J. Physiol. (1983), **339**, *pp.* 299–307
With 5 text-figures
Printed in Great Britain

THE INFLUENCE OF pH ON THE HEALING-OVER OF MAMMALIAN CARDIAC MUSCLE

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(*Received 3 September 1982*)

SUMMARY

1. The process of healing-over was followed in trabeculae from the right ventricle of guinea-pigs and in Purkinje fibres from dogs by measuring resting potential and input resistance close to a site of damage.

2. In Ca^{2+}-free bathing solution at pH 6·5 no signs of healing-over were found; at pH 5·5 healing-over was incomplete; at pH 5·0 and 4·0 healing-over occurred promptly.

3. It is concluded that transformation of low-resistance nexus membranes into high-resistance membranes separating the intracellular from the extracellular compartment normally is due to Ca^{2+}, while protons can bring about the same effect under extreme conditions.

INTRODUCTION

When heart muscle is damaged the injury potential soon vanishes (Engelmann, 1877). This process, called healing-over, is generally thought to result from the exposure of the cytoplasmic face of intercalated disks to the extracellular milieu and to a transformation of nexus membranes into high-resistance membranes. The presence of Ca (or Sr) ions in the bathing solution is essential for healing-over (Délèze, 1965, 1970; De Mello, Motta & Chapeau, 1969; Nishiye, 1977).

In certain syncytial structures the electrical resistance of nexal membranes is a sensitive function of intracellular pH (Turin & Warner, 1980; Spray, Stern, Harris & Bennett, 1982). On the assumption that cell uncoupling and healing-over are related phenomena and in order to test the hypothesis that protons may transform a nexus into a high-resistance membrane, healing-over was followed in Ca^{2+}-free solutions buffered to various levels of pH.

METHODS

Preparations

Experiments were performed on thin muscle trabeculae isolated from the right ventricle of guinea-pig hearts and on dog Purkinje fibres, 8–15 mm in length. Guinea-pigs were killed by a blow on the head and the hearts were immediately immersed in cold oxygenated Tyrode solution. Dogs

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were anaesthetized with pentobarbitone (35 mg/kg body weight). Purkinje fibres from the left ventricle were pinned into a transparent perfusion chamber.

Solutions

Preparations were maintained in Tyrode solution at 37 °C saturated with 100% O_2. The composition of this solution was the following (mM): NaCl, 138; KCl, 5·4; $CaCl_2$, 2·7; $MgCl_2$, 0·5; glucose, 5·5 and Tris-HCl, 10; the pH was adjusted to 7·3 (see Table 1). To investigate the effect of pH on the healing-over process, Ca^{2+} was removed from the solution and NaCl reduced to 7·5 mM. Sucrose was used to replace NaCl isosmotically while the concentrations of the other salts were maintained constant (see Table 1). The rationale of using a low Na solution was to avoid major and progressive depolarization caused by Ca^{2+} removal (see Délèze, 1970). The pH of the nominally zero Ca^{2+} and low Na^+ solution was varied from 7·3 to 6·5, 5·5 and 4, respectively. At pH 4 and 5 acetic acid/Na acetate was added as a buffer.

TABLE 1. Composition of solutions (mM) and their pH

Solutions	NaCl	KCl	$CaCl_2$	$MgCl_2$	Glucose	Sucrose	Tris-HCl	Na acetate/acetic acid	pH
I	138	5·4	2·7	0·5	5·5	—	10	—	7·3
II	7·5	5·4	0	0·5	5·5	261	10	—	7·3
III	7·5	5·4	0	0·5	5·5	261	10	—	6·5
IV	7·5	5·4	0	0·5	5·5	261	10	—	6
V	7·5	5·4	0	0·5	5·5	261	10	—	5·5
VI	7·5	5·4	0	0·5	5·5	241	—	10	5
VII	7·5	5·4	0	0·5	5·5	241	—	10	4

Electrical measurements

The membrane potential was measured with intracellular micro-electrodes filled with 3 M-KCl (Ling & Gerard, 1949). The voltage-recording micropipette was connected to a high-impedance DC amplifier. Voltage changes and current were displayed simultaneously on both a pen recorder and an oscilloscope. The input resistance (V_o/I_o) was measured by inserting two micropipettes close together (about 50 μm). Current pulses (400 msec duration, 1×10^{-7} A) were injected into the fibre through one of the micro-electrodes while the voltage changes were recorded with the other micro-electrode (see Fatt & Katz, 1951).

Cable analysis

This was limited to canine Purkinje fibres. There is reason to believe that injury results in a short-circuit between the inside and the outside of cardiac muscle followed by the establishment of a very high resistance over the course of a few minutes (healing-over; Engelmann, 1877; Weidmann, 1952). The input resistance to a current injected at a healed end of a cardiac Purkinje fibre extending to infinity at the other extreme is (Weidmann, 1952):

$$V_o/I_o = r_i\lambda. \tag{1}$$

If a cable is terminated by a finite resistance R_t at $x = L$ from the polarizing micro-electrode and extends to infinity at the other end, its input resistance R_{in} is (Hodgkin, A. L., cited by Délèze, 1970):

$$V_0/I_o = r_i\lambda\left\{1+\frac{(R_t/r_i\lambda)\sinh(L/\lambda)+\cosh(L/\lambda)}{(R_t/r_i\lambda)\cosh(L/\lambda)+\sinh(L/\lambda)}\right\}^{-1}. \tag{2}$$

For plotting experimental values on the theoretical curves (Fig. 1) λ and r_i were assumed to be constant. This is not entirely justifiable since membrane resistance and thus λ will change in the vicinity of a cut end as a function of membrane potential. In some experiments the influence of extracellular acidosis on the passive properties of long Purkinje fibres was studied. The space constant was determined by measuring electrotonic potentials at three different distances from the polarizing micro-electrode and plotting their amplitude against distance on semi-logarithmic paper.

The values of membrane resistance times unit length (r_m) and the intracellular longitudinal resistance per unit length (r_i) were calculated from (Hodgkin & Rushton, 1946):

$$r_m = 2\lambda\, V_o/I_o, \tag{3}$$

$$r_i = \frac{2\,X\,V_o/I_o}{\lambda}. \tag{4}$$

RESULTS

When a lesion was applied to a canine Purkinje fibre immersed in normal Tyrode solution (pH 7·3) the depolarization and the fall in input resistance elicited by damage were reversed in about 60 sec (see also Délèze, 1970). Measurements of input resistance performed 15 min after damage showed increasing values towards the cut end (Fig. 1). Indeed, this is expected when an infinite or very high resistance is

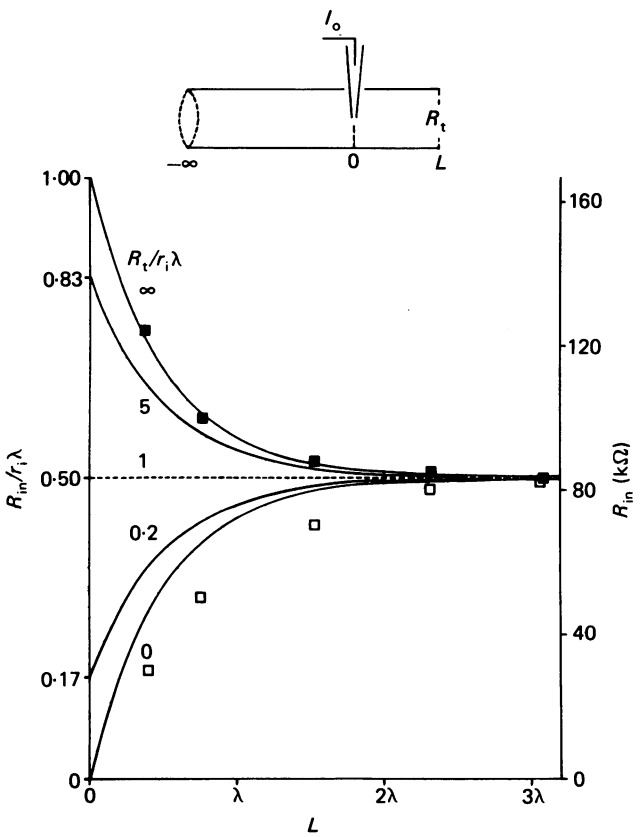

Fig. 1. Theoretical and experimental values of input resistance (R_{in}) of a cable in the proximity of a cut end. Distance is normalized in terms of space constants (λ). Theoretical lines are drawn for five ratios of terminal resistance (R_t) to characteristic resistance ($R/r_i\lambda$) of the cable, the ratios being ∞, 5, 1, 0·2 and 0. Experimental values obtained with two canine Purkine fibres are plotted in the same graph. Filled squares (■) indicate healing-over in Ca^{2+}-containing Tyrode solution; open squares (□) suggest a short circuit at $x = 0$ in nominally Ca^{2+}-free solution. The ordinate of the right-hand side gives values of input resistance actually measured. The similar value of R_{in} for the two preparations at a large distance from the cut end is purely coincidental.

established at the site of the lesion (see Weidmann, 1952; Délèze, 1970; present Fig. 1, filled quadrangles).

Effect of pH on the healing-over process

To investigate the influence of pH on healing-over it is necessary to remove Ca^{2+} from the solution. The rationale here is as follows: by suppressing the effect of Ca^{2+} on the healing process it is reasonable to assume that the exposure of the gap junctions (through the cut surface) to H^+ might increase the junctional resistance (Turin & Warner, 1977; De Mello, 1980) and consequently promote healing. The removal of Ca^{2+} from Tyrode solution is known to lead to depolarization and oscillation of the membrane potential. However, a simultaneous reduction of the extracellular Na^+ concentration avoids the gradual depolarization elicited by Ca^{2+} removal (Délèze, 1970).

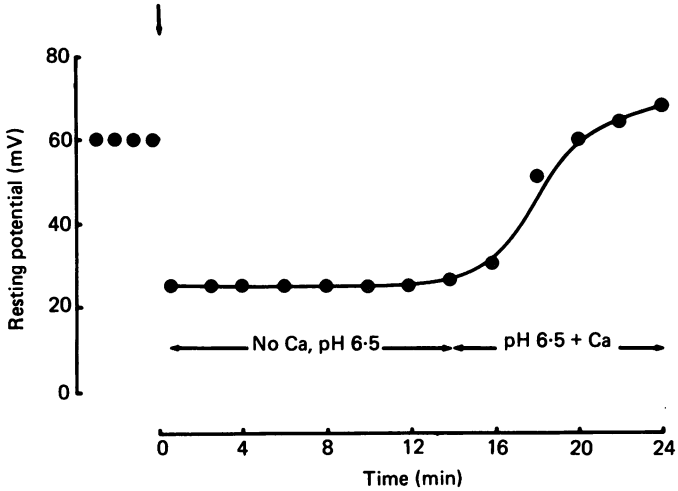

Fig. 2. Lack of healing-over in a canine Purkinje fibre previously exposed to Ca^{2+}-free solution at pH 6·5 for 20 min. The resting potential was recorded continuously at a distance of 0·05 cm from the cut end between 0 and 24 min, Ca^{2+} being re-admitted at 12 min.

In canine Purkinje fibres exposed to Ca-free low Na solution at pH 7·3, the resting potential slowly declined from 80 mV (S.D. ± 6; $n = 15$) to 60 mV (S.D. ± 8; $n = 17$) in about 15 min but was maintained at this level for several hours. The input resistance of the non-damaged fibre was well preserved in this solution. Moreover, in injured fibres, the input resistance determined 15 min after damage was found to be markedly reduced very near the cut end (Fig. 1). The deviation of experimental values from the curve drawn with $R_t = 0$ is opposite to what would be expected if $R_t = > 0$ and may reflect the fact that cardiac membrane resistance and thence λ increase upon depolarization. The findings re-confirm that in canine Purkinje fibres the removal of Ca^{2+} from the solution is sufficient to avoid the establishment of a high-resistance barrier after a lesion (Délèze, 1965. 1970; De Mello *et al.* 1969; De Mello, 1972).

Our next question is – can protons promote healing-over despite the lack of Ca^{2+}? To investigate this problem, Purkinje strands or muscle trabeculae were exposed

initially to Ca^{2+}-free solution at pH 7·3 for 20 min and then the pH was reduced to 6·5. After 20 min of equilibration in this solution the resting potential and the input resistance were measured. The resting potential of the non-damaged fibres was not altered at pH 6·5. When the fibres were cut and the membrane potential was immediately recorded near the lesion, the depolarization was not reversed. Experiments made on seven strands exposed to pH 6·5 showed low resting potential (28 mV, S.D. $\pm$5·2; n = 10) at 0·05 cm from the cut end 15 min after a lesion. The re-admission of Ca^{2+} to pH 6·5 solution led to complete recovery of the resting potential in about 7–9 min (Fig. 2). At pH 5·5 (solution II), the resting potential of the non-damaged fibres (62 mV, S.D. $\pm$6·2; n = 15) was not different from that recorded in Ca^{2+}-free solution at pH 7·3. However, the depolarization elicited by a lesion was not fully reversed. As can be seen in Fig. 3 (bottom), only 38% of the membrane potential

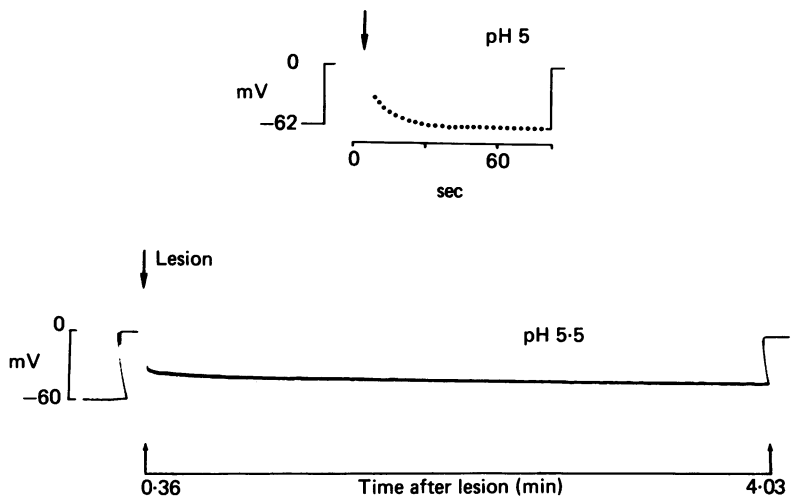

Fig. 3. Top, recovery of resting potential from a guinea-pig trabecula previously immersed in 0 Ca^{2+} solution pH 5 for 20 min. At left, membrane potential before damage. Bottom, partial recovery of membrane potential of a canine Purkine fibre previously exposed to 0 Ca^{2+} solution pH 5·5 for 30 min. Arrow indicates moment of lesion. At left, resting potential before damage.

recorded after damage was recovered in about 4 min. The significance of this result suffers from the fact that Tris-HCl has a low buffering effect at a pH of 5·5. At pH 5 and especially at pH 4 the promotion of healing-over was evident. Fig. 3 (top) shows the re-establishment of membrane potential of a guinea-pig trabecula recorded at pH 5. Although in this particular experiment healing-over was accomplished in about 60 sec, in others the rate of healing was slower and healing-over took 2–3 min.

However, at an extracellular pH of 4 the healing-over proceeded at a rate similar to that seen in normal Tyrode solution containing Ca^{2+} (See Fig. 4*A*).

Resting potentials of twenty strands exposed to Ca^{2+}-free and acid solution (pH 4) were 25 mV (S.D. $\pm$4·8 mV) immediately after the lesions and 62 mV (S.D. $\pm$5·6 mV) within 60 sec, similar to values recorded 3 or 4 mm away from the damaged end (Fig. 4*B*).

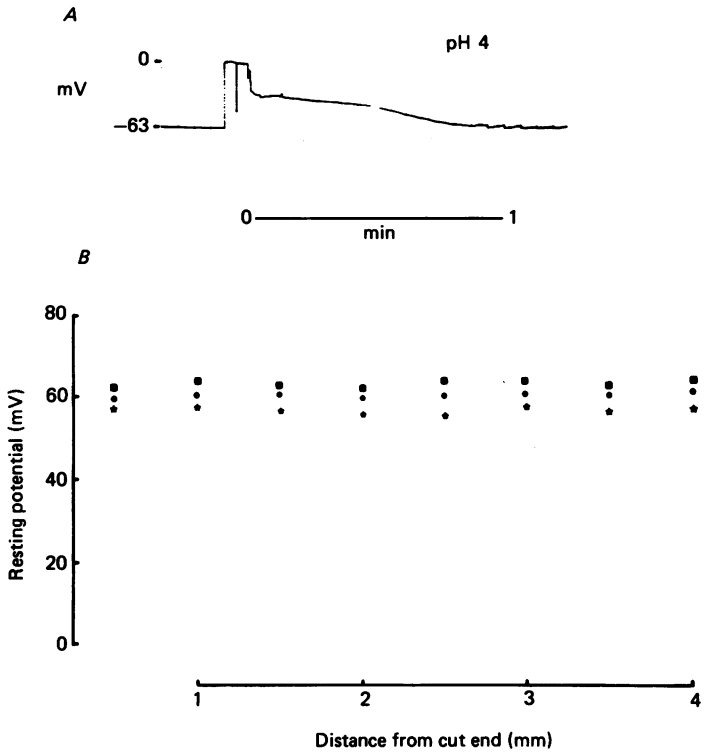

Fig. 4. Complete recovery of resting potential from a canine Purkinje fibre previously immersed in low Na^+, 0 Ca^{2+} solution, pH 4 for 30 min. *A*, resting potential recorded at 0·06 cm from the lesion, immediately after damage. At left, membrane potential before the lesion. *B*, resting potential from three strands immersed in 0 Ca^{2+} pH 4 recorded at different distances from the cross-section, 15 min after lesion. Each point represents the average from three measurements.

TABLE 2. Influence of extracellular acidosis on space constant, input resistance, r_i, and r_m of non-damaged Purkinje fibres in nominally Ca^{2+}-free solution

	λ (mm)		V_o/I_o (kΩ)		r_m (kΩcm)		r_i (MΩ/cm)	
Fibre	pH_o 7·3	pH_o 4	pH_o 7·3	pH_o 4	pH_o 7·3	pH_o 4	pH_o 7·3	pH_o 4
4	2	1·25	94·5	100	37·8	24	0·94	1·66
5	1·25	0·9	60	70	14·4	12·6	1	1·5
6	1·35	1·1	75	87	19·5	19·1	1·1	1·5

In strands exposed to acid solution the intracellular pH might fall (see Deitmer & Ellis, 1980) with a consequent increase in intracellular longitudinal resistance. The possible influence of extracellular acidosis on the passive electrical properties of Purkinje fibres was investigated. For this, the space constant and input resistance were measured initially in the mid-part of long, canine Purkinje fibres immersed in Ca^{2+}-free solution pH 7·3; the measurements were repeated in the same preparations exposed to pH 4 after 30 min. Table 2 shows the results of 3 successful experiments. As it can be seen r_i was increased by about 50 % while r_m was reduced by about 24 %. However, there was no cell uncoupling.

Table 3 gives input resistances from four additional fibres. The significance of the experimental values is best appreciated by a comparison with the theoretical curves of Fig. 1. If experimental R_{in} values fell below the dotted horizontal line (signifying R_{in} equal to characteristic resistance ($r_i\lambda$)), healing-over might be absent ($R_t/r_i\lambda = 0$) or incomplete (e.g. $R_t/r_i\lambda = 0{\cdot}2$). With experimental values well above the horizontal line, it is reasonable to conclude that R_t was a multiple of $r_i\lambda$. In other words, on the basis of both input resistance and membrane potential measurements, acid solution promoted the formation of a high terminal resistance.

In guinea-pig trabeculae the healing-over that was abolished in Ca^{2+}-free solution at pH 7·3 was re-established at pH 4 (see Fig. 5).

TABLE 3. Input resistance of canine Purkinje fibres immersed in Ca-free solution pH 4 before lesion, after healing-over and compared to theoretical values calculated from cable theory

	V_o/I_o(kΩ)			
Fibre	Before lesion mid-portion of fibre	15 min after lesion (L = 0·05 cm)	Theoretical values for a cable short-circuited at L = 0·05 cm, eqn. (2)	Theoretical values for a sealed cable at L = 0·05 cm, eqn. (2)
9	62	98	41·2–45·6*	83–124*
10	50	85	—	—
11	70	120	41·2–45·6*	124
12	84	115	—	83–124*

* Values calculated taking λ = 0·1 cm and 0·13 cm, respectively.

DISCUSSION

The present results confirm the observation that Ca^{2+} is essential for healing-over of cardiac fibres. They show that acidification to pH 6·5 is no substitute for missing Ca^{2+} but that reduction of pH below 5·5 is effective in sealing.

On the assumption that cell uncoupling and healing-over are related phenomena, the present results are in a quantitative contrast to those obtained with *Xenopus* and *Fundulus* embryonic cells (Turin & Warner, 1977; 1980; Spray *et al.* 1982) where acidification to pH 6·5 completely uncouples whereas free Ca^{2+} at a pH of 7·8 has to be increased well above 10^{-4} M until nexal resistance rises (Spray *et al.* 1982). The results presented here would tend to agree with those reported for *Chironomus* salivary glands (Rose & Rick, 1978) where nexal resistance is rather insensitive to pH but highly sensitive to internal free Ca^{2+}.

Reversible uncoupling of cardiac cells has been demonstrated following the injection into single cells of both Ca^{2+} (De Mello, 1975) and protons (De Mello, 1980). This finding, however, is qualitative in the sense that the critical intracellular range for $[Ca^{2+}]_i$ and $[H^+]_i$ has not been measured. Furthermore, the question of independent action of the two ionic species *versus* release of Ca^{2+} by acidosis is unsettled for the micro-injection experiments as it is in fact for the present healing-over experiments.

As to the non-effectiveness of pH shifts to the region of 6·5 for healing-over there is good agreement with data from cable analysis performed on sheep Purkinje fibres

(Reber & Weingart, 1982). These authors find a 30 % increase of internal longitudinal resistance when pH_i is shifted to 6·8, either by 100 mmHg CO_2 or during recovery from NH_4Cl treatment. Under comparable pH shifts pCa_i even decreases (Hess & Weingart, 1980), thus making it likely that protons and Ca^{2+} have independent effects on nexal permeability. It seems essential to stress that a conductance decrease of 30 % of a single intercalated disk per fibre would not have been detected by healing-over experiments.

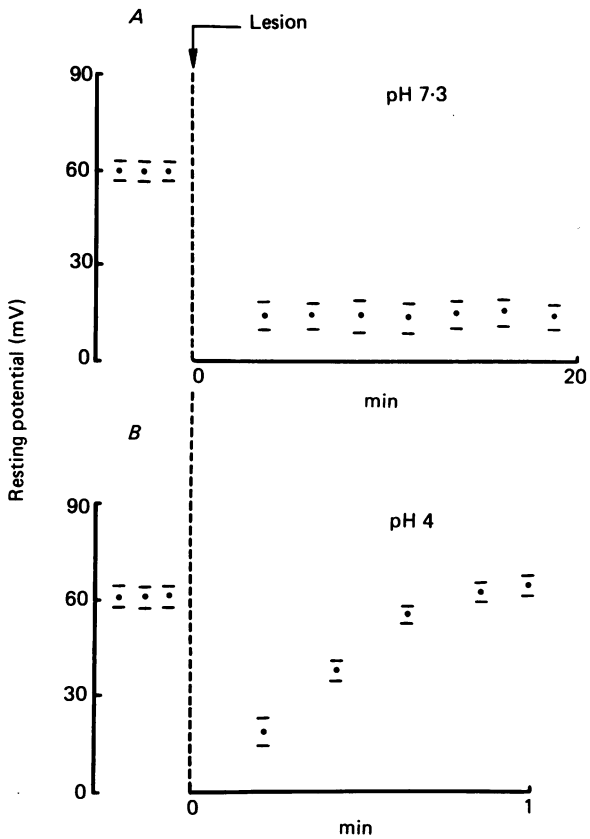

Fig. 5. *A*, lack of healing-over in guinea-pig trabeculae previously immersed in Ca^{2+}-free solution pH 7·3 (average from fifteen experiments). *B*, re-establishment of the sealing process produced by lowering the pH of Ca^{2+}-free solution to 4 (average from fifteen experiments). Distance between horizontal lines at each point is S.D.

When the effectiveness of Ca^{2+} and H^+ is compared on a molar basis, the ranges at which healing-over of cardiac tissue occurs is rather similar, namely $4·3–8·2 \times 10^{-5}$ M for Ca^{2+} (Nishiye, 1977) and $3·2–10 \times 10^{-6}$ M for protons (present results). This may be of interest with respect to possible mechanisms of conductance regulation. However, the range at which protons exert a major effect is well outside the pH values which are encountered in a living heart both under physiological and pathological conditions.

I am grateful to Professor S. Weidmann for continuing interest and for valuable discussion. I also thank Drs R. Weingart and M. Pressler for suggestions concerning the manuscript. My stay in Berne was made possible by a fellowship from the Roche Foundation for Scientific Exchange with Switzerland. Part of this work was supported by a grant from NHLI and P.R. Heart Association.

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