

tion at 1 P.M. the next day, one *dosis affectans* of atabrine having been given at midnight between the 2 examinations. Twenty-two birds survived long enough to be included in the record of this investigation; controls were injected with water alone.

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Microincineration of Active Smooth, Transitional and Skeletal Muscles.*

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The distribution of inorganic salts in the active and inactive smooth muscle of the intestine and the transitional muscle of the gizzard of birds and skeletal muscle is revealed by the technic of microincineration perfected by Scott.¹ He emphasized the distribution of ash in relatively inactive smooth and skeletal muscle by this method. He did not report in detail on the ash distribution in the active smooth, transitional, and skeletal muscles. This method of incinerating microscopic sections of tissue without disturbing the relationship of the mineral components was suggested by Liesegang² and developed by Policard.³ The recent studies of Kruszynski⁴ are on the topography of the mineral content in relatively resting muscle after microincineration.

The muscle is fixed for 24 hours in 9 parts of absolute alcohol and 1 part of neutral formalin. The mineral salts are neither increased nor decreased by this fixative. The tissue is completely dehydrated by several changes in absolute alcohol. The mineral salts remain intact by this method. It is then cleared in xylol, embedded in paraffin, and cut serially at 4 to 6 microns. Alternate sections are mounted by the usual method, and stained with hematoxylin and erythrosin, whereas, the intervening sections are mounted with liquid petrolatum to

* These investigations were carried out with the aid of a grant for Research to the Department of Anatomy, Marquette University School of Medicine, by the Committee on Scientific Research of the American Medical Association.

¹ Scott, G. H., *Comp. Rend. Acad. Sci.*, 1930, **190**, 1073 and 1323; *Bull. Hist. app.*, 1930, **7**, 251; *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 349; *Am. J. Anat.*, 1933, **53**, 243.

² Liesegang, R. E., *Biochem. Z.*, 1910, **28**, 413.

³ Policard, A., *Protoplasma*, 1929, **7**, 464.

⁴ Kruszynski, J., *Z. f. Zellforsch. und Mikros. Anat.*, 1938, **28**, 35.

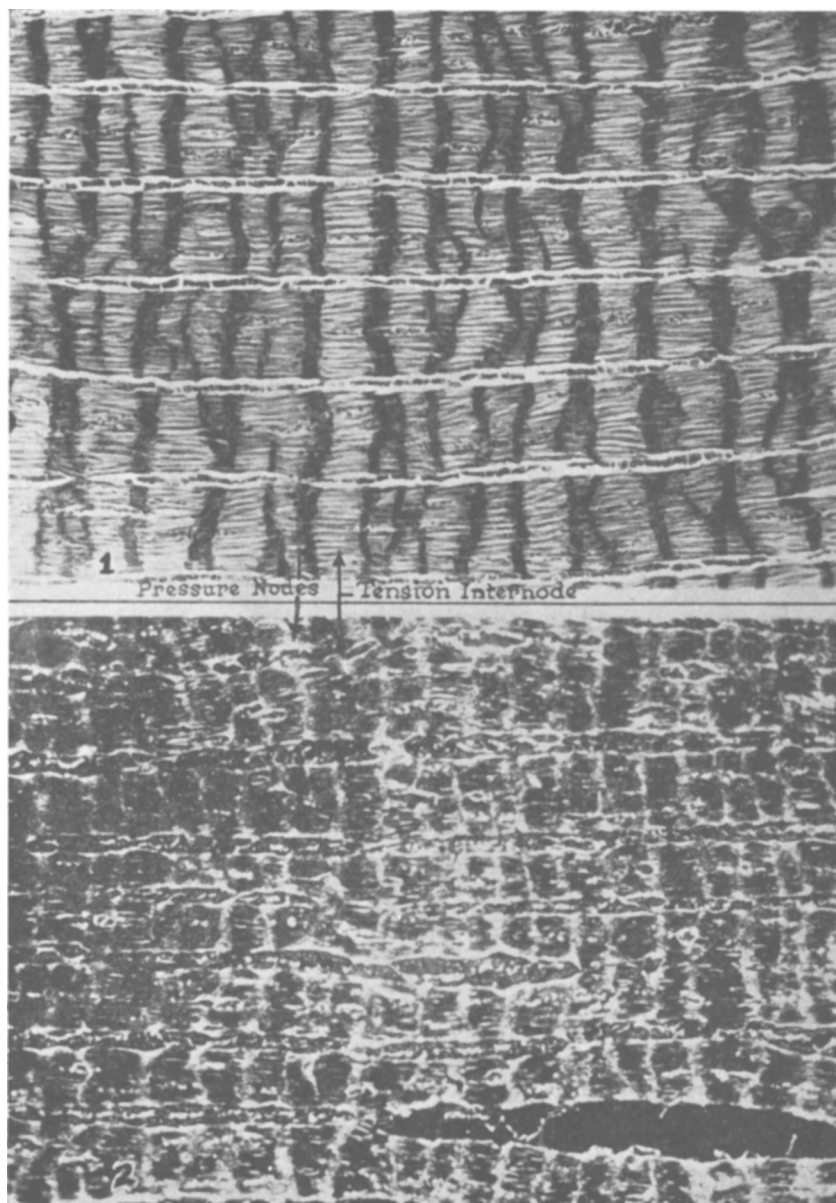
spread the tissue evenly. After the section is flat the excess liquid petrolatum is removed carefully by smooth blotting paper. The intervening sections are incinerated in a closed electric furnace at varying temperatures from 400° to 650°C.

The temperature is raised 50°C every 10 minutes until the highest temperature is reached. Sudden elevation of temperature with an excess of liquid petrolatum underlying the paraffin section results in explosive distortions due to sudden thermal agitation. The slide is cooled slowly over a period of 8 to 12 hours and the incinerated section is then covered with a number 0 cover glass around the edges of which melted paraffin is applied.

The inorganic salts that remain on the slide after microincineration of resting muscle are longitudinally distributed in the inactive cytoplasmic strands of the intestinal and gizzard muscles. In the active muscles there is a transverse orientation and concentration of the inorganic salts in the nodes of the contraction compression waves (Figs. 1 and 2). In the tension internodal spaces, between the pressure nodes, there are either less inorganic salts than in the nodes or in certain places almost a complete absence of detectable mineral salts. Iron oxide, calcium oxide, and silicates are identified in the inorganic deposit.

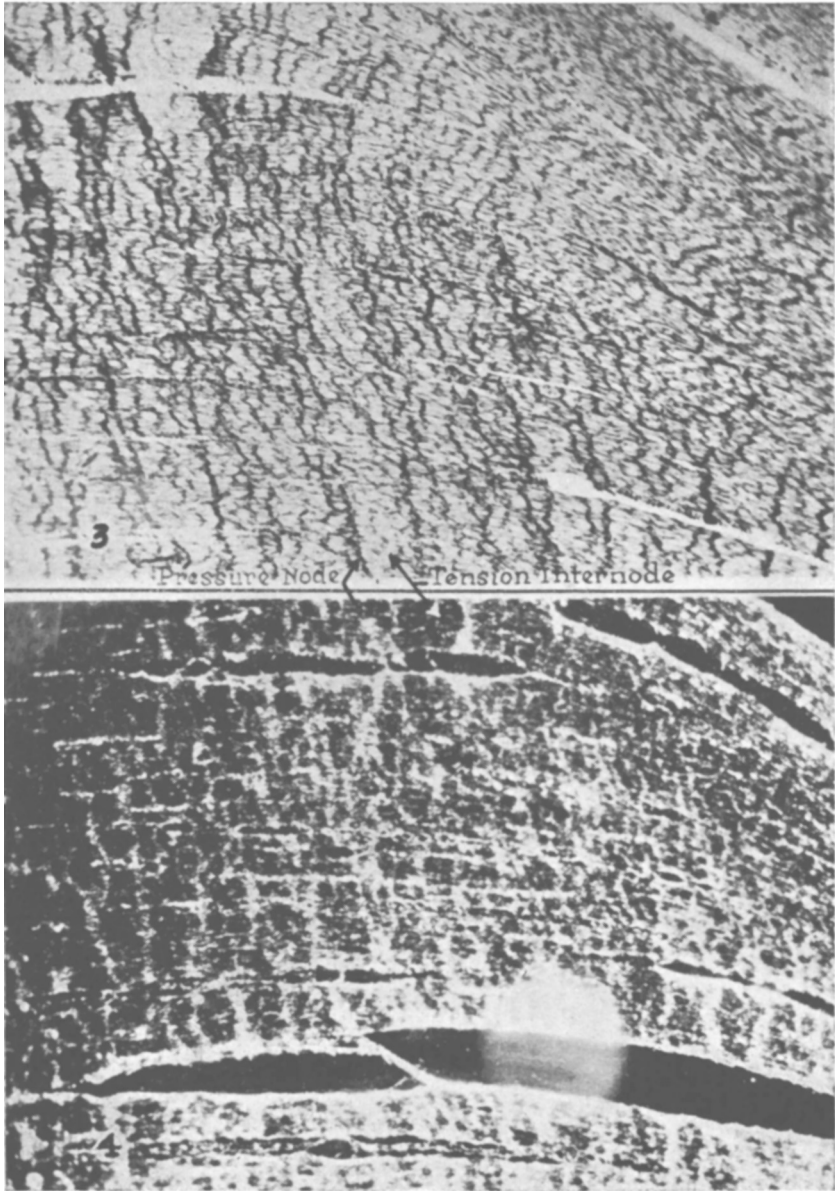
Freshly excised and ligated intestinal and uterine segments of the guinea pig one inch long and pieces of gizzard placed in Locke's solution at 42°C for 5 seconds and then 0°C for 3 minutes, followed by quick fixation, show multiplication in number of the contraction compression waves. The transversely placed nodal stripes composed of fine deposits of white and yellow-red ash are either regular or irregular in distribution. The intervening internodal space is almost clear of mineral residue. By comparing the alternate ashed and intervening stained serial sections it is apparent that the dense deposit of striped ash corresponds to the densely stained condensed cytoplasmic node in which the nuclei are rounded, compressed and densely stained. The rarefied internode in which the nuclei are elongated and the cytoplasm poorly stained corresponds to the relatively ash-free transverse band which alternates with the ash-bearing band. The granular ash that does remain in the internode is serially placed parallel to the long axis of the muscle fiber.

Phase differences in the contraction compression waves are detected by the variable degree of condensation and width of the ash bands at the nodes. In certain places the ash stripe forms either a uniformly dense, narrow, or broad band; in others there is an intervening relatively ash-free band bounded by parallel rows of a granular mineral deposit. In still other regions there is a central dense ash-



FIGS. 1 AND 2.

Stained section of active gizzard muscle of pigeon, Fig. 1. Compression nodes dark, tension internodes light. Microincinerated section, Fig. 2, ash-bearing nodes light. $\times 125$.



FIGS. 3 AND 4.

Stained section of active gizzard muscle of pigeon, Fig. 3. Closely spaced dark nodes. Microincinerated section, Fig. 4, ash-bearing nodes light. $\times 150$.

bearing band bounded on both sides by ash-free bands. All gradations of broad, widely spaced nodes 20 to 60 microns apart (Figs. 1 and 2) and narrow, finely spaced ones 3 to 20 microns apart (Figs. 3 and 4) are found by varying the intensity of the sudden application of heat, within physiologic limits, to the muscle prior to fixation.

Serial contraction compression waves in skeletal muscle fibers of the frog may be induced by either sudden elevations in temperature from 20°C to 37°C for 5 seconds or depression to 5°C, quickly followed by fixation. There is a nodal ash-bearing band composed of finely spaced and narrow Q striae alternating with an internodal band composed of diminished ash and coarse widely spaced Q striae. In skeletal muscle fibers of the frog the nuclei are compressed in the contraction node and elongated in the internode of the contraction compression waves. Scott¹ has previously demonstrated that the Q band, and Z band, when present, are ash-bearing and that the J band has diminution of mineral salts in skeletal muscle. This observation is confirmed.

In summary it may be said that the distribution of the mineral salts in the contraction compression waves induced by heat, within physiologic limits, within smooth, transitional and skeletal muscle is the same. There is a residue of ash-bearing node and a relatively ash-free internode. This is a mineral skeleton of active muscle free of organic matter. This distribution of mineral ash in active muscle fibers with serial nodes and internodes appears to correspond to the distribution of lycopodium powder when standing compression waves are produced in Kundt's tube, and to that of sand on glass slides by ultrasonic waves emanating from a crystal oscillator, demonstrated by Carey.⁵ The powder and sand are aggregated in the pressure node and relatively absent in the tension internode when standing pressure waves are produced in the glass tube.

⁵ Carey, Eben J., *Am. J. Anat.*, 1936, **59**, 175.