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*).

ISAAC HARARY
BARBARA FARLEY
Department of Biophysics and Nuclear Medicine, Laboratory of Nuclear Medicine and Radiation Biology and *Department of Physiological Chemistry, University of California, Los Angeles*

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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.

In another experiment where only the dilute dye was used, no photodynamic action could be demonstrated when the mixture was rubbed on leaves prior to illumination.

Oster and McLaren (*6*) have shown that the fluorescent dye, acriflavine, mediates a visible light inactivation of intact tobacco mosaic virus. However, to my knowledge, similar photodynamic action has not previously been demonstrated for infectious nucleic acid (*7*).

M. CHESSIN

Department of Botany,
Montana State University, Missoula

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Temperature and Charge Transfer in a Receptor Membrane

Abstract. The rate of rise and the amplitude of a mechanically elicited generator potential in a receptor membrane (Pacinian corpuscle) increases markedly with temperature. By contrast, the amplitude of the action potential of the Ranvier node adjacent to the receptor membrane remains practically unchanged over a wide range of temperature. The activation energy of the rate-limiting process in excitation of the receptor membrane is high; it indicates the existence of a high potential energy barrier for charge transfer.

Mechanical stimulation of the nerve ending of Pacinian corpuscles produces transfer of charges through its receptor membrane. The energy requirements for the transfer are markedly influenced by temperature. For example, the strength of a mechanical stimulus necessary to produce a given generator potential at 25°C may be reduced to one-third at 35°C. A temperature change alone, however, elicits no detectable transfer.

The experiments reported here were done on single intact Pacinian corpuscles of the cat and on single nerve endings isolated by dissection from the corpuscles (*1*). The receptor ending was stimulated with mechanical pulses of known strength from a piezoelectric crystal, and the resulting electrical activity was led off from the axon or directly from the nerve ending (*2*). Experiments with intact corpuscles and with denuded nerve endings yielded essentially the same results.

The main effect of a temperature change is to vary the rate of rise and the amplitude of the mechanically elicited generator potential (Figs. 1 and 2). The rate of rise and the amplitude of the generator potential in response to a mechanical stimulus of a given (submaximal) strength increase approximately linearly with temperature with a mean Q_{10} of 2.5 and 2.0, respectively (15° to 35°C). The decay time of the generator potential is not appreciably affected by temperature.

The membrane of the first node of Ranvier adjacent to the receptor membrane, the site at which the nerve impulse arises, behaves quite differently. The amplitude of the action potential of the node, like that of other membranes with regenerative excitability (*3*), remains rather constant over a wide range of temperature (20° to 40°C), and its duration increases with temperature with a Q_{10} of 3 or higher. The electrical threshold for firing of impulses at the node varies inversely with temperature; the initiation of impulses at the node fails completely below 12°C, although the receptor membrane still produces generator potentials.

It may be thought that the observed results reflect mechanical effects due to changes in viscosity or rigidity of the preparation rather than effects on the excitation process of the receptor membrane. This possibility seems, however, unlikely. The visco-elastic properties of our preparation—namely, the denuded nerve ending—are not expected to differ from those of aqueous protein jellies whose temperature coefficients of viscosity and rigidity are as low as those of water. For instance, the Q_{10}'s of viscosity of blood plasma (*4*), egg albumin (*5*), and water are all approximately 1.2 between 20° and 40°C.

The energy of activation of the rate-limiting process in receptor excitation, as calculated from the temperature dependence of the rate of rise of the generator potential, is equivalent to 16,300 cal/mole. This reveals that at least at one stage in the excitation process there is a high potential energy barrier for charge transfer. It has been shown that charge transfer increases as a function of the electrical gradients across the receptor membrane (*6*). Thus a simple and, heretofore, quite plausible model for receptor excitation is that of ions diffusing simply along their gradients through mechanically stretched "pores" of the receptor membrane. This model must now be modified. The high activation energy found in our experiments forces at least one additional element, a high energy barrier, upon this or any other model one may prefer to choose. To surmount the barrier, energy may be supplied directly through heat transfer, or indirectly through a mechanically coupled chemical reaction. In any event, the immediate source that supplies the energy to surmount this barrier cannot be identical with the one that serves to trigger the excitation process: excitation, in our experiments, could only be brought about by mechanical stimulation; heat transfer alone, however steep its gradients, was found to be ineffective in exciting charge transfer.

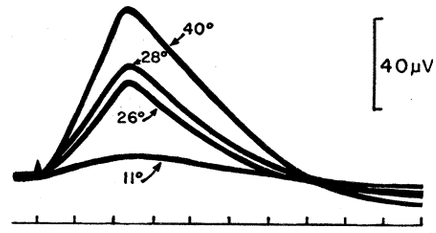

Fig. 1. Effect of temperature on charge transfer through a receptor membrane. The nerve ending is stimulated with equal mechanical pulses from a piezoelectric crystal, and the resulting generator potentials at various temperatures are superimposed. Time calibration, 1 msec.

Receptor excitation is thus thought to operate according to one of the following schemes. (i) The mechanical stimulus causes a directly coupled increase in permeability of the receptor membrane, and ions flow across the membrane along their electrochemical gradients, after overcoming an energy

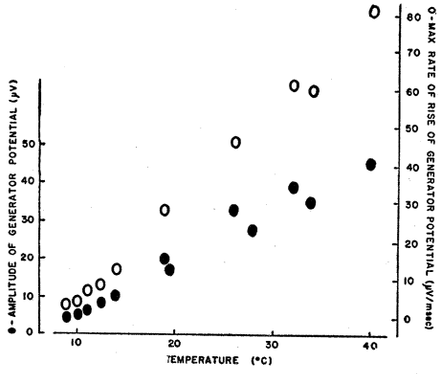

Fig. 2. Amplitude and rate of rise of generator potential as a function of temperature. The nerve ending is stimulated with equal mechanical pulses, and the mean amplitude and rate of rise of the resulting generator potential are determined at various temperatures.