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Studies on Isolated Smooth Muscle Cells. I. Continuous Observation of Contraction of Single Smooth Muscle Cells Isolated from *Vas Deferens* of Guinea Pig

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Simple technique for isolation and continuous observation of contractile response of single smooth muscle cells from *vas deferens* of guinea pig was examined for further investigation of contractile mechanism of smooth muscle instead of use of whole tissue. Cells were isolated by digestion with collagenase followed by mechanical agitation. Intact cells which were not stained by trypan blue and spindle shape were capable of contraction by application of calcium in a medium containing higher concentration of potassium. For continuous observation of contracting cells, the cells were perfused continuously with incubation medium on silicon-coated slide glass at 37° and degree of contraction was determined photographically with use of phase contrast microscope. By this procedure, many isolated cells could be observed before and through contraction with the same preparation.

Keywords——smooth muscle; *vas deferens*; calcium contraction; isolated cell; guinea pig; isolation procedure

For studies on contractile mechanism of smooth muscle, responses of whole tissue have been investigated. However, the procedure is inadequate for the investigation of intrinsic mechanism of contraction, since this is the method for recording the change in tonus of smooth muscle as whole tissue. As a countermeasure for this limitation, introduction of isolated smooth muscle cells in these experiments might be of use for further investigation of muscle contraction. In this paper, authors introduce a simple technique for isolation and continuous observation of contractile response of a single cell from *vas deferens* of guinea pig.

Smooth muscle cells were isolated by modified procedure for isolation of single muscle cells from *Bufo marinus* stomach.[2-5] *Vas deferens* from male albino guinea pig (300—350 g) was suspended in calcium-free modified Tyrode solution (2.7 mM KCl, 137 mM NaCl, 1.0 mM $MgCl_2$, 5.6 mM glucose and 10 mM Tris HCl, pH 7.4) for 60 min and in the incubation medium (140 mM KCl, 1.0 mM $MgCl_2$, 5.6 mM glucose and 10 mM Tris HCl, pH 7.4) for another 30 min at 37° with aeration. Then the tissue was sliced and digested in 2—3 ml of the incubation medium in the presence of 0.2% collagenase (Sigma, Type I) and 1.0% bovine plasma albumin (Armour Pharmaceutical Co. Fraction V) for 60 min. After the digestion, the tissue suspension was diluted with 7—8 ml of the incubation medium containing 1.0% albumin and centrifuged at $100 \times g$ for 5 min. The pellets were resuspended in 10 ml of the incubation medium (albumin free) and the suspension was centrifuged. The washing procedure was repeated and the tissue was suspended in 5 ml of the incubation medium. The digested tissue was preserved as clots at 37° with gentle stirring and cells were dispersed prior to examination by pipetting with wide bore Pasteur pipette in the presence of 0.5—1 mg/ml DNAse-II. Preservation of cells as clots was useful to maintain the ability of contraction. The cell preparation was used within 90 min after the digestion.

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The cell preparation contained various cells of several shapes and cell fragments. Intact cells, which were not stained by trypan blue and spindle shape of length 158±5 μm (n=144) and diameter approximately 10 μm (Fig. 1), were capable of contraction by the application of calcium into the medium containing higher concentration of potassium chloride in the presence of magnesium ion and Tris hydroxyaminomethane. Calcium contraction of the intact cells was reversible and contracted cells were relaxed to the initial length by washing with the incubation medium. Yield of the intact cells was increased by addition of bovine plasma albumin into the incubation medium during the digestion.

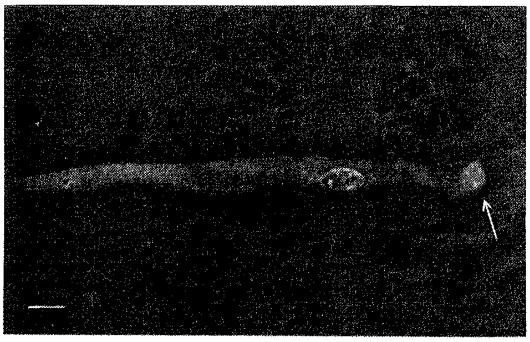

Fig. 1. Isolated Smooth Muscle Cell from *Vas Deferens* of Guinea Pig

Scale indicates 10 μm and arrow indicates a site where the cell adheres to the slide glass.

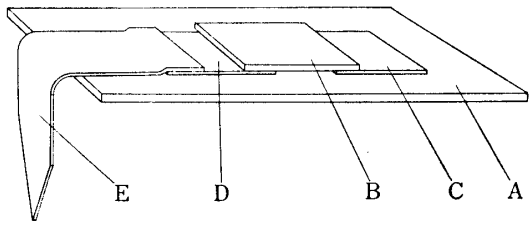

Fig. 2. An Apparatus for Continuous Observation of Contracting Single Smooth Muscle Cells

A: slide glass coated with dichlorodimethylsilane, B: cover glass, C and D: filter paper (Toyo Roshi, No. 6), E: strip filter paper.
Incubation medium was supplied onto C continuously through pipette and oozed medium onto D was blotted by E.

For continuous observation of contracting cells, an apparatus illustrated in Fig. 2 was designed. The slide glass was coated with dichlorodimethylsilane and the apparatus was placed on temperature-controled stage of phase contrast microscope. Space between the cover and slide glass was filled with the incubation medium, then 0.1 ml of cell suspension was introduced into the space. After a few seconds, these cells were perfused continuously at 37° with the incubation medium and calcium was applied with the perfusate. Since some of the intact cells adhered to the silicon-coated slide glass loosely and could remain on it against

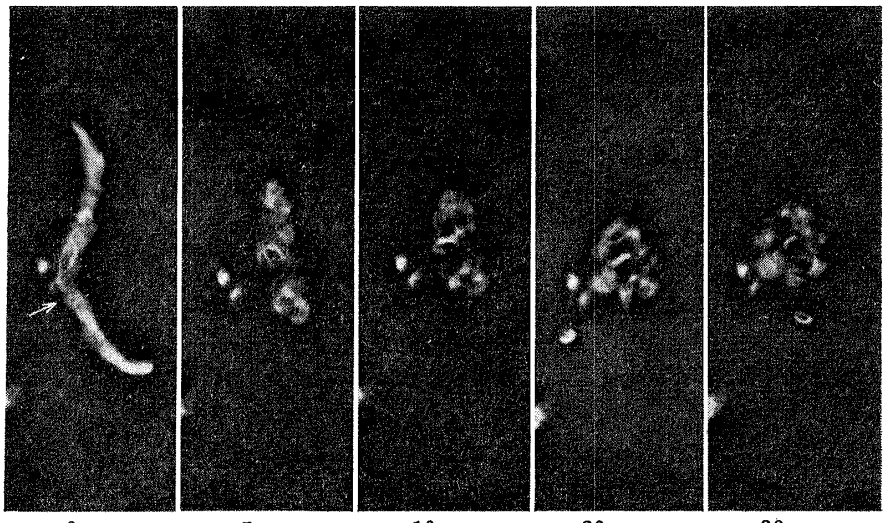

Fig. 3. Phase Contrast Micrograms Showing Time Course of Contracting Single Smooth Muscle Cell of *Vas Deferens*

The cell was perfused at 37° with incubation medium consisted of 140 mM KCl, 1.0 mM $MgCl_2$, 5.6 mM glucose and 10 mM Tris HCl, pH 7.4. Calcium contraction was started by addition of 56 mM $CaCl_2$ to the perfusate. Arrow indicates a site where the cell adheres to the slide glass.

flow of the incubation medium, continuous observation could be carried out on such cells until the contractile responses were completed. With such procedures, the contraction of isolated cells could be observed continuously and the degrees of contraction were determined photographically with use of phase contrast microscope (Fig. 3). Although cells adhered to the slide glass and remained on it against flow of the perfusate, contraction was not disturbed when silicon-coated slide glass was used. On the other hand, when uncoated slide glass was used, cells adhered so tightly that contraction was not observed even if the experiment was carried out under the best condition for the contraction.

The calcium contraction of the cells was not accelerated by ATP and almost of the cells contracted little or slightly by calcium in normal Tyrode solution. These results suggested that, in contrast to the case of glycerinated muscle cells and skinned fibers, membrane of the isolated cells in this experiment was not damaged.

According to this simple procedure, many isolated smooth muscle cells could be continuously observed before and through contraction with the same preparation. This technique could be applicable to studies on contraction mechanism of smooth muscle.

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Extraction-Spectrophotometric Determination of Berberine in Pharmaceutical Preparations with 2,6-Dichlorophenolindophenol[1]

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A new application of monoacidic dye is reported for the determination of berberine. The method is based on solvent extraction into nitrobenzene of an ion-pair compound formed between 2,6-dichlorophenolindophenol and berberine. Berberine is determined by measuring absorbance of the extracts over the range of $(1-5)\times10^{-6}$ M (0.408—2.04 μg/ml) at 650 nm. The molar absorptivity was 3.8×10^4 l, mol^{-1}, cm^{-1}. Continuous variations plots have a maximum at 0.5 mol fraction of DCIP, indicating a 1:1 DCIP-berberine ratio.

Keywords——spectrophotometry; solvent extraction; berberine; 2,6-dichlorophenolindophenol; pharmaceuticals

Introduction

Several reagents such as bromothymol blue[3] or bromophenol blue[4] have been suggested for the spectrophotometric determination of onium compounds. However, in the case of such diprotic acids, the acidity gave a complicated effect on the extraction because stepwise dissociation occurred in the aqueous phase. Therefore, a singly charged tetrabromophenolphthalein

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