mum oxygen concentrations of 10 to 20 percent were used since preliminary experiments had shown that neuronal responses were not affected by increases in oxygen concentration above 10 percent. An attempt was made to test all olfactory bulb units at oxygen concentrations of 10, 5, 3, 2, 1, and 0 percent, but in many cases the cell could not be held long enough to examine satisfactorily its response at all of these concentrations.

Figure 1 shows the responses of four olfactory bulb units to x-ray as a function of nasal oxygen concentration. The results are representative of all data collected on 17 different units. In all cases, the response to a standard x-ray exposure was the same for all oxygen concentrations above 10 percent. In a few instances there appeared to be a slight depression of response with 5 percent oxygen, but clear response depression occurred only at oxygen concentrations of 2 percent or less. With 2 percent oxygen the mean response of 12 neurons studied was 70 percent of the control response with 10 percent oxygen. At an oxygen concentration of 1 percent the mean response for 13 units was 55 percent of the mean control response for these same units. A mean response amounting to only 30 percent of control values was obtained at 0 percent oxygen (15 units). The curves shown in Fig. 1 closely resemble the "oxygen effect" curves which have been obtained in a variety of other radiobiological experiments (*7*).

With three units the x-ray dose rate, as well as the oxygen concentration, was systematically varied. Representative results obtained from one of these cells are shown in Fig. 2. At any given oxygen concentration the response is approximately a linear function of the logarithm of the dose rate. As the oxygen concentration is reduced, the response function shifts to the right with no appreciable change in slope.

Depressions of normal resting activity of olfactory nerve fibers and olfactory bulb neurons, as well as very small depressions of olfactory nerve response to odor, have been observed with nasal oxygen concentrations of 1 percent or less (*2*, *8*). Therefore, to control for the effects of lowered oxygen concentrations on normal receptor function I examined the response of ten of the units described above to amyl acetate (1/100 of vapor saturation at 30°C). Responses to odor stimulation remained essentially constant at all oxygen concentrations.

We have proposed that ionizing radiation affects the olfactory system through the production of a hypothetical stimulus substance in or near the olfactory mucosa. If this is so, then the data of Figs. 1 and 2 suggest that, within limits, the concentration of this substance is linearly related to the dose rate. As shown in Fig. 2, the neuronal response is approximately a linear function of the logarithm of the dose rate. Since this semilogarithmic relationship between stimulus intensity and response is a rather common property of peripheral sensory systems, we may therefore tentatively conclude that there might be a direct relationship between the dose rate and any hypothetical agent mediating stimulation produced by the ionizing radiation. The data of Figs. 1 and 2 also suggest that the concentration of this hypothetical stimulus substance is positively related to nasal oxygen concentration at very low concentrations. The presence of oxygen within the nasal cavities apparently is not essential. However, the oxygen derived from vascular sources might be quite important or even essential if, as seems probable, ionizing radiation produces an effective olfactory stimulus within the olfactory mucosa itself. I infer from these data that ozone or some other short-lived oxidizing substance probably is the agent mediating olfactory stimulation by ionizing radiation.

G. P. COOPER
Departments of Environmental Health and Physiology, College of Medicine, University of Cincinnati, Cincinnati, Ohio 45219

References and Notes

1. J. Garcia, N. A. Buchwald, B. H. Feder, R. A. Koelling, L. Tedrow, *Science* **144**, 1470 (1964); C. D. Hull, J. Garcia, N. A. Buchwald, B. Dubrowsky, B. H. Feder, *Nature* **205**, 627 (1965); G. P. Cooper and D. J. Kimeldorf, *Int. J. Radiat. Biol.* **9**, 101 (1965); H. I. Dinc and J. C. Smith, *Physiol. Behav.* **1**, 139 (1966); H. Brust-Carmona, H. Kasprzak, E. L. Gasteiger, *Radiat. Res.* **29**, 354 (1966); G. P. Cooper and D. J. Kimeldorf, *Experientia (Basel)* **23**, 137 (1967); H. L. Taylor, J. C. Smith, A. H. Wall, B. Chaddock, *Physiol. Behav.* **3**, 929 (1968).
2. G. P. Cooper, D. J. Kimeldorf, G. C. McCorley, *Radiat. Res.* **29**, 395 (1966).
3. J. C. Smith, paper presented at the meeting of the Eastern Psychological Association, April 1967 [cited in H. L. Taylor *et al.* (*1*)].
4. G. P. Cooper, *Amer. J. Physiol.* **215**, 803 (1968).
5. E. L. Gasteiger and S. A. Helling, *Science* **154**, 1038 (1966).
6. S. A. Helling and E. L. Gasteiger, *Radiat. Res.* **31**, 658 (1967).
7. T. Alper, in *Mechanisms in Radiobiology*, M. Errera and A. Forssberg, Eds. (Academic Press, New York, 1961), vol. 1, p. 353.
8. D. Tucker, *J. Gen. Physiol.* **46**, 453 (1963).
9. I thank J. Lemons and Mrs. S. Anderson for technical assistance. Supported by AEC contract No. AT(11-1)-1669 (report No. COO-1669-7) and by PHS grant P510-ES-00159-04.

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Spontaneous Rhythmic Contraction of Separated Heart Muscle Cells

Abstract. *Muscle cells that contract spontaneously and rhythmically can be obtained from adult mouse myocardium. Contractions are observed immediately after homogenization in a solution that is ionically similar to intracellular fluid. Contraction frequency varies directly with temperature and decreases as a function of time after homogenization. At 16°C rhythmic relaxation and contraction occur for about 20 minutes. Contractions are dependent on the presence of adenosine triphosphate in the homogenization medium.*

Although partially differentiated rat heart muscle cells in culture show spontaneous contractile activity (*1*) and separated adult muscle fibers can be induced to contract (*2*), spontaneous rhythmic contraction and relaxation by separated mature mammalian cardiac muscle fibers has not been reported. I now describe a method for producing such preparations and comment on the relation of spontaneous contractile behavior to the components of the medium.

When mouse hearts are homogenized in simulated extracellular fluid (*3*) or in a buffered isotonic sucrose solution, microscopic examination reveals the presence of subcellular particles and dense clumps of cell fragments, intact cells, and groups of undissociated cells.

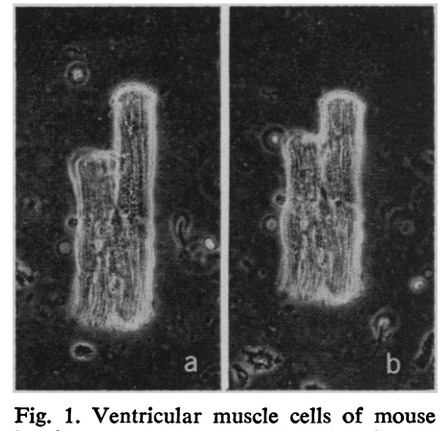

Fig. 1. Ventricular muscle cells of mouse beating spontaneously. Relaxed (a) and contracted (b) state of same cells. These photographs were taken with a high-intensity stroboscopic light source. The condenser was adjusted to be slightly eccentric, and a red filter was used in order to enhance the cross striations. The large size and irregular shapes of this muscle fragment indicate that it is composed of several cells. The contracted cells are about 15 percent shorter than the relaxed cells (both × 400).

Marked contracture of all muscle cells is apparent, and there is no rhythmic relaxation and contraction. If, however, homogenization is carried out in a medium more like intracellular fluid, many of the intact cell clusters will show rhythmic contraction and relaxation.

In a typical experiment, a mouse is killed by cervical dislocation, and its beating heart is quickly removed and washed in cold solution (homogenization medium, HM) that contains (mmole/liter) KCl, 104; $MgCl_2$, 5; disodium adenosine triphosphate (ATP), 5; potassium oxalate, 2.0; glucose, 7; potassium-phosphate buffer at *p*H 7.0, 18. Upon contact with this solution, the heart quickly arrests in diastole. The ventricles are immediately dissected free and homogenized for 10 seconds in a blender (Virtis-23) at 100 volts in 20 ml of cold HM. The homogenate is kept on ice, but samples are removed at intervals and examined microscopically at room temperature. When a drop is first placed on the slide, there is no contractile activity, and the cells remain in an extended, or relaxed configuration. In about 5 seconds, occasional muscle cells show sporadic contractions (*4*). The number of contracting cells increases, as does the frequency and rhythmicity of contraction, until about 15 seconds, when more than 50 percent of the intact muscle cells and cell groups show this activity. Each beating cell or cell cluster is rhythmically independent of the others. The degree of shortening is also variable, from a barely perceptible twitch to a 20 percent overall decrease in length (Fig. 1). This compares to a maximum change in length of 30 percent found in muscle cells of intact hearts (*5*). Rhythmic contraction and relaxation continue for a variable period of time, depending on the temperature and the period of time the homogenate was stored on ice prior to warming for microscopic observation. A sample brought to 20°C immediately after homogenization shows contracting cells for 10 to 20 minutes (drop survival time, DST). As the time between homogenization (with the homogenate on ice) and warming for examination is increased, the DST decreases, as does the number of contracting cells and their rhythm of contraction. The time interval over which an ice-cold homogenate continues to provide more than one contracting cell per two low-power fields (×4 objective and ×10 oculars) is referred to as the homogenate survival time (HST).

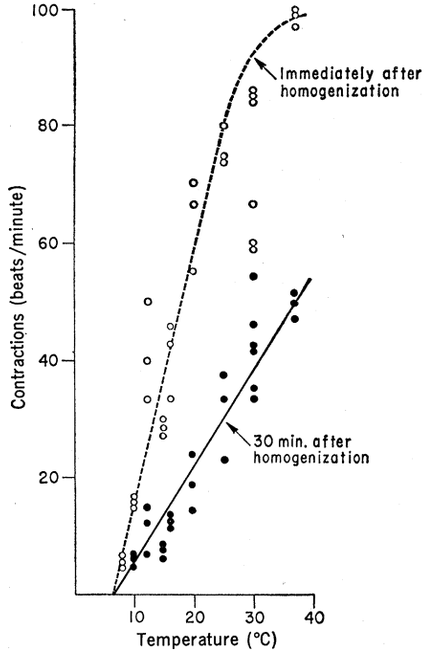

Fig. 2. Frequency of contraction as a function of temperature. Mouse hearts were homogenized in ice-cold HM, and a sample was immediately transferred to a glass slide on a temperature-regulated microscope stage. The most rapidly beating cell was singled out, and the number of contractions in 1 minute (*beats/minute*) was determined. Thirty minutes after homogenization (with the homogenate on ice) another sample was taken and contraction frequency was determined as before.

The relation between frequency of contraction and temperature was studied with a Leitz "Heating and Cooling Microscope Stage 80." This was maintained at constant temperature with a refrigerated-heated fluid circulator. The entire microscope was jacketed by a thin polyvinyl sac so as to protect the stage from drafts. The temperature of a drop of homogenate could be controlled to within 0.4°C, as verified by a thermistor with a probe within the drop. Since beating cells are rhythmically independent, the most rapidly beating cell in a given sample was used as the index of temperature responsiveness. Contractions do not occur below 6°C (Fig. 2). Above 6°C, the frequency of contraction rises linearly with temperature. It is also apparent that a sample kept on ice for 30 minutes after homogenization shows a lower frequency of contraction, at any given temperature, than a sample studied immediately after homogenization.

Experiments designed to show the dependence of contractions on ATP were conducted in HM with tris buffer (18 m*M*, *p*H 7.0) in place of phosphate buffer, and in the absence of glucose, so as to minimize the possibility of synthesis of high-energy phosphorus compounds. The HST is directly proportional to the ATP content of the medium up to a maximum of about 10 mmole/liter (Fig. 3). These data confirm reports that suggest that the cell membrane of heart muscle is permeable to ATP (*6*).

We have maintained groups of cells for up to 4 hours in a continuously beating condition. This is accomplished by bathing the cells in fresh medium at room temperature in a specially built flow cell (*7*).

Perhaps the most remarkable quality of the system described in this report is that rhythmic contractions occur in the absence of added calcium and with a very low concentration of sodium. Although the homogenizing medium contains 10 m*M* sodium, this can be omitted by the use of dipotassium ATP, with no change in the HST, DST, or rhythm of contraction. Likewise, small amounts of calcium, below 10 μmole/

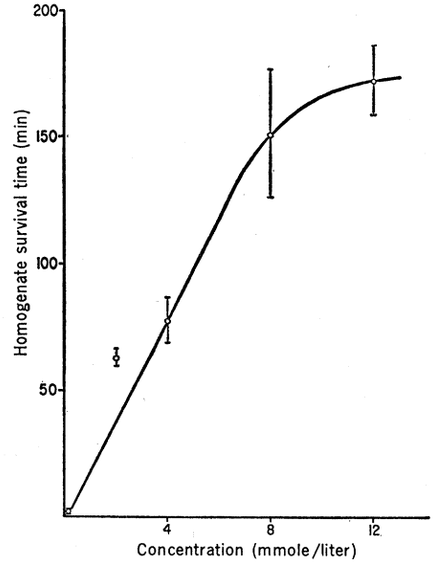

Fig. 3. Homogenate survival time (HST) as a function of ATP concentration. Mouse ventricles were homogenized in 20 ml of HM with tris buffer instead of potassium-phosphate buffer. The medium was also modified by the exclusion of glucose, and by the variations in ATP concentration. The homogenates were kept on ice, and samples were periodically withdrawn and examined at room temperature for contracting cells. The HST is the period of time over which a homogenate continues to provide contracting cells. All points are the average of three determinations. Brackets indicate standard error of the mean.

liter, exert no apparent effect on the behavior of the contracting cells. Larger amounts of calcium (above 100 μmole/liter) cause contracture. In addition to this unusual ionic milieu, the present system differs in several other potentially significant ways from the cardiac muscle cell cultures in which spontaneous contractions have been observed. Thus, the cells described here have not been exposed to trypsin, they initiate contraction immediately following disaggregation, and they are completely dependent on added (rather than endogenously produced) ATP. These qualities may prove of value for the study of heart muscle cell physiology and pathology.

SHERMAN BLOOM

Department of Pathology, University of Utah College of Medicine, Salt Lake City 84112

References and Notes

1. I. Harary and B. Farley, *Exp. Cell Res.* **29**, 451 (1963).
2. E. H. Wood, R. L. Heppner, S. Weidman, *Circ. Res.* **24**, 409 (1969).
3. J. R. Robinson, *Biochem. J.* **45**, 68 (1949).
4. The term "muscle cells" is used here to indicate small fragments of muscle. These fragments include groups of undissociated cells as well as single cells, as indicated by examination of stained preparations and measurements of contracting cells.
5. S. Bloom and P. A. Cancilla, *Circ. Res.* **24**, 189 (1969).
6. I. Harary and E. C. Slater, *Biochim. Biophys. Acta* **99**, 227 (1965); M. Fedelesova, A. Ziegelhotter, E. Krause, A. Wollenberger, *Circ. Res.* **24**, 617 (1969).
7. S. Bloom, in preparation.
8. Supported by PHS grant 10962 (AHI). I thank J. Allison for her assistance.

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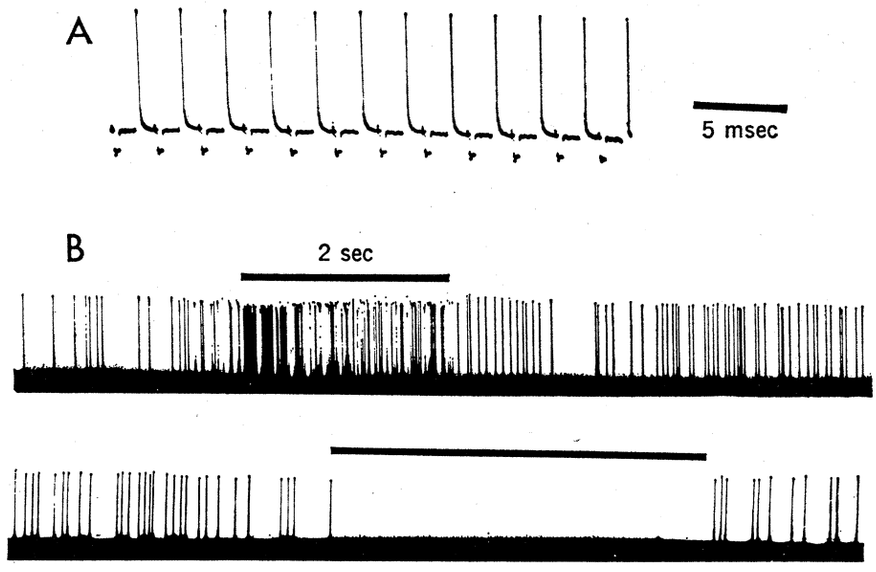

Fig. 1. Microelectrode recording from a direct somatovisceral fiber. (A) Electrical stimulation (20 volts, 0.5 msec) at C_1. Stimulus repetition rate, 250 per second. Note constant latency. (B) Typical excitatory and inhibitory responses to physiological stimuli. Bar above trace indicates duration of applied stimulus. (Top) Light brushing of hairs over contralateral thigh; (bottom) rapid injection of 2 ml of normal saline (37°C) into the gall bladder.

Somatovisceral Pathway: Rapidly Conducting Fibers in the Spinal Cord

Abstract. *Fibers that respond to distension of hollow viscera and to mechanical stimulation of somatic structures were found primarily near the ventromedian fissure of the upper lumbar spinal cord. These fibers could be directly excited by electrical stimulation at C_1. The average conduction velocity for these fibers was 68.6 meters per second.*

The locations of ascending exteroceptive and proprioceptive pathways in the mammalian spinal cord are well known. Both crossed and uncrossed tracts in dorsal, lateral, and the lateral part of the ventral columns have direct projections to higher centers. The locations of ascending visceral or interoceptive pathways, however, are not well known. With one exception (*1*), visceral pathways have been localized mainly in the dorsal and lateral columns.

Responses to visceral stimulation can be recorded from axons located throughout the ventral part of the spinal white matter (*2*). The present study was undertaken to determine whether these fibers project directly or indirectly to supraspinal loci. We now present neurophysiological evidence suggesting that in addition to propriospinal systems, there are rapidly conducting somatovisceral fibers which project directly to supraspinal loci. These fibers are located primarily in the ventral funiculus of the spinal cord along the median fissure.

The preparations (125 units obtained in 10 cats) were spinalized at the cervicomedullary junction and anemically decerebrated. Muscular paralysis and artificial ventilation were employed. Blood pressure, carbon dioxide concentration in expired air, and body temperature were monitored and maintained within physiological limits. Electrical stimuli were delivered through a transverse array of four electrodes placed in the cord just below the transection (C_1). Recordings were made at L_2 to L_4 with microelectrodes which contained 3*M* KCl and had impedances of about 20 megohm. The conduction distance from C_1 to the recording site was about 20 cm. Microelectrode tips were cut off in place and the tracts subsequently identified in frozen sections. Visceral stimulation

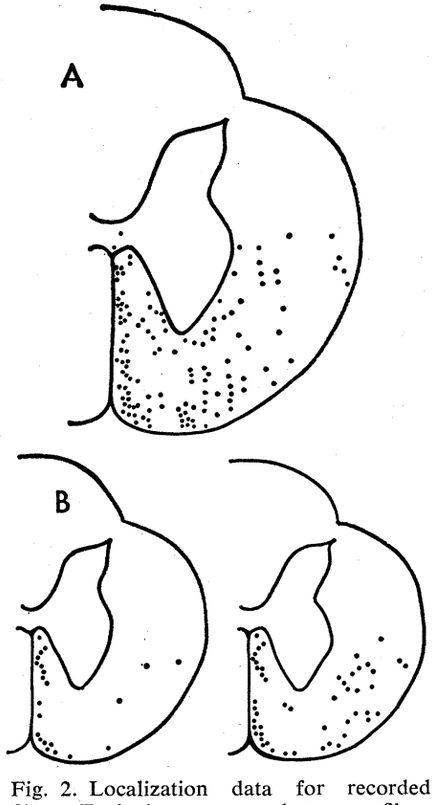

Fig. 2. Localization data for recorded fibers. Each dot corresponds to one fiber. The position was approximated by superimposing a photomicrograph of the section containing the microelectrode tip over a standardized L3 spinal cord cross section. (A) Total fiber sample. (B) (Left) Antidromically activated fibers with visceral inputs; (right) all antidromically activated fibers with detectable receptive fields or spontaneous activity, or both.