In vitro Organization of Single Beating Rat Heart Cells into Beating Fibers

Abstract. Single, separate rat-heart cells in culture beat at different rates. When they grow into physical contact the beating becomes synchronous. Increase in cell number leads to the formation of beating, fiber-like masses. It appears that direct physical contact is necessary for attainment of synchronous contractions.

A previous report (*1*) from this laboratory concerned the isolation of single beating cells from the hearts of young rats (2 days to 6 weeks old). Several interesting phenomena have been observed as these single cells developed into confluent sheets. The techniques were essentially those previously reported (*1, 2*). Further investigation has led to experiments in which essentially all of the single cells attached to the glass are beating. In the early stages of the culture, these cells are separate from each other and beat at independent rates, varying from slow to fast and from intermittent to regular beats. The rate varies in general from about 30 to 80 beats per minute. Figure 1 is a photomicrograph of independent cells, with different rates of beating. In the single separate cell cultures, from 2 to 10 percent of the cells beat.

As these cells spread out and increase in diameter, they become very irregular in outline and put out long protoplasmic extensions. Eventually these processes touch each other to form a network of joined cells which lose their independence and beat synchronously. This is accompanied by an increase in the number of beating cells from a small percentage to essentially 100 percent. Figure 2 shows a group of cells attached through extended processes. These cells are all beating at the same rate and in unison. A similar phenomenon was observed by Fischer (*3*), who found that heart tissues from embryonic chick, grown in vitro, showed at first their own rhythmic rate and became synchronous after the two adjacent cultures had fused.

Continued growth is a result of an increase in the number of cells (Fig. 3). As these cells become more confluent, centers of beating are established which consist of many crowded cells. Some of these cells, as shown in Fig. 4, are flat and attached to the glass; others are rounded and attached only to other cells. Beating occurs in the areas indicated by arrows. The flattened cells seem to be oriented in their long axes directed toward the center of beating.

Eventually, as the areas of beating cells grow and become more crowded, some develop into long, thick, fiber-like masses of tissue. These fibers beat strongly at about the same rate as the original beating cells and develop from beating centers by an orientation of the cells along a long axis during their growth, as shown in Fig. 5. A more developed fiber is pictured in Fig. 6. All of the beating on the plate seems to be concentrated in these fibers, as the flattened areas around the fibers do not appear to contract.

More information as to the nature of the communication of beating centers was obtained through a study of the effect of temperature. The beating rate of the connected cells is extremely temperature-dependent. In one representative case, starting at 180 beats per minute at 34°C, the rate fell steadily until it reached 60 beats per minute at 23°C. In another dish, cells at 34°C, beating at a rate of 78, dropped to a level of 24 beats per minute as the temperature dropped to 23°C. These results indicate a Q_{10} of approximately 3. The beating at the higher temperature was a regular, strong contraction in which the whole cell participated. As the temperature was lowered the beating became not only slower but also more irregular. In one representative case the beating started at 78, and when it reached 40 beats per minute a faint after-contraction was observed immediately after each main contraction. This slight twitch of the cellular protoplasm was oriented in a different plane from that of the main contraction and seemed localized in one part of the cell. As the rate further slowed to 25 beats (at 23°C, where it was maintained), a more irregular beating echo occurred which, coupled with the weakened main beat, gave the whole cell an irregular contraction pattern.

The relevant aspect of this change is that the same rhythmic relation of main beat to after-beat occurs in every in-

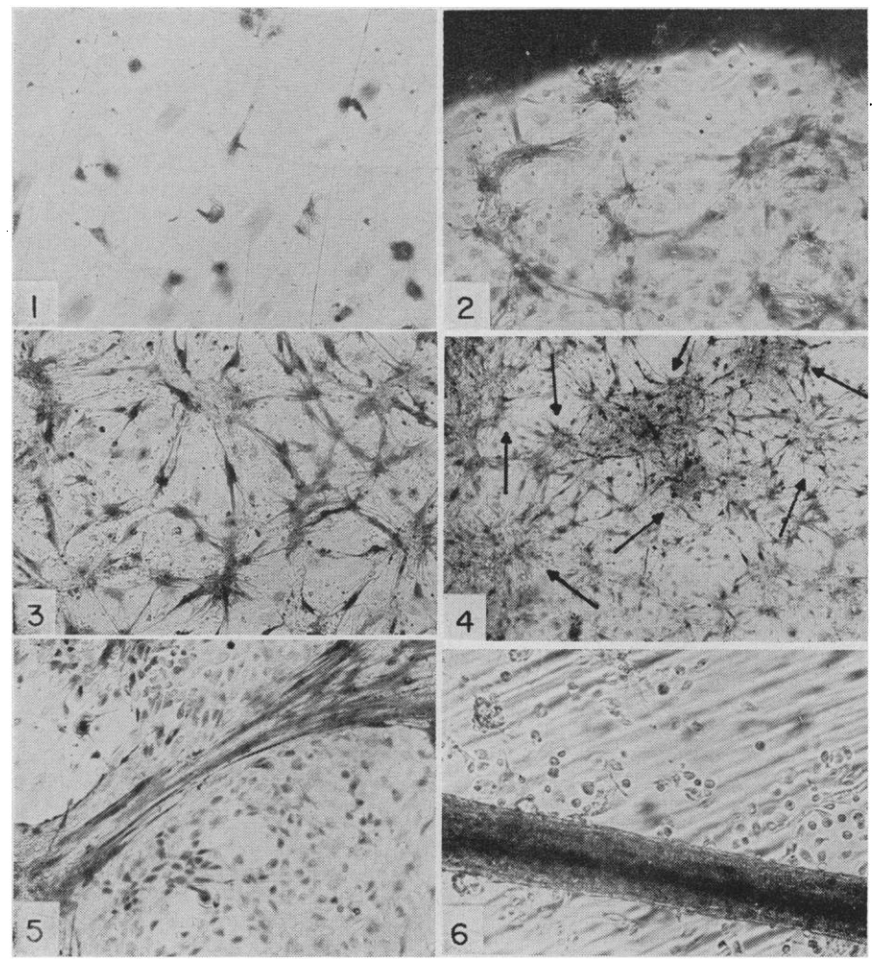

Fig. 1. Single cells beating at independent rates (stained with hematoxylin and eosin) (about × 170). Fig. 2. Individual cells joined through protoplasmic extensions (stained with methylene blue) (about × 170). Fig. 3. Further growth of connected cells (stained with methylene blue) (about × 170). Fig. 4. Beating centers of heart cells (stained with methylene blue) (about × 85). Fig. 5. Beginning of fiber formation (stained with methylene blue) (about × 170). Fig. 6. Beating fiber, unstained (about × 85).

dividual within a group of connected cells, indicating that a system exists which communicates the rate, strength, frequency, and location of the contraction. The pattern of beating may vary from one preparation to another. It may involve one strong and one weak beat, or one strong and two or three weak beats, but in all cases where the cells are joined, the beating of the cells is identical. The intercommunication between cells within such a group of cultured cells does not appear to be of the neuromuscular synaptic type. Flaxedil, a curare-type drug, has no effect on either the rate or the synchrony of the connected beating cells, nor do eserine and acetylcholine affect the synchrony of such preparations.

There are clear indications that physical contact plays a role in the communication of beating. A preparation of freshly suspended heart cells was divided into three unequal aliquots and cultured in equal volumes of media. The first contained X number of cells, the second $3X$, and the third $9X$ number of cells. The dilute or X culture contained single separated cells beating independently. The more concentrated or $3X$ culture contained single cells which were in physical contact. Many areas were examined and found to exhibit beating cells in synchrony, but these areas were not all in synchrony with each other. In the most concentrated, or $9X$, culture all the cells were in contact. Many areas, chosen at random, were examined; they exhibited the same rate of beating, and all these areas were beating in synchrony with each other. It appeared in this culture that all the cells were beating in unison.

The cells in the $9X$ culture were divided into two separate parts by running a needle down the middle of the dish to clear a swath of all cells and dividing the remainder into two groups, both in the same dish and in contact with the same media but not in contact with each other. In two cases, when this was done, it was seen that all the cells within each group beat synchronously but not with those of the other group. The beating rate in one case was 90 on one side and 75 on the other, and in another case 35 on one side and 10 on the other. The two sides were inhibited to different extents by acetylcholine, although the synchrony within each group was not affected.

The fact that single heart cells may be prepared from young heart tissue indicates that the heart exists, at the least, as a tissue potentially composed of mononucleated cells. On the other hand, the development of single cells in culture into sheets of tissue which, when stained, appear to be a syncytium indicates that the dissociation process must be, to an extent, reversible. The communication of physical or chemical events from one beating center to another would be facilitated by the absence of cell membrane barriers. It is also clear that physical contact may also serve to communicate the contractile event. It is obvious, however, that even if neuromuscular synapse mechanisms do not operate, and even though the syncytial form would facilitate communication, we have neither ruled out other synaptic mechanisms, nor explained, on the basis of the syncytium, the extreme rapidity of the communication of beating from one center to another.

The ability of single rat heart cells to grow into beating sheets of cells is, in this case, a process which is not accompanied by loss of function. The continued growth into a beating, fiber-like mass may indicate that these cells have an inherent potential related to the functioning of the intact heart.

The expression of cellular function in a manner which can be quantitatively evaluated provides a system in which such matters as cellular communication, organization, and differentiation may be studied (*4*).

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References and Notes

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4. This work was supported in part by research grant No. A-2135 from the National Institutes of Health, U.S. Public Health Service, and under contract No. AT-04-1-GEN-12 between the U.S. Atomic Energy Commission and the University of California, Los Angeles.

27 June 1960

Photodynamic Inactivation of Infectious Nucleic Acid

Abstract. Tobacco mosaic virus–infectious nucleic acid causes a color shift when combined with acridine orange, methylene blue, and safranine. A high concentration of acridine orange inactivates infectious nucleic acid even in darkness, while a mixture of nucleic acid with a low concentration of the dye must be exposed to visible light prior to inoculation for inactivation to occur.

Ribonucleic acids can combine with a variety of basic dyes. The combination is assumed to occur between the positively charged chromophore of the dye and the negatively charged phosphate groups of the ribonucleic acid, and can result in a shift in the wavelength of the maximum visible light absorption by the dye. Michaelis (*1*) demonstrated these effects with yeast nucleic acid and the dyes phenosafranine, toluidine blue, thionine, and pyronin, while Oster and Grimsson (*2*) demonstrated a color shift when toluidine blue, methylene blue, and safranine were combined with the nucleic acid released when tobacco mosaic virus is heated above 90°C.

I have recently observed a similar color shift, readily visible to the naked eye, when the dyes acridine orange, methylene blue, and safranine are combined with infectious nucleic acid from tobacco mosaic virus, prepared by the phenol method of Gierer and Schramm (*3*). Since acridine orange and methylene blue fluoresce in visible light and are therefore potentially capable of photodynamic action (*4*), it seemed of interest to test the effect of visible light on dye-bound infectious nucleic acid.

In a typical experiment, nucleic acid, at 0.1 mg/ml was bound to acridine orange at $6.4 \times 10^{-4}M$ and at $1.65 \times 10^{-5}M$. Dilutions of the nucleic acid and dye were made in O.1M, pH 7 phosphate buffer. This mixture was either exposed to light of 2300 ft-ca from white fluorescent tubes for 30 minutes or else kept in the dark. The mixtures were kept in iced water throughout, including the period when the test plants of *Nicotiana glutinosa* were inoculated. Each inoculum, containing 50 mg of celite per milliliter as abrasive, was rubbed on at least 72 half-leaves in an incomplete block design (*5*). Local lesions were counted 3 days later, and the results are shown in Table 1.

Table 1. Lesions produced on leaves inoculated with nucleic acid and nucleic acid-dye mixture.

Inoculum	Treatment	Lesions (total No.)
Nucleic acid	Light	3043
Nucleic acid	Dark	2381
Nucleic acid and $6.4 \times 10^{-4}M$ dye	Light	0
Nucleic acid and $6.4 \times 10^{-4}M$ dye	Dark	36
Nucleic acid and $1.65 \times 10^{-5}M$ dye	Light	0
Nucleic acid and $1.65 \times 10^{-5}M$ dye	Dark	3190

Even at the high dye concentration, which obviously caused a marked reduction of infectivity in the dark, there is a noticeable additional effect of light. However, at the low dye concentration used, the photodynamic action is extremely striking, since at this concentration there is no demonstrable dark effect, while visible light resulted in a complete abolition of infectivity.