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Distribution of Mineral Ash in Striated Muscle Cells.

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This report is a brief account of one phase of a study of the distribution of inorganic salts, in various tissues and cell types, as revealed by the technique of microincineration. The method of incinerating thin sections of tissue without disturbing the topography of the mineral components was suggested by Liesegang,[1] but its advantages were never realized until the researches of Policard.[2] Until recently the technique has been largely a gross histologic one because of inherent difficulties in making observations with the higher powers of the microscope. However, it was discovered by the writer[3, 4, 5] that the use of the dark field condenser of Zeiss removed to a large extent these difficulties and rendered it possible to follow through even the changes of the fixed minerals of the nucleus during mitosis.

It is necessary to use a fixative which neither removes nor increases the inorganic elements of the tissue. Absolute alcohol is best adapted for this purpose, but gives poor fixation even under the most favorable circumstances. It was found after some experimentation that a mixture of 9 parts of absolute alcohol to 1 part of neutral formalin (Will Corporation) gives rather good cytologic detail and yet, for all practical purposes, does not change the original inorganic constitution of the tissue. After fixation for 24 hours the tissue is passed through several changes of absolute alcohol to complete the dehydration. The specimens are then cleared in xylol, embedded in paraffin and cut serially at 4 microns. Alternate sections are mounted according to the usual technique and stained with haematoxylin and eosin. The intervening sections of the series are mounted on glass slides with liquid petrolatum as a medium in which to spread the tissue. Sections prepared in this manner are incinerated in an open electric quartz tube furnace. The highest temperature of incineration, approximately 650°C., is reached by slow steps covering from 25 to 40 minutes. When the incineration is completed

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1 Liesegang, R. E., *Biochem. Z.*, 1910, **28**, 413.
2 Policard, A., *Protoplasma*, 1929, **7**, 464.
3 Scott, G. H., *Comp. Rend. Acad. Sci.*, 1930, **190**, 1073.
4 Scott, G. H., *Comp. Rend. Acad. Sci.*, 1930, **190**, 1323.
5 Scott, G. H., *Bull. Hist. app.*, 1930 **7**, 251.

the slide is cooled slowly, and carefully covered with a number 0 coverglass, the edges of which are then sealed with melted paraffin.

The preparation may be examined with the darkfield condenser (Zeiss Cardioid) and oil immersion objectives. Under favorable conditions photographs can be taken with a Zeiss achromatic objective 90 N.A. 1.25 (equipped with an iris diaphragm) and a 10x ocular (see figure 1).

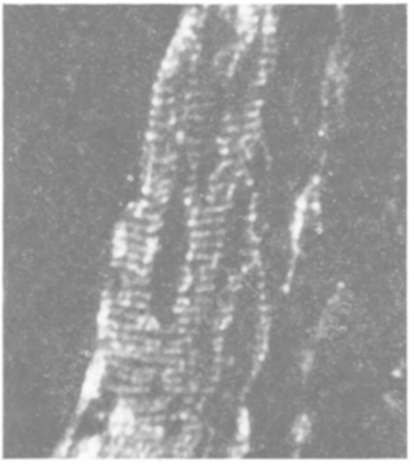

FIG. 1.
Photograph of incinerated longitudinal section of rectus muscle of cat. Zeiss achromatic 90 N.A. 1.25 (with iris diaphragm) 10x ocular and Zeiss Cardioid condenser.

The inorganic salts which are not volatilized at 650°C. retain the same topographical distribution as in the fixed tissue. The residue is then essentially a mineral skeleton of the cells free from organic materials. With some practice tissues may be identified quite as well by their ash deposits as by their appearance when prepared by the ordinary histologic methods. Iron can be identified by its color since it forms iron oxide under the influence of the incineration. Calcium is in all probability present as an oxide. It is difficult to say in what form the remaining salts are found. Silicates can be detected by their birefringence when viewed with polarized light.

When skeletal and cardiac muscles are subjected to incineration, the resulting ash is arranged in a distinctly characteristic manner. The striations are preserved with startling clearness (Fig. 1). Crossing the individual fiber may be seen stripes which are composed of small deposits of whitish ash, in some places tinged with the yellowish red which denotes the presence of iron. Alternating with these stripes of ash deposit are areas almost clear of mineral residue. Comparisons with the adjacent stained serial sections show that these cross bands of heavy ash deposit correspond very well with the Q, or anisotropic discs seen in ordinary preparations. The striations which are ash-free are in all probability the remains of the J, or isotropic bands.

In favorable preparations a fine line of ash may be seen traversing the J band. This disc corresponds to the Z, or intermediate band of the J striation. In some instances a differentiation of the Q band of ash can be distinguished. When this happens there is a definite row of twin deposits of mineral material. This appearance is especially striking in cardiac muscle. In skeletal muscle the sarcolemma shows as quite a distinct line of whitish ash in which there are some deposits of silicates. Cardiac muscle does not present as sharp an outline of each muscle fiber. In many instances it is possible to find isolated myofibrils with their characteristic bands of ash-bearing and of ash-free substances.

Thus far it has not been possible to distinguish any deposits of ash similar in form to the intercalated discs of cardiac muscle. Structures which are in all probability motor end plates show as a fine, heterogenous mass of mineral material with little or no orientation of the deposit. The nuclear ash of the skeletal muscle cell shows as a dense deposit at the periphery, while in heart muscle it is found in the center of the fiber. Simple chemical tests indicate that the nuclear ash is largely calcium. No striking differentiation of the ash deposit of the Purkinje fibers has yet been noted.

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Evidence of Possible Occurrence of Anaphylactic Phenomena in Poliomyelitis Immune Monkeys.*

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In connection with a series of experiments whereby monkeys are injected intrasplenically with poliomyelitis virus and subsequently develop an appreciable immunity, my attention has been drawn to a phenomenon which may be evidence of an anaphylactic reaction.

Monkeys convalescent from an experimental infection and there-

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