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## Chloride Activity and its Control in Skeletal and Cardiac Muscle [and Discussion]

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## Chloride activity and its control in skeletal and cardiac muscle

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Ion-selective microelectrodes have been used to compare the mechanisms controlling intracellular  $\text{Cl}^-$  activity in skeletal and cardiac muscle. In frog Sartorius skeletal muscle fibres,  $\text{Cl}^-$  levels are low (about 3 mM) and are determined mainly passively. The effect of any  $\text{Cl}^-$  transport system will be quickly short-circuited through the high membrane  $\text{Cl}^-$  conductance. In contrast, the sheep-heart Purkinje fibre, like other cardiac tissues, contains higher than passive levels of intracellular  $\text{Cl}^-$  (20–30 mM). Many  $\text{Cl}^-$  movements occur, not through  $\text{Cl}^-$  channels (the permeability for  $\text{Cl}^-$  is low), but by a  $\text{Cl}^-$ – $\text{HCO}_3^-$  countertransport system. High internal  $\text{Cl}^-$  levels are achieved by an exchange of extracellular  $\text{Cl}^-$  for intracellular  $\text{HCO}_3^-$ , which acidifies the fibre by 0.3 pH. Anion exchange in heart differs from that proposed for other excitable cells in that it is not specialized to compensate for an intracellular acidosis. Instead, it can prevent the fibres from becoming too *alkaline* by promoting a bicarbonate efflux and a chloride influx whenever internal bicarbonate levels rise. Possible reasons for this are briefly discussed.

## 1. INTRODUCTION

It is frequently assumed that  $\text{Cl}^-$  ions are distributed passively across muscle membranes. This is largely because of work on the frog Sartorius skeletal muscle fibre (e.g. Boyle & Conway 1941; Adrian 1956; Hodgkin & Horowicz 1959). It was concluded that the membrane of this cell was freely permeable to  $\text{Cl}^-$  ions and that at equilibrium the intracellular level of  $\text{Cl}^-$  could be predicted from the Nernst equation by making the  $\text{Cl}^-$  equilibrium potential ( $E_{\text{Cl}^-}$ ) equal to the resting membrane potential. Hence for a fibre with a resting potential of about –90 mV (Adrian 1956) one would expect to find a very low level of intracellular  $\text{Cl}^-$  (about 3 mM). The idea of passive  $\text{Cl}^-$  distribution was later extended to include cardiac muscle (Hutter & Noble 1961), a tissue that is normally electrically active. It was pointed out that because there is not normally a stable resting potential in heart,  $E_{\text{Cl}^-}$  might lie passively somewhere between peak systolic and diastolic membrane potentials, producing a much higher level of intracellular  $\text{Cl}^-$ . Furthermore, chemical analysis seemed to indicate a high level of Cl in cardiac muscle (about 20–30 mM) (see for example, Lamb 1961). It should be noted, however, that if  $\text{Cl}^-$  is simply distributed passively then it will fall to low levels in quiescent cardiac tissue because the resting potential will now be similar to that of skeletal muscle.

With the advent of ion-selective microelectrodes it became possible to measure  $\text{Cl}^-$  directly inside muscle fibres, permitting a re-evaluation of the mechanisms controlling intracellular  $\text{Cl}^-$  activity (Bolton & Vaughan-Jones 1977; Vaughan-Jones 1979*a, b*, 1981, 1982). In this paper I briefly review some of the results of these studies together with preliminary results of more recent experiments. First of all I compare the regulation of  $\text{Cl}^-$  inside a skeletal muscle fibre (the frog Sartorius) with that in a cardiac fibre (the sheep cardiac Purkinje fibre). The comparison is made by using microelectrode measurements of intracellular  $\text{Cl}^-$  activity,  $a_{\text{Cl}^-}^i$ .

The rest of the paper is then devoted to a study of the cardiac fibre, because the measurements here clearly indicate a non-passive distribution of  $\text{Cl}^-$ . Much of the net  $\text{Cl}^-$  movement across the cardiac membrane appears to be carrier-mediated in exchange for  $\text{HCO}_3^-$  ions. This means that the system controlling intracellular  $\text{Cl}^-$  activity has an important influence on the control of intracellular pH in heart.

## 2. COMPARISON OF INTRACELLULAR $\text{Cl}^-$ ACTIVITY IN SKELETAL AND CARDIAC MUSCLE

Figure 1*a* shows a direct measurement of the intracellular  $\text{Cl}^-$  activity,  $a_{\text{Cl}^-}^i$ , of a frog Sartorius skeletal muscle fibre. The measurement was made by using a liquid ion-exchanger  $\text{Cl}^-$ -sensitive microelectrode (Walker 1971) in conjunction with a separate conventional intracellular microelectrode to record the membrane potential (for details of the method see

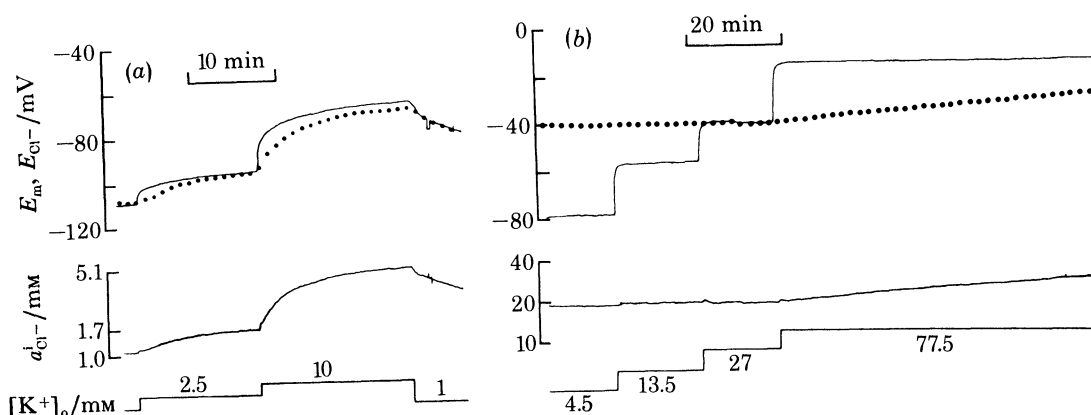


FIGURE 1. Intracellular  $\text{Cl}^-$  activity,  $a_{\text{Cl}^-}^i$ , is low in skeletal muscle but high in cardiac muscle. (a) Pen recording of an experiment to measure the effects of raising the external  $\text{K}^+$  level (isosmotic substitution of  $\text{KCl}$  for  $\text{NaCl}$ ) on  $a_{\text{Cl}^-}^i$  (lower trace) and the membrane potential,  $E_m$  (upper trace) of a frog Sartorius skeletal muscle fibre.  $E_{\text{Cl}^-}$  (dots) has been plotted over the voltage trace.  $a_{\text{Cl}^-}^i$  is measured as the difference signal between a conventional voltage microelectrode and a  $\text{Cl}^-$ -microelectrode when both are intracellular. Ringer, 15 mM Tris- $\text{Cl}$  + 100%  $\text{O}_2$ ;  $\text{pH}_o$  7.20; temperature, 25 °C;  $a_{\text{Cl}^-}^o$ , 76 mM. (b) Pen recording of an experiment to test the effects of raising the external  $\text{K}^+$  level (isosmotic substitution of  $\text{KCl}$  for  $\text{NaCl}$ ) on  $a_{\text{Cl}^-}^i$  (lower trace) and  $E_m$  (upper trace) of a sheep cardiac Purkinje fibre.  $E_{\text{Cl}^-}$  (dots) is plotted over the voltage trace.  $a_{\text{Cl}^-}^o$ , 100 mM; Tyrode buffered with 20 mM HEPES + 100%  $\text{O}_2$ ;  $\text{pH}_o$  7.40; temperature 36 °C. Sources: (a) modified from Bolton & Vaughan-Jones (1977); (b) modified from Vaughan-Jones (1979*a*).

Bolton & Vaughan-Jones (1977) and Vaughan-Jones (1979*a, b*)). The  $\text{Cl}^-$  equilibrium potential,  $E_{\text{Cl}^-}$ , was then calculated from the Nernst equation by using the measured level of intracellular and extracellular  $\text{Cl}^-$ . This has been plotted on the voltage trace (filled circles). Intracellular  $\text{Cl}^-$  levels are low (about 1 mM). In the steady state the computed values of  $E_{\text{Cl}^-}$  are virtually coincident with the membrane potential. Depolarization produced by raising external  $[\text{K}^+]_o$  results in a relatively rapid rise of  $a_{\text{Cl}^-}^i$  such that as a new steady state is approached,  $E_{\text{Cl}^-}$  is again quite close to the membrane potential. This behaviour can now be contrasted with the situation in the cardiac Purkinje fibre (figure 1*b*). In this experiment, as in all those that follow, the preparation was allowed to remain quiescent. This was to eliminate the possibility that electrical activity might raise  $a_{\text{Cl}^-}^i$  passively. Despite this  $a_{\text{Cl}^-}^i$  is about 20 mM, so that  $E_{\text{Cl}^-}$  is -40 mV, about 40 mV positive to the resting membrane potential. Furthermore,  $a_{\text{Cl}^-}^i$  is insensitive to modest changes of membrane potential induced by raising external  $[\text{K}^+]_o$ ,

although very large depolarizations in high  $[K]_o$  produce a slow rise of  $a_{Cl^-}^i$ . These measurements therefore confirm that  $Cl^-$  is distributed essentially passively in frog skeletal muscle and that the membrane permeability to  $Cl^-$  is high. A change of membrane potential is therefore followed by a relatively rapid redistribution of  $Cl^-$  across the membrane. The  $Cl^-$  permeability  $P_{Cl^-}$  (constant field theory), estimated from the rates of rise of  $a_{Cl^-}^i$  is in broad agreement with the original estimates of *ca.*  $5 \times 10^{-6} \text{ cm s}^{-1}$  (Hodgkin & Horowicz 1959). However, in the quiescent Purkinje fibre, intracellular  $Cl^-$  is about four times higher than that predicted passively, and the membrane permeability to  $Cl^-$  is clearly very low: changes of membrane potential only slowly affect  $a_{Cl^-}^i$ . The  $Cl^-$  permeability,  $P_{Cl^-}$ , is probably less than  $3 \times 10^{-8} \text{ cm s}^{-1}$  (see Vaughan-Jones (1979*b*) for a discussion of this). Furthermore, high values for  $a_{Cl^-}^i$  have been measured in other cardiac tissues, and these appear to be unaffected by the presence or absence of normal electrical activity (Ladle & Walker 1975; Vaughan-Jones 1979*a*; Spitzer &

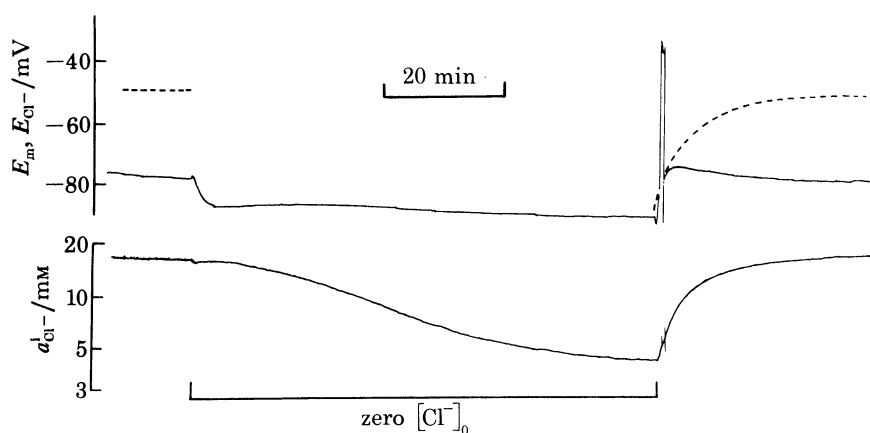


FIGURE 2.  $Cl^-$  can be pumped back into a Purkinje fibre. Effect of removing and re-adding external  $Cl^-$  on  $a_{Cl^-}^i$  and  $E_m$  of a sheep cardiac Purkinje fibre ( $Cl^-$  replaced by glucuronate and gluconate; for details of this, see Vaughan-Jones (1979*a*)). Broken line indicates estimated position of  $E_{Cl^-}$ . Tyrode buffered with  $22 \text{ mM HCO}_3^- + 5\% \text{ CO}_2 + 95\% \text{ O}_2$ ; pH 7.40. This  $\text{HCO}_3^-$  concentration is used in all subsequent figures unless otherwise specified.

Walker 1980). It is clear, then, that the behaviour of  $Cl^-$  inside cardiac muscle does not conform to a simple passive distribution. In the sections that follow I shall consider the reasons for this.

### 3. CHLORIDE TRANSPORT IN THE CARDIAC PURKINJE FIBRE

Chloride is capable of crossing the Purkinje fibre membrane. Hence it leaves the fibre in  $Cl^-$ -free solution. The experiment shown in figure 2 demonstrates this, with glucuronate used as a  $Cl^-$  substitute. After about 90 min  $a_{Cl^-}^i$  has fallen to about 4 mM. It is not known whether this residual level is really  $Cl^-$  that has not yet left the fibre or whether it represents a background level of interference from unidentified intracellular anions. This is because the  $Cl^-$ -sensitive microelectrode is not perfectly selective for  $Cl^-$  (Walker 1971; Bolton & Vaughan-Jones 1977). However, even assuming that the residual level is caused by unwanted interference, then the initial resting level of  $Cl^-$  would be overestimated by only 4 mM. Such an error would not alter the conclusion that, in heart,  $Cl^-$  levels are high and non-passive (see Vaughan-Jones (1979*a*) for a discussion of this problem).

Upon readmitting normal extracellular  $Cl^-$ , the intracellular  $Cl^-$  activity recovers rapidly

to its original, high level (figure 2).  $E_{\text{Cl}^-}$  becomes positive to the membrane potential. This is direct evidence that  $\text{Cl}^-$  can be accumulated against an electrochemical gradient.

Figure 3 shows an experiment where different amounts of extracellular  $\text{Cl}^-$  were readmitted after the fibre had been depleted of its intracellular  $\text{Cl}^-$ . The relation observed between internal and external  $\text{Cl}^-$  activities has been replotted in figure 4 (filled squares). In the steady state

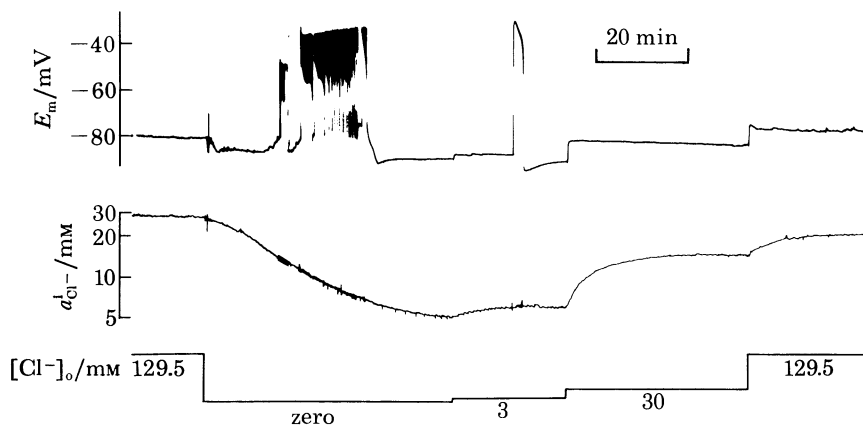


FIGURE 3. Effect of changing the extracellular  $\text{Cl}^-$  ( $\text{Cl}_o^-$ ) level on  $a_{\text{Cl}^-}^i$  and  $E_m$  of a sheep cardiac Purkinje fibre. At 20 min after removing  $\text{Cl}_o^-$ , there was a spontaneous burst of action potentials. These have been attenuated on the voltage trace because of the slow time-constant of the pen recorder. Glucuronate + gluconate used as a  $\text{Cl}^-$  substitute;  $\text{CO}_2$ - $\text{HCO}_3^-$ -buffered Tyrode.

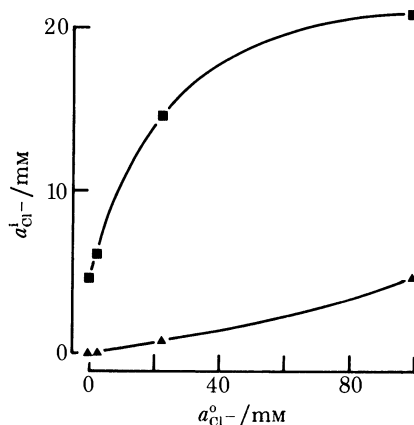


FIGURE 4. Relation between steady-state intracellular and extracellular  $\text{Cl}^-$  activities,  $a_{\text{Cl}^-}^i$  and  $a_{\text{Cl}^-}^o$ , for a sheep cardiac Purkinje fibre. Extracellular  $\text{Cl}^-$  concentrations can be converted to activities by the relation  $a_{\text{Cl}^-}^o = \gamma[\text{Cl}^-]_o$ , where  $\gamma$  is the activity coefficient ( $\gamma = 0.76$ ). Data taken from experiment shown in figure 3 (filled squares). The points are derived from the recovery of  $a_{\text{Cl}^-}^i$  after the readdition of  $\text{Cl}_o^-$ . The relation predicted for a passive  $\text{Cl}^-$  distribution in accordance with the measured membrane potential is also shown (filled triangles). Lines fitted by eye.

there is a roughly hyperbolic dependence of intracellular  $\text{Cl}^-$  activity on extracellular  $\text{Cl}^-$  activity. Typically,  $a_{\text{Cl}^-}^i$  is half-maximal when  $a_{\text{Cl}^-}^o$  is about 10 mM. An important point to note in figure 4 is that if  $\text{Cl}^-$  were conforming to a passive distribution in accordance with the measured membrane potential, the relation described by the lower curve (filled triangles) would be predicted. This indicates that for all the levels of external  $\text{Cl}^-$  tested,  $\text{Cl}^-$  was accumulated inside the fibre to a level in excess of that expected passively. In addition, estimates can be made of the initial rate of rise of  $a_{\text{Cl}^-}^i$  upon readdition of different amounts of external  $\text{Cl}^-$ ,

in each case to a fully depleted fibre. This is a measure of the initial  $\text{Cl}^-$  influx. The initial rate increases with increasing levels of  $\text{Cl}_o^-$  and then saturates at higher levels of  $\text{Cl}_o^-$  (not shown). The rate of uptake is half-maximal when  $a_{\text{Cl}^-}^o$  is about 40 mM. In other words, the  $\text{Cl}^-$  reuptake system in heart displays saturation kinetics with respect to external  $\text{Cl}^-$ . This feature, coupled with the fact that  $\text{Cl}^-$  is rapidly reaccumulated to high non-passive levels within the cell, suggests that much of the  $\text{Cl}^-$  entry into the Purkinje fibre is carrier-mediated on an ion-transport system.

#### 4. DEPENDENCE OF $\text{Cl}^-$ TRANSPORT ON $\text{HCO}_3^-$ : A $\text{Cl}^-$ - $\text{HCO}_3^-$ EXCHANGE

The  $\text{Cl}^-$  reaccumulation in the cardiac Purkinje fibre could be coupled to the movement of another ion or it might exist simply as an uncoupled electrogenic uptake. Much of the evidence for the participation of other ions in  $\text{Cl}^-$  transport has been considered previously (Vaughan-Jones 1979*b*, 1981, 1982). Therefore I shall only briefly summarize the evidence here. Recent experiments suggest that  $\text{Na}^+$  or  $\text{K}^+$  ions are not directly involved. In the absence of internal and external  $\text{Na}^+$ , the reuptake of  $\text{Cl}^-$  proceeds at about the same rate, achieving the same high level of  $a_{\text{Cl}^-}^i$  ( $\text{Na}_o^+$  substituted with bis(2 hydroxyethyl)dimethylammonium  $\text{Cl}^-$ ). Similarly, removing external  $\text{K}^+$  does not impair  $\text{Cl}^-$  reuptake. Bicarbonate ions, however, *do* appear to be involved. This is because  $\text{Cl}^-$  reaccumulation is slowed nearly 18-fold if  $\text{HCO}_3^-$ -free solutions are used (i.e. 20 mM HEPES, 100%  $\text{O}_2$  at  $\text{pH}_o$  7.40) (Vaughan-Jones 1979*b*). Under these conditions both the internal and external levels of  $\text{HCO}_3^-$  will be extremely low, although probably they will still be finite because of the metabolic production of  $\text{CO}_2$  by the cell.

Further evidence for the participation of  $\text{HCO}_3^-$  comes from direct microelectrode measurements of intracellular pH,  $\text{pH}_i$ . Such an experiment is shown in figure 5:  $\text{pH}_i$  measured with a recessed-tip pH-sensitive microelectrode (Thomas 1978) slowly becomes more alkaline in  $\text{Cl}^-$ -free solution (an increase of 0.33 pH). Upon readding external  $\text{Cl}^-$ ,  $\text{pH}_i$  returns to its more acid level. This suggests that  $\text{HCO}_3^-$  may be exchanging for  $\text{Cl}^-$  across the membrane. If  $\text{CO}_2$  is assumed to be in equilibrium across the membrane, then a movement of  $\text{HCO}_3^-$  out of the cell in exchange for an influx of  $\text{Cl}^-$  will decrease  $\text{pH}_i$ . The exact change of  $\text{pH}_i$  will depend on the net membrane flux of  $\text{HCO}_3^-$  and also on the intracellular proton buffering power. Conversely, if  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange can work in reverse, for example when external  $\text{Cl}^-$  is removed, then a departure of  $\text{Cl}^-$  from the fibre in exchange for the entry of  $\text{HCO}_3^-$  will increase  $\text{pH}_i$ . The observed changes in  $\text{pH}_i$  in figure 5 are therefore important because they suggest that the loss of intracellular  $\text{Cl}^-$  during  $\text{Cl}^-$  depletion as well as its subsequent reuptake is via a  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange rather than through membrane channels. Movement of some  $\text{Cl}^-$  through channels cannot be excluded but it is thought to be small, especially during the rapid  $\text{Cl}^-$  reaccumulation phase, because the membrane permeability to  $\text{Cl}^-$  is so low (figure 1*b*).

Finally, addition of the disulphonic stilbene drug SITS (4-acetamido-4'-isothiocyanato-stilbene-2,2'-disulphonic acid), which inhibits  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange in the red blood cell, largely inhibits both the fall and the subsequent reaccumulation of intracellular  $\text{Cl}^-$  (Vaughan-Jones 1979*b*). As shown in figure 5, SITS also inhibits the simultaneous changes of  $\text{pH}_i$ , indicating that the changes of  $a_{\text{Cl}^-}^i$  and  $\text{pH}_i$  are interrelated.

### 5. $\text{Cl}^-$ - $\text{HCO}_3^-$ EXCHANGE AND THE REGULATION OF INTRACELLULAR pH IN THE PURKINJE FIBRE

The existence of a  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchanger in cardiac tissue is perhaps not surprising. Already it has been proposed for a variety of other excitable cells where it forms part of an ion-transport system that removes excess acid, or its ionic equivalent, from the cell (see, for example, Russell & Boron 1976; Thomas 1977). To achieve this, the system promotes a net  $\text{Cl}^-$  efflux in exchange

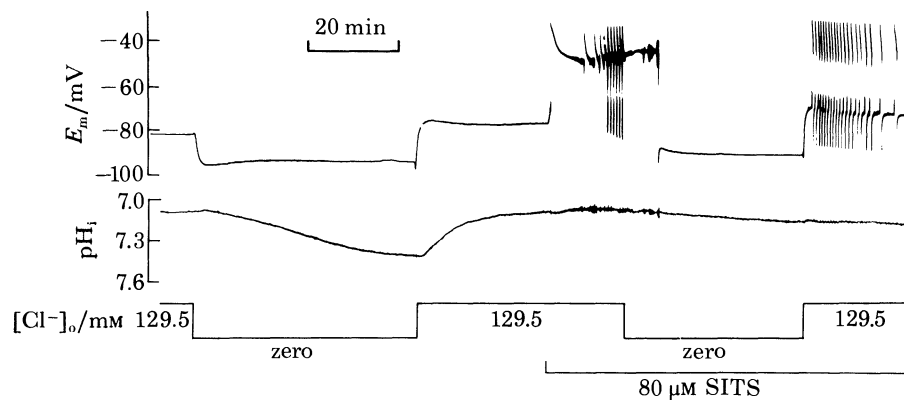


FIGURE 5. Removal of  $\text{Cl}^-$  makes intracellular pH more alkaline. Effects of removing and adding back external  $\text{Cl}^-$  on the intracellular pH ( $\text{pH}_i$ ) and membrane potential ( $E_m$ ) of a sheep cardiac Purkinje fibre. After the application of SITS (4-acetamido-4'-isothiocyanatostilbene-2,2'-disulphonic acid),  $E_m$  spontaneously depolarized and then repolarized during subsequent removal of  $\text{Cl}^-$ , but this had no effect on  $\text{pH}_i$ .  $\text{CO}_2$ - $\text{HCO}_3^-$ -buffered Tyrode.

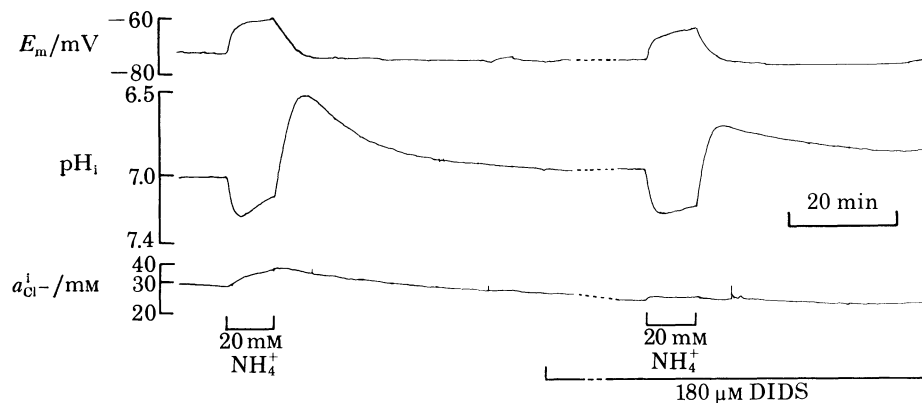


FIGURE 6. Experiment to investigate the participation of  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange in the regulation of intracellular pH. Simultaneous measurement of membrane potential,  $\text{pH}_i$  and  $a_{\text{Cl}^-}^i$  in a sheep cardiac Purkinje fibre. For this experiment three microelectrodes were inserted. 20 mM  $\text{NH}_4\text{Cl}$  was added (isosmotic substitution for  $\text{NaCl}$ ) and then removed, first in the absence and then in the presence of the drug DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid). The broken lines indicate an interval of 45 min. Tyrode 10 mM  $\text{HCO}_3^-$  (3%  $\text{CO}_2$  + 97%  $\text{O}_2$ );  $\text{pH}_o$  7.4.

for a net  $\text{HCO}_3^-$  influx. It is therefore important to see if anion exchange plays a similar role in heart. One way of testing this is to change  $\text{pH}_i$  experimentally and then observe whether this 'switches on'  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange. Figure 6 shows such an experiment. External  $\text{NH}_4\text{Cl}$  is applied while  $a_{\text{Cl}^-}^i$  and  $\text{pH}_i$  are measured simultaneously. There is a rapid intracellular alkalization. This presumably is because  $\text{NH}_4^+$  is a weak base ( $\text{p}K_a \approx 9.0$ ) so that molecular  $\text{NH}_3$  will cross the membrane rapidly and take up  $\text{H}^+$  ions to form intracellular  $\text{NH}_4^+$  (see

Roos & Boron (1981) for a discussion of this). The  $\text{pH}_i$  then starts to recover from its alkaline level and it is notable that  $a_{\text{Cl}^-}^i$  rises. If the external  $\text{NH}_4\text{Cl}$  is now removed, all the  $\text{NH}_4^+$  that has been formed inside the cell disappears as  $\text{NH}_3$  across the membrane, leaving behind  $\text{H}^+$  ions, hence acidifying the cell. Intracellular  $\text{pH}$  then recovers from the acid level and  $a_{\text{Cl}^-}^i$  falls. Protons are not normally in passive equilibrium across the membrane: for a passive distribution  $\text{pH}_i$  would be about 6.2. Hence this recovery of  $\text{pH}_i$  has been attributed to the active extrusion of acid or its ionic equivalent (Ellis & Thomas 1976; Deitmer & Ellis 1980; Vaughan-Jones 1982). Such a recovery is seen in many excitable cells (see Roos & Boron (1981) for a review). The addition and removal of  $\text{NH}_4\text{Cl}$  can now be performed in the presence of the disulphonic stilbene DIDS (4,4'-diisothiocyanostilbene-2,2'-disulphonic acid) to inhibit  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange. This is shown in figure 6. The recovery of  $\text{pH}_i$  from the alkaline level is severely slowed and there is now no rise of  $a_{\text{Cl}^-}^i$ . Because the recovery of  $\text{pH}_i$  is impeded, less  $\text{NH}_4^+$  will have been formed inside the cell. Consequently, the acidification produced by removing external  $\text{NH}_4^+$  is smaller than in the control experiment. The rise of  $a_{\text{Cl}^-}^i$  in the presence of  $\text{NH}_4^+$  does not occur if  $\text{CO}_2$ -free,  $\text{HCO}_3^-$ -free solutions are used to remove most of the external and internal  $\text{HCO}_3^-$  (unpublished observations). These experiments therefore provide strong evidence that the recovery of  $\text{pH}_i$  from the alkaline level is partly mediated by the cell's  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange:  $\text{Cl}^-$  enters and  $\text{HCO}_3^-$  leaves, hence raising  $a_{\text{Cl}^-}^i$  and decreasing  $\text{pH}_i$ .

The evidence for the participation of  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange in the recovery of  $\text{pH}_i$  from an intracellular *acid* load is far less compelling, i.e. a  $\text{Cl}^-$  efflux in exchange for a  $\text{HCO}_3^-$  influx. This is because DIDS does not slow the recovery of  $\text{pH}_i$  from acid levels. This is perhaps unclear in figure 6, because, in the presence of DIDS,  $\text{pH}_i$  is recovering to a slightly more acid level after the removal of  $\text{NH}_4\text{Cl}$ . However, the time course of this recovery is virtually identical to that measured in the absence of DIDS. Furthermore, in other experiments complete recovery of  $\text{pH}_i$  from an acid load has often been seen in the presence of DIDS: in these cases there is again no evidence of inhibition (see, for example, Vaughan-Jones 1981). Lastly, DIDS does not inhibit the recovery of  $a_{\text{Cl}^-}^i$  that usually accompanies the recovery of  $\text{pH}_i$  (not shown). This may therefore be a passive loss of  $\text{Cl}^-$  via some other membrane pathway.

In summary, at a constant  $p_{\text{CO}_2}$ , an intracellular alkaline load switches on  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange whereas an intracellular acidosis probably does not. Ellis & Thomas (1976) have also reported a lack of effect of SITS on 'acid' extrusion from Purkinje fibres.

The participation of  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange in the recovery of  $\text{pH}_i$  from an alkaline level is of interest. Such recovery is usually attributed to the passive entry of  $\text{NH}_4^+$  perhaps through  $\text{K}^+$  channels or even on the  $\text{Na}^+$ - $\text{K}^+$  pump (see Roos & Boron 1981). Although there is evidence for  $\text{NH}_4^+$  entry into the Purkinje fibre (Vaughan-Jones, unpublished results) its effect on  $\text{pH}_i$  is often much slower than that produced by  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange.

Recovery of  $\text{pH}_i$  from alkaline levels can also be demonstrated by adding and then removing a weak acid such as acetate. This is shown in figure 7. Removing external acetate (50 mM) raises  $\text{pH}_i$  which then returns to its initial, more acid level. Once again this recovery is inhibited by DIDS. The way in which a weak acid initially alters  $\text{pH}_i$  is analogous to the situation with a weak base such as  $\text{NH}_4\text{Cl}$ . In this case, however, adding the weak acid decreases  $\text{pH}_i$  as the undissociated form of the acid crosses the membrane. Conversely, removing it increases  $\text{pH}_i$ . The reason for the recovery of  $\text{pH}_i$  in the presence of acetate (figure 7) is not yet known and may involve entry of acetate ions into the cell (Sharp & Thomas 1981). However, the recovery of  $\text{pH}_i$  after the removal of acetate is a simpler case to consider. Under these conditions any



intracellular acetate should have been lost across the membrane as undissociated acid, so that the recovery of  $\text{pH}_i$  then proceeds in the absence of acetate. The inhibitory effect of DIDS on this recovery therefore suggests that once again  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange may be switched on by the alkalosis.

This behaviour is so far unique. In other excitable cells  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange is thought to switch off or at least revert to  $\text{Cl}^-$ - $\text{Cl}^-$  exchange when  $\text{pH}_i$  is alkaline (Boron *et al.* 1978; Roos & Boron 1981, p. 351). Moreover, if anion exchange in heart does not assist acid extrusion, there must be some other mechanism that does, since  $\text{pH}_i$  recovers efficiently from an intracellular acidosis. It is possible that a  $\text{Na}^+$ - $\text{H}^+$  countertransport fulfils this function (Deitmer & Ellis 1980; Vaughan-Jones 1982), or that an exchange of  $\text{H}^+$  for  $\text{Ca}^{2+}$  at intracellular sites

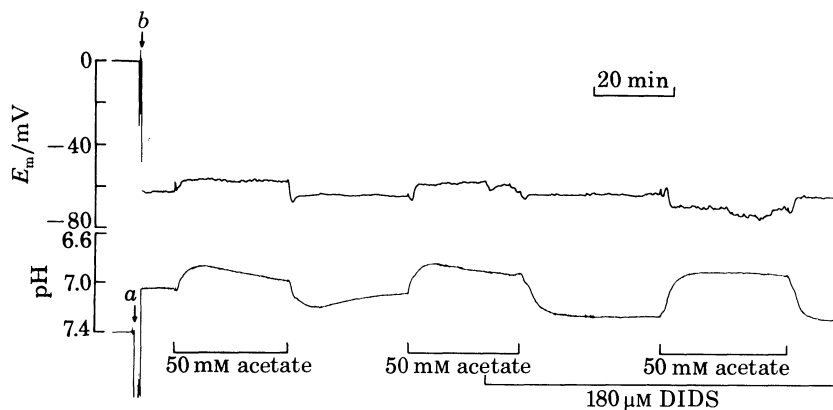


FIGURE 7. Effect on  $\text{pH}_i$  and  $E_m$  of a Purkinje fibre of adding and then removing external Na acetate (isosmotic substitution with NaCl; similar results are observed if  $[\text{Cl}^-]_o$  is kept constant during the addition and removal of acetate). At the beginning of the pen recording both microelectrodes are extracellular. The pH electrode is inserted at arrow *a* and the conventional microelectrode at arrow *b*. Because  $\text{pH}_i$  is measured as a differential signal between both electrodes, there is an upwards deflexion of the pH trace coincident with arrow *b*.  $\text{CO}_2$ - $\text{HCO}_3^-$ -buffered Tyrode.

such as mitochondria may under some circumstances assist the removal of acid from the cytoplasm (Bers & Ellis 1982; Eisner *et al.* 1982). We are left then with the question of the role of anion exchange in heart and also with the task of explaining its apparently novel properties.

#### 6. POSSIBLE MECHANISMS FOR $\text{Cl}^-$ - $\text{HCO}_3^-$ EXCHANGE

The  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange carrier has undoubtedly been characterized most fully in the red blood cell (see, for example, Cabantchik *et al.* 1978) although it is notable that, unlike in the Purkinje fibre, the  $\text{Cl}^-$  distribution here is in equilibrium with the resting membrane potential. Nevertheless, there may still be many similarities between anion exchange in the two cells. For example, if, in the Purkinje fibre, there is competition between  $\text{Cl}^-$  and  $\text{HCO}_3^-$  for attachment to carrier sites on either face of the membrane as there is in the red blood cell, then much of the behaviour of  $\text{Cl}^-$  in heart might be explained by assuming a low apparent affinity of the system for  $\text{HCO}_3^-$ . Under normal conditions of high levels of intracellular and extracellular  $\text{Cl}^-$ , the carrier would bind mainly  $\text{Cl}^-$ , promoting  $\text{Cl}^-$ - $\text{Cl}^-$  exchange. There is indeed evidence of such exchange in cardiac tissue (Polimeni & Page 1980). At a constant  $p_{\text{CO}_2}$  an intracellular alkalosis would elevate intracellular  $\text{HCO}_3^-$  levels.  $\text{HCO}_3^-$  might then compete more successfully for attachment to the carrier's internal site, promoting a  $\text{HCO}_3^-$  efflux in exchange for a  $\text{Cl}^-$

influx and hence a recovery of  $\text{pH}_i$  from an alkaline level. On the other hand, an internal acidosis (constant  $p_{\text{CO}_2}$ ) would reduce intracellular  $\text{HCO}_3^-$  levels, and  $\text{Cl}^-$ - $\text{Cl}^-$  exchange would merely continue to operate. In other words net movements of  $\text{Cl}^-$  and  $\text{HCO}_3^-$  would occur only if intracellular  $\text{HCO}_3^-$  levels rose and not if they fell, which is what is observed experimentally. The observed activation of  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange in  $\text{Cl}^-$ -free solution would occur because under these conditions only  $\text{HCO}_3^-$  would bind to the external membrane site so that a  $\text{HCO}_3^-$  influx in exchange for  $\text{Cl}^-$  would occur and this would be reversed by adding back external  $\text{Cl}^-$ .

At present such a model is speculative but it does at least provide a useful working hypothesis that can be tested experimentally. However, many factors have been ignored that eventually must be taken into account. For example, it is not known whether anion exchange in heart is a one-for-one exchange as it is in the erythrocyte. Estimates of  $\text{Cl}^-$ - $\text{HCO}_3^-$  coupling in heart suggest that this might be so but they are based upon assumptions about the intracellular  $\text{H}^+$  buffering power and also require that ions such as  $\text{H}^+$  or  $\text{OH}^-$  are not involved in  $\text{Cl}^-$  transport (Vaughan-Jones 1979*b*). The possible existence in the Purkinje fibre of passive leakage pathways for  $\text{Cl}^-$  and  $\text{HCO}_3^-$  must also be considered. As indicated earlier (figure 1*b*) these are probably very small, at least for  $\text{Cl}^-$ , but nevertheless they will produce a slow outward leak of  $\text{Cl}^-$  and  $\text{HCO}_3^-$ . Finally the energy source for  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange is not known. If, for example, ATP is involved, as has been proposed in the squid axon (Boron & de Weer 1976), then this could dramatically alter any quantitative interpretation of  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange that is based simply on thermodynamic considerations of transmembrane ion gradients.

## 6. CONCLUSION

In conclusion, we can return to a comparison of  $\text{Cl}^-$  activity regulation in skeletal and cardiac muscle. In the frog Sartorius fibre at least,  $a_{\text{Cl}^-}^i$  is low and determined mainly passively (figure 8*a*). It should be noted, however, that this does not preclude the presence of an active transport of  $\text{Cl}^-$ . Rather the effects of such a system will be largely short-circuited through the membrane simply because of its high  $\text{Cl}^-$  permeability. Indeed there is some evidence of  $\text{Cl}^-$  transport in the Sartorius fibre (Hutter & Warner 1967; Bolton & Vaughan-Jones 1977; see also Macchia 1982). In addition,  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange has been proposed to assist  $\text{pH}_i$  regulation in mouse Soleus muscle, although there have so far been no direct measurements of its effects on  $a_{\text{Cl}^-}^i$  (Aickin & Thomas 1977).

In contrast the sheep cardiac Purkinje fibre has a low membrane permeability to  $\text{Cl}^-$  so that carrier-mediated  $\text{Cl}^-$  transport can easily build up a non-passive level of  $a_{\text{Cl}^-}^i$ . The intracellular  $\text{Cl}^-$  level is high (figure 8*b*) and determined largely by a  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange mechanism. Evidence for the participation of  $\text{Na}^+$  or  $\text{K}^+$  ions has so far proved negative. Unlike that proposed for other excitable cells,  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange in heart is not specialized to compensate for an intracellular acidosis. Instead it can prevent the cell from becoming too alkaline by promoting a  $\text{HCO}_3^-$  efflux and a  $\text{Cl}^-$  influx whenever internal  $\text{HCO}_3^-$  levels rise. It is not known whether this is a specific function of anion exchange in heart. For example, it might reflect the need in cardiac muscle for a more precise control of  $\text{pH}_i$  under alkaline conditions. Alternatively the response of  $a_{\text{Cl}^-}^i$  to changes of  $\text{pH}_i$  may be a consequence of the fact that the system is specialized to establish a high  $a_{\text{Cl}^-}^i$ . The  $\text{Cl}^-$  gradient might then be used for other purposes. For example, it is still not clearly established whether  $\text{Cl}^-$  ions are important in

generating certain ionic currents in the heart (see, for example, Seyama 1979). Nevertheless irrespective of its primary function,  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange undoubtedly influences intracellular pH under a variety of conditions. Finally, it is interesting to note that a  $\text{HCO}_3^-$ -sensitive  $\text{Cl}^-$  accumulation mechanism has recently been demonstrated in smooth muscle (Aickin & Brading 1982), so that the system may not be unique to the cardiac Purkinje fibre. Instead it is tempting to speculate that it may be a basic property of many muscle tissues.

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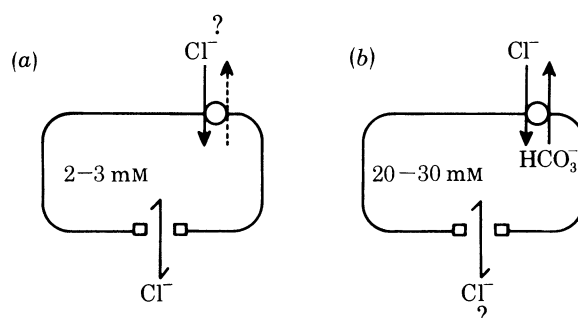


FIGURE 8. Diagrams summarizing the proposed regulation of  $\text{Cl}^-$ -level in skeletal and cardiac muscle. Both pumps and leaks have been included. (a) Frog Sartorius skeletal muscle. The intracellular  $\text{Cl}^-$ -level is low (2–3 mM). The effects of the  $\text{Cl}^-$  channel and the membrane potential dominate, producing a near-passive distribution. It is possible that  $\text{Cl}^-$  is also transported, but the details are not known (see text). (b) Sheep cardiac Purkinje fibre. Intracellular  $\text{Cl}^-$ -level is high. The effects of  $\text{Cl}^-$  transport dominate. The  $\text{Cl}^-$  permeability of the membrane is low; its exact value is uncertain. The anion exchange depicted reverses in  $\text{Cl}^-$ -free solution. It is also suggested that under resting conditions it indulges mainly in  $\text{Cl}^-$ - $\text{Cl}^-$  exchange (see text).

#### REFERENCES

- Adrian, R. H. 1956 The effect of internal and external potassium concentration on the membrane potential of frog muscle. *J. Physiol., Lond.* **133**, 631–658.
- Aickin, C. C. & Brading, A. F. 1982 The effect of  $\text{CO}_2/\text{HCO}_3^-$  and DIDS on  $\text{Cl}^-$  movements in guinea-pig vas deferens. *J. Physiol., Lond.* (In the press.)
- Aickin, C. C. & Thomas, R. C. 1977 An investigation of the ionic mechanism of intracellular pH regulation in mouse soleus muscle fibres. *J. Physiol., Lond.* **273**, 295–316.
- Bers, D. & Ellis, D. 1982 Intracellular calcium and sodium activity in sheep heart Purkinje fibres: effect of changes of external sodium and intracellular pH. *Pflügers. Arch. Eur. J. Physiol.* **393**, 171–178.
- Bolton, T. B. & Vaughan-Jones, R. D. 1977 Continuous direct measurement of intracellular chloride and pH in frog skeletal muscle. *J. Physiol., Lond.* **270**, 801–833.
- Boron, W. F. & De Weer, P. 1976 Active proton transport stimulated by  $\text{CO}_2/\text{HCO}_3^-$ , blocked by cyanide. *Nature, Lond.* **259**, 240–241.
- Boron, W. F., Russell, J. M., Brodwick, M. S., Keifer, D. W. & Roos, A. 1978 Influence of cyclic AMP on intracellular pH regulation and chloride fluxes in barnacle muscle fibres. *Nature, Lond.* **276**, 511–513.
- Boyle, P. J. & Conway, E. J. 1941 Potassium accumulation in muscle and associated changes. *J. Physiol., Lond.* **100**, 1–63.
- Cabantchik, Z. A., Knauf, P. A. & Rothstein, A. 1978 The anion transport system of the red blood cell: the role of membrane protein evaluated by the use of 'probes'. *Biochim. biophys. Acta* **515**, 239–302.
- Deitmer, J. W. & Ellis, D. 1980 Interactions between the regulation of the intracellular pH and sodium activity of sheep cardiac Purkinje fibres. *J. Physiol., Lond.* **304**, 471–488.
- Ellis, D. & Thomas, R. C. 1976 Direct measurement of the intracellular pH of mammalian cardiac muscle. *J. Physiol., Lond.* **262**, 755–771.
- Eisner, D. A., Lederer, W. J. & Vaughan-Jones, R. D. 1982 The effects of extracellular calcium on the intracellular pH and Na activity of sheep cardiac Purkinje fibres. *J. Physiol., Lond.* (In the press.)
- Hodgkin, A. L. & Horowitz, P. 1959 The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol., Lond.* **148**, 127–160.
- Hutter, O. F. & Noble, D. 1961 Anion conductance of cardiac muscle. *J. Physiol., Lond.* **157**, 335–350.

- Hutter, O. F. & Warner, A. E. 1967 The pH sensitivity of the chloride conductance of frog skeletal muscle. *J. Physiol., Lond.* **189**, 403–425.
- Ladle, R. O. & Walker, J. L. 1975 Intracellular chloride activity in frog heart. *J. Physiol., Lond.* **251**, 549–559.
- Lamb, J. F. 1961 The chloride content of rat auricle. *J. Physiol., Lond.* **157**, 415–425.
- Macchia, D. D. 1982 Chloride self exchange in toad skeletal muscle in vivo and in vitro. *Am. J. Physiol.* **11**, C207–217.
- Polimeni, P. I. & Page, E. 1980 Chloride distribution and exchange in rat ventricle. *Am. J. Physiol.* **7**, C169–176.
- Roos, A. & Boron, W. F. 1981 Intracellular pH. *Physiol. Rev.* **61**, 296–434.
- Russell, J. M. & Boron, W. F. 1976 Role of chloride transport in regulation of intracellular pH. *Nature, Lond.* **264**, 73–74.
- Seyama, I. 1979 Characteristics of the anion channel in the sino-atrial node cell of the rabbit. *J. Physiol., Lond.* **294**, 447–460.
- Sharp, A. P. & Thomas, R. C. 1981 The effects of chloride substitutes of intracellular pH in crab muscle. *J. Physiol., Lond.* **312**, 71–80.
- Spitzer, K. W. & Walker, J. L. 1980 Intracellular chloride activity in quiescent cat papillary muscle. *Am. J. Physiol.* **238**, H487–H493.
- Thomas, R. C. 1977 The role of bicarbonate, chloride and sodium ions in the regulation of intracellular pH in snail neurones. *J. Physiol., Lond.* **273**, 317–338.
- Thomas, R. C. 1978 *Ion sensitive intracellular microelectrodes. How to make them and use them.* (Academic Press Biological Techniques Series.)
- Vaughan-Jones, R. D. 1979a Non-passive chloride distribution in mammalian heart muscle: microelectrode measurement of the intracellular chloride activity. *J. Physiol., Lond.* **295**, 83–109.
- Vaughan-Jones, R. D. 1979b Regulation of chloride in quiescent sheep heart Purkinje fibres studied using intracellular chloride and pH-sensitive microelectrodes. *J. Physiol., Lond.* **295**, 111–137.
- Vaughan-Jones, R. D. 1981 Ammonia stimulates chloride–bicarbonate exchange in sheep cardiac Purkinje fibres. *J. Physiol., Lond.* **320**, 33P.
- Vaughan-Jones, R. D. 1982 Chloride-bicarbonate exchange in the sheep cardiac Purkinje fibre. In *Intracellular pH: its measurement, regulation and utilization in cellular functions* (ed. R. Nuccitelli & D. Deamer), pp. 239–252. New York: Alan Liss.
- Walker, J. L. 1971 Ion-specific liquid ion exchanger microelectrodes. *Analyt. Chem.* **43**, 89A–93A.

### Discussion

C. CLAIRE AICKIN (*University Department of Pharmacology, Oxford, U.K.*). I should like to enlarge on Dr Vaughan-Jones's comment about smooth muscle. We have shown that the intracellular  $\text{Cl}^-$  level in the guinea-pig vas deferens is five times higher than that predicted for a passive distribution (at an activity of about 42 mM or concentration of about 50 mM) when measured with  $\text{Cl}^-$ -selective microelectrodes, ion analysis or  $^{36}\text{Cl}^-$  efflux (Aickin & Brading 1982a). It appears that the  $\text{Cl}^-$  permeability is extremely low (around  $4\text{--}5 \times 10^{-9} \text{ cm s}^{-1}$ ) and that a reversible  $\text{Cl}^-$ – $\text{HCO}_3^-$  exchange system, which can also operate in a  $\text{Cl}^-$  self-exchange mode, is responsible for most transmembrane  $\text{Cl}^-$  movements. Like Dr Vaughan-Jones, we have found that  $\text{Cl}^-$  reaccumulation in  $\text{Cl}^-$ -depleted tissues is inhibited by the nominal absence of  $\text{CO}_2$  and  $\text{HCO}_3^-$  or by the presence of DIDS (Aickin & Brading 1982b). Under these conditions the normal level of intracellular  $\text{Cl}^-$  is slowly restored, the inhibition not being as dramatic as that seen in the Purkinje fibre. Furosemide also slows the reaccumulation and it enhances the inhibition seen in either of the above conditions. The loss of intracellular  $\text{Cl}^-$  on removal of extracellular  $\text{Cl}^-$  ( $\text{Cl}_o^-$ ) is considerably slowed by the nominal absence of  $\text{CO}_2$  and  $\text{HCO}_3^-$ . This effect is more pronounced than in the Purkinje fibre. Complete removal of extracellular  $\text{Na}^+$  has small effects on both the accumulation and loss of  $\text{Cl}^-$  but these could result from changes in intracellular pH. Our preliminary experiments with the pH-selective ion exchanger of Ammann *et al.* (1981) have shown that removal of  $\text{Cl}_o^-$  causes a substantial intracellular alkalization, and replacement of  $\text{Cl}_o^-$  causes a rapid acidification, qualitatively similar to results in Purkinje fibres. Further experiments with pH-selective microelectrodes may reveal

whether  $\text{Na}^+$  plays a primary role in  $\text{Cl}^-$  transport or whether it is involved in compensation for intracellular acidosis.

*References*

Aickin, C. C. & Brading, A. B. 1982a *J. Physiol., Lond.* **326**, 139–154.

Aickin, C. C. & Brading, A. B. 1982b *J. Physiol., Lond.* **327**, 74–75P.

Ammann, D., Lanter, F., Steiner, R. A., Schulthess, P., Shijo, Y. & Simon, W. 1981 *Analyt. Chem.* **53**, 2267–2269.