscle, obtained soon after death, was chosen for this study. A rectangular section of muscle, *ca.* $1 \times 2 \times 4$ cm, was fixed in Zenker-5% acetic acid solution for 12 hr, washed overnight in several changes of distilled water at room temperature, and carried consecutively through dehydrating mixtures of 50, 75, and 95% alcohol, absolute alcohol and finally xylol. The tissue was taken from xylol, embedded in paraffin, and cut on an ordinary microtome in sections of 5μ thickness. For study with the quartz microscope the sections were transferred to a quartz slide to which they were fixed with a trace of albumin. The paraffin was removed with xylol which in turn was removed with absolute alcohol. While still moist a portion of the tissue was covered with glycerol and a quartz cover slip was attached. An area of each section was allowed to dry when removed from the absolute alcohol in order to study the tissue in the desiccated state.

Fig. 1 is a reproduction of a photomicrograph made in the ultra-violet, at 2200 magnification, of an unstained longitudinal section of human striated muscle. Fig. 4 is the same section at 1100 magnification. Two sets of transverse bands at regular intervals are seen, the dark area corresponding to the anisotropic portion and the light area to the isotropic portion of muscle. An enlargement of an area of this fiber to 7000 is shown in Fig. 8, and reveals in addition to the classical line of Krause in the isotropic zone, 2 additional bands in the anisotropic zone, which absorb light selectively and divide the anisotropic segments of the fibril into at least 3 distinct areas. Longitudinal columns of intense absorption, similar in character to those pointed out in the micrograms made by Richards and associates³ are clearly seen.

The nuclei of the individual muscle cells are shown scattered along the fibers at irregular intervals, immediately adjacent to the sarcolemma. A zone of undifferentiated

cytoplasm appears around each nucleus. In the ultra-violet photomicrographs the sheath of the sarcolemma reveals structure which is not ordinarily seen in stained sections studied in visible light. Fig. 2 and 5, made in ultra-violet light, represent portions of the sections from which Fig. 1 and 4 were made, but which were allowed to dry and studied without the addition of glycerol. The distortion and disruption of structure due to drying of the section is clearly revealed. Fig. 3 and 6 are photomicrographs of sections taken from the same block of tissue and stained with hematoxylin and eosin, and photographed in visible light. Fig. 9 and 12 are photomicrographs of transverse sections of muscle made in the ultra-violet at magnifications of 2200 and 1100 respectively. Fig. 7 is a photomicrograph enlarged to 7000. Structural arrangement of the sarcolemma which make up a single muscle fibril can be observed clearly in the transverse sections. This internal structure of muscle and the structure of the surrounding sarcolemma cannot be seen in similar sections (Fig. 11 and 14) stained with hematoxylin and eosin and photographed with visible light. Again, the marked distortion of structure due to drying is revealed in the transverse sections shown in Fig. 10 and 13.

To obtain a clue to the chemical composition of the absorbing areas of muscle, preliminary studies have been made with a quartz spectrograph attached to the quartz microscope in such a manner that various areas of the section of tissue can be studied with a continuous light source. Results of this work will appear in a later communication.

Summary. By means of an improved technique of ultra-violet light microscopy described in an earlier communication, it has been possible to obtain photomicrographs of muscle which reveal structures heretofore not seen by technics which employed stained sections and photography in visible light. The photomicrographs have been produced from sections of muscle fixed and embedded in the manner usually employed by cytologists and pathologists for microscopy of stained tissue, and cut at 5μ on an ordinary laboratory microtome.

* Kindly made for us by Mr. Paul Nisley of A. D. Mackay Co., New York City.