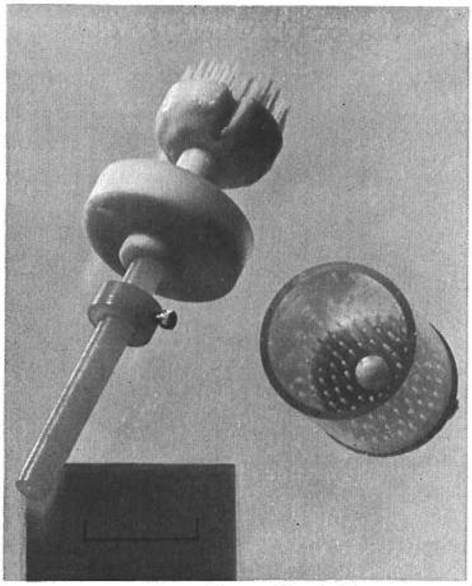

Fig. 1. The device (finger apparatus) used in mechanical treatment of heart tissue. Scale bar is 2 cm long. Detailed description in text.

fabricated apparatus which removes enzyme-loosened cells by a gentle abrading action of silicone elastomer fingers. Fig. 1 shows the device (finger apparatus) used in the isolation procedure.* Two disks with four circular rows of round finger-like projections are cast from Dow-Corning type 382 Medical Grade Elastomer (Dow Corning Corp., Medical Products Division, Midland Michigan, U.S.A.). One (lower) disk is held stationary being glued (Silastic Medical Adhesive, Silicone Type A, Dow Corning Corp., Medical Products Division, Midland Michigan, U.S.A.) fingers-up into one end of a 25 mm diameter glass tube with which it forms a liquid-tight vessel. The other (upper) disk mounted on a nylon shaft is rotatable and fits freely, fingers down, into the vessel. The nylon shaft passes through a teflon piece which serves as a bearing and as a tightly fitting cap for the vessel. The rows of fingers on the upper and lower disks interdigitate when the apparatus is assembled, and the lower disk has a central hub-like projection which fits into a complementary fingerless area of the upper disk. The hub prevents tissue from accumulating in the central region where the finger speed is lowest. The upper disk and teflon cover respectively have a segmental slot and a hole which when aligned permit insertion of a teflon tube for injection or withdrawal of solution. A nylon collar clamped to the shaft with a set screw allows the degree of interdigitation of fingers to be set. The shaft of the apparatus is flexibly coupled to a reduction gear train driven by a variable speed motor.

Preparative procedure. A Wistar or Sprague Dawley rat is decapitated and the thoracic cavity is opened to expose the heart. Using aseptic technique, about one-third to one-half of the apical end of the heart is cut off with a scissors. The excised tissue is transferred to a flat glass plate submerged in a dish of Saline A (22) at 21 °C. The tissue is minced into 2–4 mm fragments by vertical chopping strokes of an unsterilized but freshly unwrapped double edge razor blade. Minced tissue is rinsed briefly in a second dish of Saline A and placed in the apparatus. Four milliliters of enzymatic medium are added to the vessel. The medium consists of

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* A detailed drawing is available from the author. Inquiries about the availability of fabricated apparatuses may be directed to A.A. Raffler, Science Workshop, Carleton University, Ottawa, KlS 5B6, Canada.

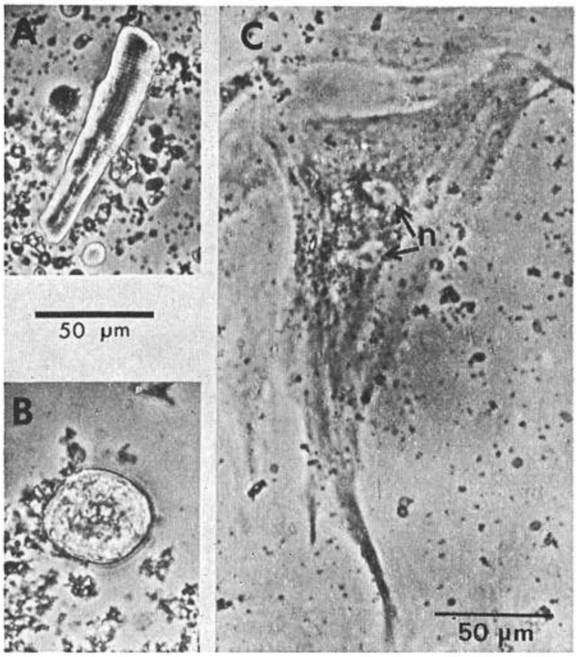

Fig. 2. Phase contrast photomicrographs of three of the stages in development of spontaneously contractile loci derived from isolated adult rat myocardial cells. A, Elongated cell shortly after plating. B, Cell 3 days in culture after completion of elongate to round transition. C, Well developed binucleate locus after 13 days in culture. n, nucleus. Cells were contracting spontaneously when photographed. Granular material in background is cell debris.

appreciable shortening of the cell. Instead it is manifest as a wave-like propagation of a narrow, optically distinct, transverse region of the cell. The wave-regions arise spontaneously at various points along the cell and can propagate in either longitudinal direction. A similar phenomenon termed "escalation" has been described for cultured skeletal muscle of chick embryos (6). Contractility of established loci is unlike either type one or two. Here contraction is a forceful tremulous motion in a region of cytoplasm within the locus.

Contractile frequency of cells in a single culture or of the same cell at different times can vary over a wide range. Frequencies from 0.02 to 5 Hz have been observed. Higher rates may occur but are virtually impossible to measure without a special detector. A contractile frequency of 3 to 5 Hz is often precursive to sudden cell deterioration.

Electrical and pharmacological sensitivity. A preliminary study indicates that isolated cells are sensitive to electrical stimulation and to application of L-epinephrine. Cells prepared from 35 to 112 day old rats were tested 2 days after being placed in culture. Electrical stimulation was accomplished by passing 0.1 msec current pulses of 10^{-7} to 10^{-5}A between two Ag-AgCl electrodes. The electrodes contacted Saline A salt bridges immersed in the culture medium. The stimulating (negative) salt bridge was a glass micropipette of about 1.5 μm tip diameter and the positive salt bridge was a glass tube plugged with a Saline A-soaked cotton wick. The cell being stimulated was positioned between the salt bridges 1 to 3 μm from the micropipette. Eleven of 18