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An Electron Microscope Study of Calcium and Magnesium in Smooth Muscle.*

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Recent investigations of electrolytes in Thyone muscle by Steinbach[1] have led him to the conclusion that Ca is present in cells in lower concentration than that of the surrounding medium. Since these results are at variance with our own in a number of respects we have studied again the localization of Ca and Mg in mammalian and frog smooth muscle by the electron microscope as described by Scott and Packer.[2] Naturally, the results obtained cannot be compared directly with those of Steinbach as the same form was not used. Furthermore, our interest lay in determining the localization in smooth muscle cells which had been preserved as nearly as possible in their normal physiological state.

Thin strips of smooth muscle were taken from the duodenum and stomach wall of cats and frogs. These pieces were frozen in liquid air while still actively contracting from the mechanical stimulation of the manipulation. The frozen tissues were dehydrated *in vacuo* at −63°C and infiltrated in water-free paraffin without breaking the vacuum. This procedure precludes any salt shift during dehydration and the addition of water from outside sources at any time and thus maintains the spatial relations of the element in question. Thin sections (8μ) of muscle were prepared and examined by heating on a surfaced cathode in the electron microscope.

The technic of preparation of tissues for examination and the method of localizing Ca and Mg are believed to preserve the exact topical cellular relationships. Unfortunately, it is impossible by the method used to differentiate Ca and Mg, since both activate the cathode surface. Quantities of these elements in less than 1×10^{-12} g are easily detected. In no instance was either Ca or Mg found outside the cell wall. Smooth muscle cells of the frog duodenum show a dense concentration of Ca and Mg in the cell. Occasionally delicate threads of mineral traverse the tissue spaces to connect adjacent cells. When stained sections are examined one finds that

* Aided by a grant from the Rockefeller Foundation.

1 Steinbach, *J. Cell. and Comp. Physiol.*, 1940, **15**, 1.

2 Scott and Packer, *Anat. Rec.*, 1939, **74**, 17, 31.

there are protoplasmic extensions connecting the cells and that these filaments are surrounded by clearly demonstrable cell walls. In mammalian smooth muscle this particular situation does not exist insofar as could be ascertained. The tissue spaces are extremely small and difficult to locate. One of the striking features in mammalian muscle is the clearness with which cell boundaries can be determined. Yet Ca and Mg were by no means, either in frog or mammalian smooth muscle, limited to the periphery of the cell.

These findings do not imply that there is no Ca or Mg in the tissue spaces. The conclusion is apparent, however, that under conditions of normal existence the Ca and Mg concentration is many times greater within the smooth muscle cells of the forms studied than in their surrounding tissue fluid environment.

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Protective Effect of Sulfamethylthiazol* on Experimental *Salmonella enteritidis* Infection in Mice.

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In a previous report[1] the effect of sulfamethylthiazol upon the course of experimental staphylococcus infection in mice was presented. In further studies of the protective effect of this drug against experimental infections in mice a series of experiments have been made wherein the infecting organism was a strain of *Salmonella enteritidis*.† The organism had been previously recovered as the etiological agent in an epizootic disease among the mice of our colony.

Preliminary studies regarding the pathogenicity of this organism for mice of a selected strain, known to be free from Salmonella organisms, indicated that animals succumbed to the organism as a result of a progressive infection rather than to the presence of pre-formed toxin factors present in the inoculum. A dose of one million cells, injected intraperitoneally, brought about the death of a majority of

* Supplied through the courtesy of the Department of Medical Research of the Winthrop Chemical Co., New York.

1 Carroll, G., Kappel, L., Jones, L., Gallagher, F. W., and DiRocco, F. W., *South. Med. J.*, 1940, **33**, 83.

† We are indebted to Dr. P. R. Edwards for determining the serological character of this organism.