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# Cell-to-Cell Diffusion of Procion Yellow in Sheep and Calf Purkinje Fibers

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*Summary.* Sheep and calf Purkinje fibers (false tendons) were cut near one end and exposed to a solution containing no calcium and the dye Procion Yellow (M4RS, molecular weight near 700). Fifteen minutes later the damaged end was sealed by applying calcium ions (Tyrode solution). Traces of Procion Yellow were detected within the intracellular compartment at a distance of 2.4 mm from the site of damage when the preparations had been washed in dye-free solution for 4 hr. This indicates that the dye had diffused through about 20 cells in succession. There was no detectable uptake of Procion Yellow through intact surface membranes. Visual curve fitting to quantitative data on concentration *vs.* distance gives an apparent diffusion coefficient (cell junctions and myoplasm in series) of $3 \times 10^{-8}$ cm$^2$ sec$^{-1}$, as against $1 \times 10^{-6}$ cm$^2$ sec$^{-1}$ in an agar gel. It is concluded that specialized contact areas between neighboring cardiac cells represent a considerable yet not an absolute hindrance to the movement of this particle.

Cardiac muscle is made up of single cells (Sjöstrand & Anderson, 1954) which are electrically coupled by low-resistance pathways (*cf.* Weidmann, 1952, 1969). Diffusion measurements using $^{42}$K have indicated that small ions can move from cell to cell (Weidmann, 1966). Procion Yellow, a dye of molecular weight near 700, has successfully been used to study the ramifications of nerve fibers (Stratton & Kravitz, 1968; Mulloney, 1970). It has also been used to demonstrate a diffusion pathway between segments of crayfish giant axon (Payton, Bennett & Pappas, 1969) and between neighboring horizontal cells of the dogfish retina (Kaneko, 1971). The present work concerns the question whether or not this fairly large molecule would diffuse from cell to cell in a cardiac preparation. This problem has been resolved by showing (a) that the dye does not noticeably enter intact

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cells, while (b) it spreads to neighboring cells when introduced into the intracellular space. The results have been demonstrated to the Swiss Physiological Society on the occasion of its fall meeting in 1970 (*unpublished*).

## Materials and Methods

Sheep and calf "false tendons" of 0.5 to 0.8 mm diameter and 1.5 to 2 cm in length were obtained from the slaughterhouse. The muscle chamber consisted of two half-chambers separated by an interchangeable rubber membrane. The false tendon containing several Purkunje fibers was pulled through a tight-fitting hole in the membrane. At the beginning of the experiment, action potentials were recorded from various sites using Tyrode as the bathing solution, to make sure that the preparation was in a good condition.

To introduce Procion Yellow (ICI, M4RS) into the intracellular compartment, use was made of a phenomenon described in detail by Délèze (1970): Purkinje fibres, when damaged in a $Ca^{2+}$-free solution, do not heal over. Upon addition of $Ca^{2+}$ to the extracellular solution the undamaged part of the preparation promptly seals itself off from the extracellular space by a barrier of high electric resistance.

In the present experiments the chamber containing the shorter part of the false tendon was perfused by a solution containing no calcium, little sodium (12.3 mM NaCl), and Procion Yellow (20 mM), the solution being made isotonic with Tyrode by additional sucrose. The end of the false tendon was cut across with a fine scissors. A time of 15 min was allowed for the dye to enter and then $Ca^{2+}$-containing Tyrode solution was substituted for the dye solution. For the remainder of the experiment both chambers were perfused with normal Tyrode solution, bubbled by 95% $O_2$ and 5% $CO_2$. Temperature was 37 °C.

An estimate of the distance by which Procion Yellow had moved at the end of 4 hr was obtained in the following way. The preparations were removed from the chamber and frozen on the $CO_2$ attachment of a microtome. Transverse sections of 60 μ were cut and mounted under glycerol-gelatine, without further processing. The slices were examined using a UV microscope with a photographic attachment. In a second series of experiments the results were quantitated by comparing 200-μ-thick sections of false tendons with 200-μ agar standards. The latter were prepared as follows. Known amounts of Procion Yellow were dissolved in agar solution (30 g/liter of purified Agar-Agar, Merck). The warm solutions were sucked into 500-μ capillary tubes. When solidified at room temperature the agar rods were blown out and sliced.

## Theory

Consider a cable sealed at $x=0$ and extending to infinity at $x>0$. Assume that over a distance $h$ (cm) from the sealed end a tracer is introduced with the concentration $C_0$ (mmoles/liter). At time $t=0$ the tracer will start to diffuse along the cable with a diffusion coefficient of $D$ ($cm^2\ sec^{-1}$). Assuming no loss of tracer through the surface membrane of the cable the concentration ($C$) as a function of time and distance is given by Crank

[1964, Eq. (2.15)] as

$$C=\frac{1}{2}C_0\left[\operatorname{erf}\frac{h-x}{2\sqrt{Dt}}+\operatorname{erf}\frac{h+x}{2\sqrt{Dt}}\right]. \quad (1)$$

If tracer is lost from the inside of the cable through its surface and if the amount lost per unit time is proportional to the inside concentration this expression extends to

$$C=\frac{1}{2}C_0\left[\operatorname{erf}\frac{h-x}{2\sqrt{Dt}}+\operatorname{erf}\frac{h+x}{2\sqrt{Dt}}\right]e^{-kt} \quad (2)$$

where $k$ is the rate constant ($\sec^{-1}$) for transmembrane efflux. A more detailed treatment is given by Weingart (1974).

## Results

### *Control Experiments*

It was found that Procion Yellow does not enter the cells to any appreciable extent unless the cells are injured. Fifteen experiments were performed, applying the dye-solution up to 4 hr. As shown by color photographs, the connective tissue of such cross-sections was intensely yellow while the intracellular compartment was free of dye.

It seemed advisable to check the method used to quantitate concentration by using a relatively simple diffusion system. Agar-filled polythene tubes were brought into contact with dye for a short time and sliced at the end of 30 and 60 min. The values in Fig. 1 have been fitted by assuming a diffusion coefficient (at room temperature) of $1\times10^{-6}$ cm$^2$ sec$^{-1}$ and a labeled portion from $x=0$ to $x=0.012$ cm.

### *Diffusion of Procion Yellow in Purkinje Fibers*

The values plotted in Fig. 2 are average concentrations as estimated at various distances at time 3 hr (12 experiments) and 4 hr (12 experiments). Purkinje fibers had been allowed to take up Procion Yellow for 0.25 hr in $Ca^{2+}$-free solution and were sealed at $t=0$ hr by $Ca^{2+}$. It was evident from examination of the experimental values that the dye not only diffused along the fibers but partially left the intracellular compartment. For, if there were no transmembrane efflux, the area underneath both curves ought to be the same. The theoretical curves used to fit the experimental points

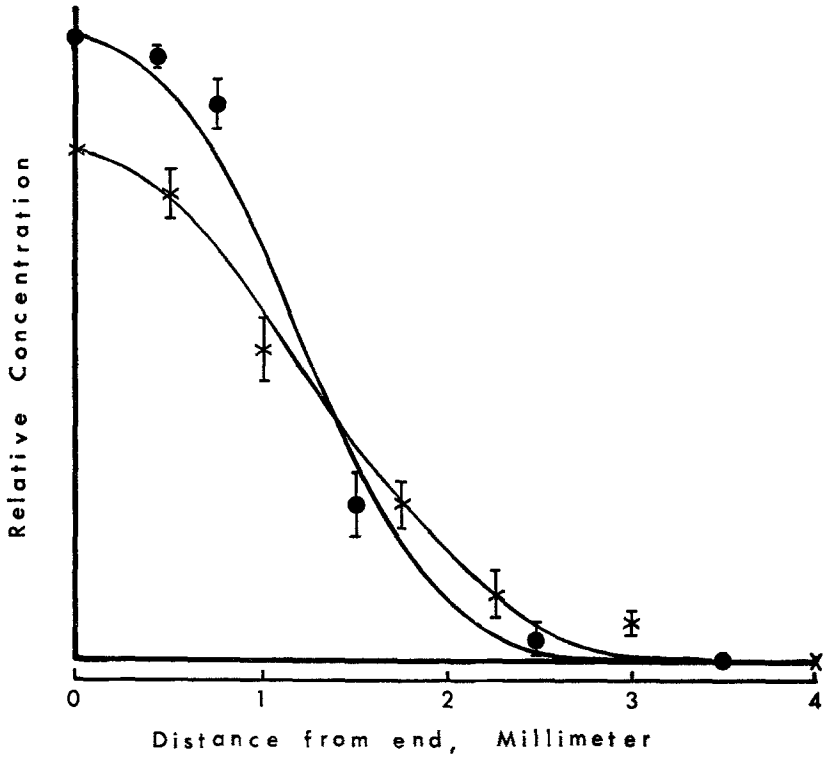


Fig. 1. Distribution of Procion Yellow along an agar rod, 30 min (circles) and 60 min (crosses) after application of the dye at $x=0$. Curves are drawn through mean values from 15 estimates ($\pm$SD) to give a good overall fit on the basis of Eq. (1)

of Fig. 2 were chosen from a plot-out of Eq. (2). It was rather difficult to find a satisfactory overall fit. The lines of Fig. 2 are those for an initial label ($h$) of 0.01 cm, a longitudinal diffusion coefficient of $D=3\times10^{-8}$ $cm^2\ sec^{-1}$, and a loss of dye between hours 3 and 4 from 100% to 43%, corresponding to a rate constant of $2.36\times10^{-4}\ sec^{-1}$.

Inspection of 60-μ slices indicated that traces of dye had moved as far as 2.4 mm from the cut end at the end of 4 hr.

### *Effects of Stimulation*

The boundary as judged in a somewhat arbitrary way between "no fluorescence" and "fluorescence" was followed in the course of 30 additional diffusion experiments. Distance for "just visible" was plotted

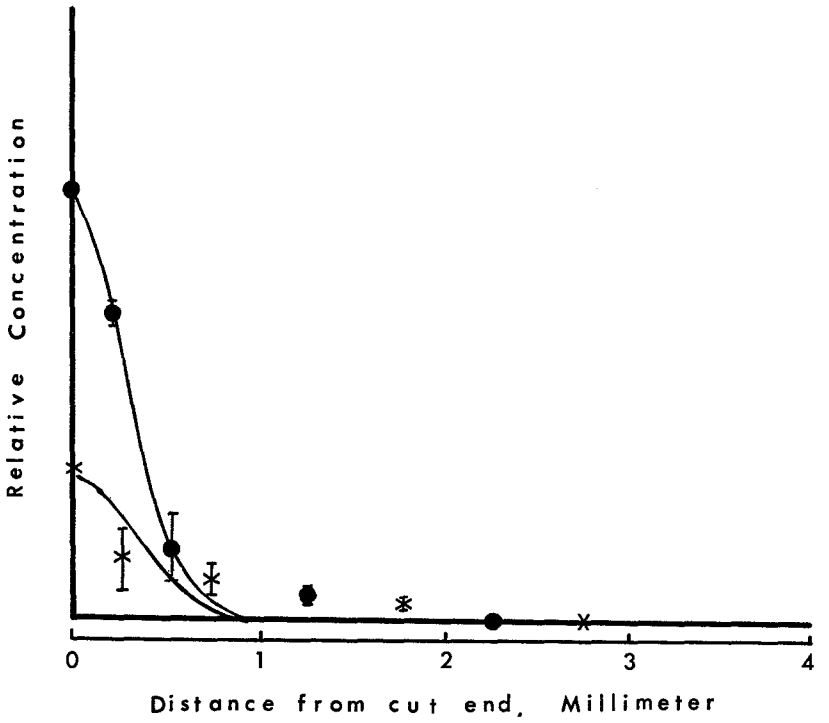


Fig. 2. Distribution of Procion Yellow along sheep Purkinje fibers, 3 hr (circles) and 4 hr (crosses) after sealing the damaged end at $x=0$. Curves are drawn on the basis of Eq. (2), with the main intention to fit the values as obtained 3 hr after sealing. Vertical bars indicate SD

against time. Taking the average values for 10 false tendons at rest and 20 false tendons stimulated at a frequency of 0.5/sec the data indicated no significant difference.

### *Chicago Blue*

This dye having a molecular weight of about 1,000 was applied, in the same manner as Procion Yellow, by the cut-and-seal method. After many hours there was no indication that this dye had moved by more than 100 to 200 μ. It is assumed that staining of the very end signifies some binding to intracellular constituents of damaged cells.

## Discussion

Four hours after the start of the experiments traces of Procion Yellow are seen at a distance of about 2,400 μ. With a cell length of the order of

100 μ for sheep Purkinje fibers (Mobley & Page, 1972), this result suggests that the dye must have crossed about 20 cells in succession. A semi-quantitative evaluation indicates that the longitudinal diffusion coefficient for cells and discs coupled in series is about 300 × lower than the diffusion coefficient of the same molecules in an agar gel. This is interpreted to mean that intercalated discs offer a considerable yet not an infinitely high resistance to the movement of Procion Yellow. Electrical (and, therefore, a slight degree of mechanical?) activity might tend to assist longitudinal diffusion through cytoplasm by having a convection effect. The result that rhythmical stimulation does not assist longitudinal diffusion to a marked extent is in line with the conclusion that the main hindrance is located in the specialized membrane structures.

The present results suggest that Procion Yellow can leave the cells but hardly enters resting fibers. This might be expected since Procion Yellow at a pH of 7.0 carries three negative charges. The resting membrane potential (inside negative) would, therefore, act against passive influx while aiding passive efflux. Weidmann (1966) postulated cell-to-cell diffusion of $^{42}K$ but discussed a possible criticism, namely movement of $^{42}K$ within extracellular space and uptake of $^{42}K$ by isolated cells. The fact that Procion Yellow hardly enters intact cells makes it unnecessary to consider this possibility.

The permeability of the surface membrane of Purkinje cells to Procion Yellow may be calculated by using the constant field approximation [Goldman, 1943; Carmeliet, 1961, Eqs. (2.9) and (2.14)]. Taking a volume/surface ratio of 2.56 μ (Mobley & Page, 1972), a membrane potential of −85 mV, a temperature of 37 °C, and the estimated rate constant for the efflux of a particle with three negative charges ($k = 2.36 \times 10^{-4}$ sec$^{-1}$, this paper) the permeability works out to $6.3 \times 10^{-9}$ cm sec$^{-1}$.

In the salivary gland of *Drosophila*, fluoresceine has been shown to move rather freely from one cell to the next (Loewenstein & Kanno, 1964). Furthermore, there are indications for a critical pore size (Kanno & Loewenstein, 1966), since serum albumin (mol wt 69,000) passes while polylysine (mol wt 127,000) does not. In the salivary gland of *Chironomus*, experiments with various tracers suggest the existence of smaller pores, permeable to molecules with an upper molecular weight between 1,000 and 10,000 (*see* review by Socolar, 1973). For mammalian heart, McNutt and Weinstein (1973) and Matter (1973) report on pores as part of the nexus structures having a diameter of 10 to 15 Å. The findings that $^{42}K$ (Weidmann, 1966), tetraethylammonium (Weingart, 1974), and Procion Yellow (mol wt 700, present paper) diffuse from cell to cell while Chicago Blue (mol wt 1,000)

does not, would again be in support of the concept of a critical pore size. It seems necessary, however, to use a variety of tracers of different molecular weight, different configuration, and different electrical charge to define more closely the conditions under which a particle can get from one cell to its neighbor.

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