basic requirement should already be taken into account in instructions for game damage inventories.
The present study tries to put assessment criteria for game browsing on an objective basis and to adapt them to the specific growth conditions of high altitude forests. Its prime purpose was to find a suitable method of investigation, and it only gives a first indication of the silviculturally permissible browsing limit for sycamore maple. Further repetitions and different site conditions are needed to complement the above analyses.

Acknowledgment: The present paper is part of a comprehensive research project on the silviculturally acceptable browse impact in mountain forests. We are indebted to the Committee of the Swiss Fund for the Promotion of Forest and Wood Research for financial assistance.

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Cardiac cellular electrophysiology: past and present

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Summary. The time-course of the cardiac action potential can be accounted for in terms of ionic currents crossing the cell membranes. Depolarizing current is carried by Na^+ or Ca^{2+} entering the cells, repolarizing current by K^+ leaving the cells. Membrane permeability for the passive movement of these ions is thought to be voltage-dependent as well as time-dependent. Net transfer of charge may also result from active transport, 2 Na^+ out against 1 K^+ in; or coupled exchange, 3 or 4 Na^+ in against 1 Ca^{2+} out. This review follows the path by which present-day knowledge has been reached. It also gives a few examples to illustrate that electrophysiology has provided concepts useful to clinical cardiology.
Key words. Cardiac potentials; membrane currents, heart; cell coupling, heart; electrophysiology, heart; ion flux, heart; active transport; pacemaker.

The present review is addressed to the non-specialist biologist. Accordingly, an effort has been made to simplify, rather than to point out difficulties and uncertainties. It is hoped that the historical approach used will enable the reader to distinguish between experimental facts and interpretations. Facts should remain reproducible, whereas interpretations have changed in the past and will change in the future.

Early monophasic recordings

The construction of the Lippmann capillary electrometer[86] as a means of recording voltage fluctuations with a reasonably high degree of fidelity opened the way for studying the so-called 'injury action potential' (Burdon-Sanderson and Page, 1883, fig. 1). Two electrodes were placed on the exposed surface of a frog's heart. By injuring the tissue underneath one of these electrodes a record was obtained showing a rapid upstroke (a negative swing at the undamaged site), a long-lasting plateau, and a relatively well-defined downstroke, the whole deflection lasting for 2 s.

Cardiac muscle when damaged has a remarkable tendency to 'heal over' i.e. to set up an electrical barrier between undamaged and damaged tissue[34]. It is the general experience in student practicals that a monophasic record obtained by injury near one of the electrodes returns to a biphasic record within a few minutes. A major step towards stable monophasic recording was made in the 1930s by Schütz and his colleagues[123] who

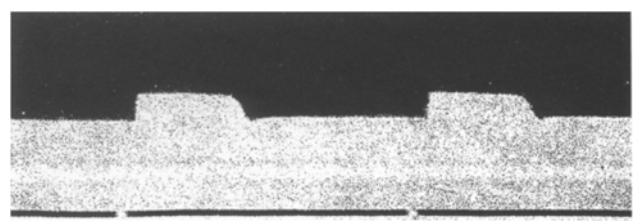

Figure 1. Voltage fluctuations of a frog's heart as recorded by a capillary electrometer. One electrode is placed at the base of the ventricle. The other electrode is placed near the apex, where the tissue is injured by a hot wire. Breaks in the black line are 5 s apart and mark direct ventricular stimulation[13].

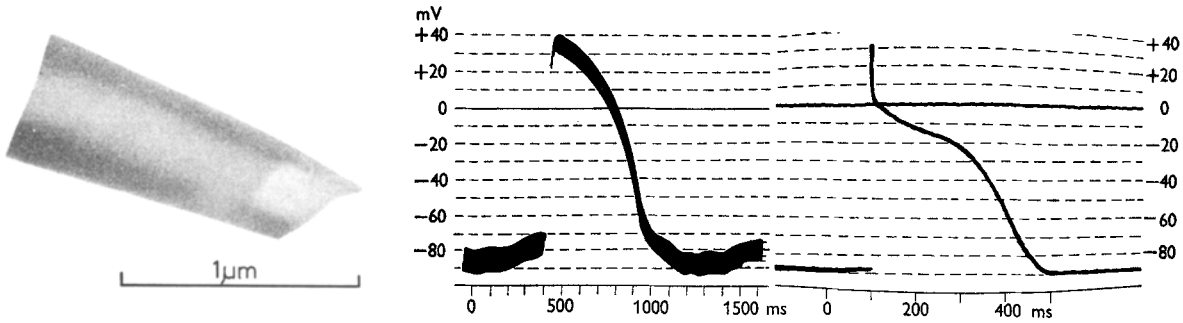

Figure 2. Left: electron micrograph of the tip of a glass micropipette used for intracellular recording[96]. Middle: intracellular record from a frog's ventricle[149]. Right: record from a 'false tendon' of a dog's ventricle[20].

combined tissue compression by a knot with suction. Extensive reviews of various effects on the 'injury action potential', mainly from in situ dog ventricles[122, 123] contain much material that apparently had to be re-discovered by means of intracellular electrodes.

Intracellular recording

In the summer of 1949 the time was ripe to introduce the tip of a capillary microelectrode into cardiac cells (fig. 2). For Coraboeuf, Draper and myself the saying held true that it is important to be in the right place at the right time. The know-how for this technique, inaugurated in Chicago for skeletal muscle[85], was handed on to Salt Lake City by J. W. Woodbury and to Cambridge, England by A. L. Hodgkin (see fig. 2).

From the beginning, we looked for a mammalian preparation which would survive in a tissue bath without the necessity for vascular perfusion[20, 30]. Thin bundles from the free-running part of the dog's ventricular conductive system (false tendons) proved to be an excellent preparation, surviving for many hours. Preparations from sheep, goat or calf, which in general can be obtained at a slaughterhouse, contain so-called Purkinje-fibers[111], which are 100–300 µm in diameter and practically do not contract, and are therefore well-suited for long-lasting impalements.

Representative preparations for auricular and ventricxular muscle are thin bundles (trabeculae) running freely through the cavities of frogs or mammals, as well as the papillary muscles of kittens, rabbits, guinea pigs or ferrets. An attempt to perfuse the vascular bed is up against considerable difficulties but is the only way to keep the deeper layers of tissue sufficiently oxygenated[72].

The excitable membrane and the flow of ions

For the early work in Cambridge my daily contact with Hodgkin and Huxley was of the utmost importance. The 'sodium hypothesis' of the action potential had first been mentioned publicly in 1947 by Hodgkin and Katz[57]. The first voltage clamp results with squid giant axons were obtained by G. Marmont[88], a pupil of K. S. Cole on the American side of the Atlantic. The interpretation, however, of membrane current changing as a function of imposed voltage and time is the achievement of the Cambridge group. During the latter part of 1949 a whole series of experiments was done at the Marine Biological Laboratory, Plymouth, which led to the papers that won the Nobel prize[56].

In retrospect it seems odd that while Bernstein in 1912 had expressed very clearly that changes of membrane voltage (his negative swing during activity) must depend on the movement of ionic charge through the then hypothetical surface membrane[6], nobody thus far had asked the question, what species of ions carry charge in which phase of the action potential. From a knowledge of the intra- and extracellular concentrations for Na, K and Cl, the Nernst equilibrium potentials could be calculated. Since during a nerve action potential the membrane voltage is brought from near the K equilibrium potential to near the Na equilibrium potential, it seemed logical for Hodgkin and Katz[57] to speculate that an increase of sodium permeability (P_{Na}) is the reason for the depolarization. This was confirmed by comparing the anticipated effects of lowering extracellular Na on the upstroke velocity and on the amplitude of the action potential, and finding satisfactory agreement with predictions (fig. 3). From earlier work it had been known[54] that crab nerve fibers lose K in the course of repetitive firing. However, the conclusion that there is a delayed *increase* of K permeability assisting the rapid *re*polarization in nerve

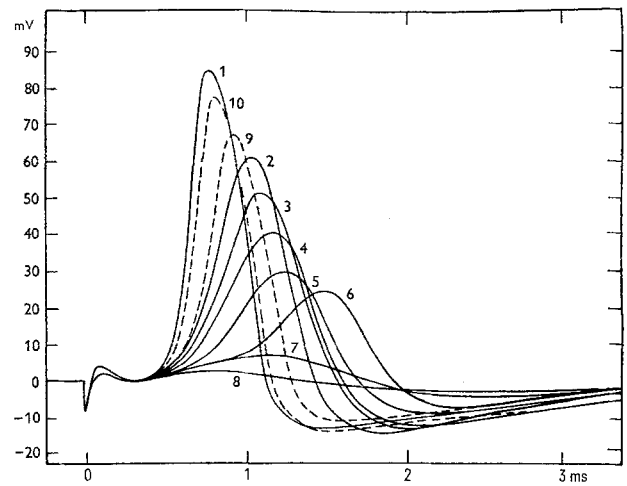

Figure 3. Action potentials of a squid giant axon. The bathing solution is changed from sea water to an increasingly Na-deficient solution (isosmotic sucrose). External sodium concentration decreases as a function of time from record 2 to record 8. Record 9 is taken 30 s after reapplication of sea water, record 10 at 90 s. Upward deflections signify depolarization from the resting potential[57].

could not be reached until the method of voltage-clamping had been developed.

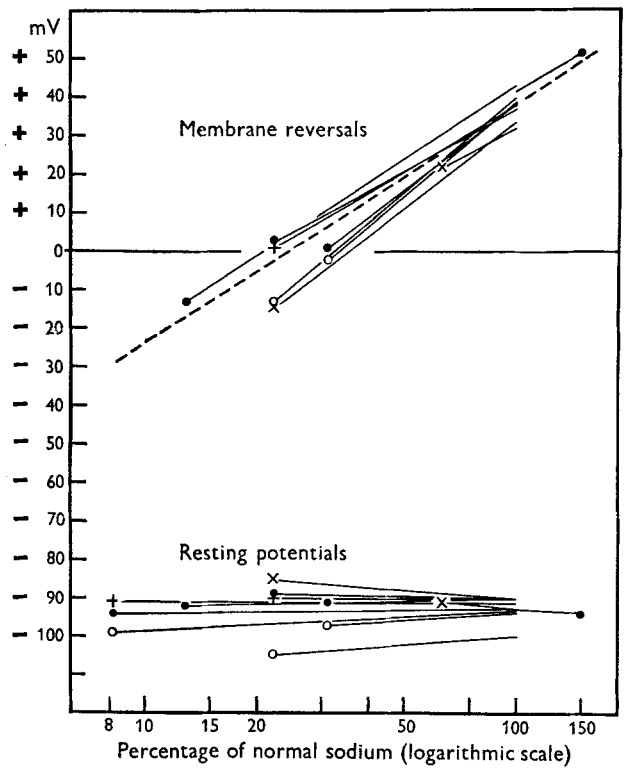

Figure 4. Resting potentials and membrane reversals as a function of extracellular Na concentration. False tendons of dogs (×, +) and kids (○, ●). Sodium concentrations lower than 100% are obtained by mixing Tyrode's solution with isosmotic sucrose; 150% refers to a solution made hypertonic by addition of NaCl. The broken line indicates the slope expected if the membrane at the peak of the action potential were exclusively permeable to Na ions and the internal Na concentration constant: 61.5 mV per decade[30]. These assumptions are not entirely fulfilled and therefore the good agreement is fortuitous[33].

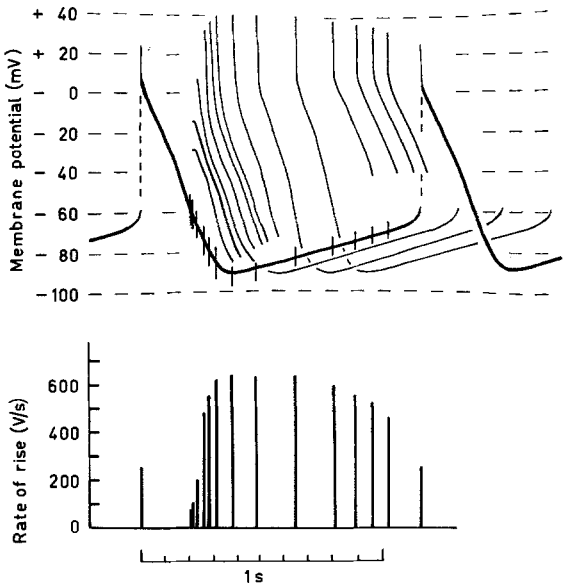

Figure 5. Upper graph: Superimposed tracings of action potentials recorded from a pacemaker region of a sheep's Purkinje fibre. Extra-responses obtained by stimulating at various times. Lower graph: Rates of rise of corresponding extra-responses[137].

Application of the ionic theory to heart: early findings

Here we start with experiments intended simply to extend to heart what was known for nerve. To show that the upstroke of the cardiac action potential depends on an increased Na permeability needed only a few experiments. As with nerve, upstroke velocity was close to being proportional to the extracellular Na concentration; the height of the overshoot (mV) was in satisfactory agreement with expectations assuming a predominantly Na-permeable membrane[30] (fig. 4). Also, it had been shown[55] that the peak Na influx in nerve critically depends on the level of the membrane potential prior to activation. This property of excitable tissue was readily verified for heart[137]. Displacing the membrane potential by current or by the application of K^+-rich solutions gave an S-shaped relationship: a maximal rate of rise (dV/dt max) with 'resting' potentials of −80 mV or more; a half-maximal rate of rise with a 'resting' potential of about −70 mV, and a very low rate of rise prior to conduction block with a resting potential near −60 mV. The same relationship could be demonstrated in the course of a free-running action potential (fig. 5). Upstroke velocities are low whenever the 'take-off' potential is low; this is the case either before final repolarization or when the pacemaker has depolarized below about −80 mV. There has been much controversy about the question whether dV/dt max of a propagated action potential is directly proportional to or simply 'a measure of' maximal Na inward current[127]. For various reasons a direct proportionality is unlikely to hold.

The long duration of the cardiac action potential

The explanation for the long-lasting plateau of the cardiac action potential was a problem between 1950 and 1960. There seemed to be no reason in the early 1950s to assume that different ionic mechanisms were operative in heart from those recognized in nerve. Thus, the general opinion was that Na permeability is only partially inactivated at the end of the spike of a Purkinje fiber, leaving a small amount of Na inward current in balance with a small amount of K outward current to hold a plateau between the K and the Na equilibrium potential. The idea that P_K *must* fall as a consequence of depolarization in order to explain the long-lasting overshoot in ventricular preparations was advanced in 1960, by three different groups independently[11, 18, 65]. This assumption became the basis for explaining the relatively high membrane slope resistance (fig. 6) during the plateau of both ventricular preparations and Purkinje fibers[135]. As to the nature of this potential-dependent fall of permeability (anomalous rectification) it seems best to admit that we have no idea. The common statement according to which an increase of outward driving force on K ions is associated with a decrease of K^+ permeability is a convenient way to camouflage our ignorance. Nevertheless, the fact that depolarization decreases K efflux is well established, even at the level of tracer efflux studies[43, 73, 134].

There were many brave attempts to measure the flux of radioactively labeled ions, ^{42}K and ^{24}Na, in the course of a cardiac cycle[126, 146]. Some pictures showing ^{42}K efflux in the course of a single cycle of activity were just too nice to

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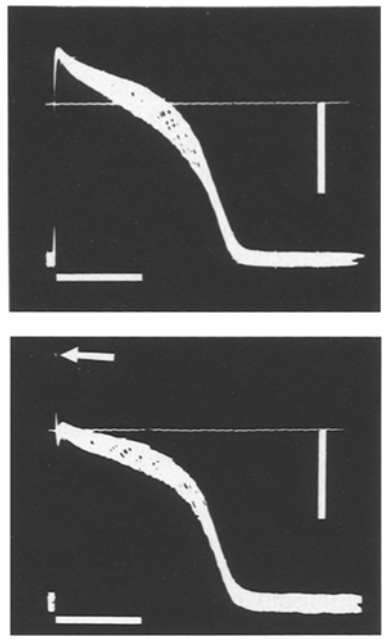

Figure 6. Changes of slope resistance in the course of an action potential showing a relatively high resistance (low conductance) during the plateau phase. Above: from sheep ventricle; below: from sheep Purkinje fibre (crest of the spike just visible, +43 mV). Some 20 action potentials are superimposed in each case. A square wave of current is forced through the internal longitudinal resistance and the surface membrane. The amplitude of the resulting change of membrane potential is a measure of the membrane slope resistance. The voltage recording microelectrode is inserted about one resting space constant from the site of maximal voltage deflection, with the intention of displaying resistance changes in a sensitive way. Calibration marks: 200 ms and 50 mV, respectively. Unpublished records by J. Délèze, Poitiers, France.

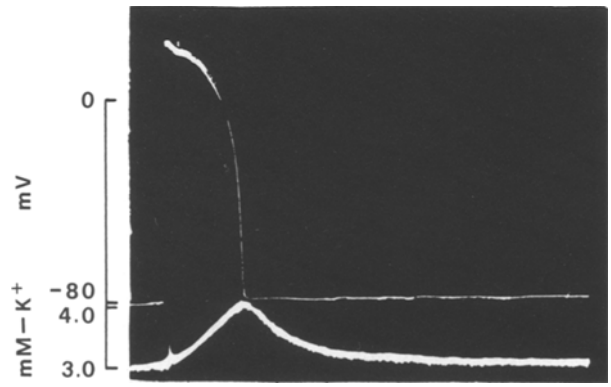

Figure 7. Action potential of a frog's ventricular strip (upper trace) and K^+ activity in the interspace (lower trace). K^+ activity increases from 3 mM to 4 mM over the duration of the action potential[74].

be true. Using the cavity of frog ventricles and rapid flow it was shown that any 'indifferent' small ion distributing in the interspace will follow a similar washout curve as ^{42}K allowing the conclusion that many if not all of such curves reflect a squeezing artifact[79]. This statement has killed for many years all attempts to measure ionic fluxes associated with single action potentials. It was not until K-selective microelectrodes became available that a small but distinct rise of $[K^+]_o$ in narrow extra-cellular clefts could be demonstrated throughout the plateau phase of the cardiac action potential[74] (fig. 7).

A word is necessary with respect to the so-called independence principle of ionic movement. This essentially means 1) that all ions cross the membrane at sites which are highly selective for a given species and 2) that fluxes in opposite directions for a given species are mutually independent. While this was a convenient assumption in 1952[56], one exception was published as early as 1955[58]: interference between K influx and K efflux. With respect to heart it had been shown[138] that a sudden rise of $[K^+]_o$ brought about by KCl injection into the coronary perfusate of a turtle could initiate a premature repolarization. This was explained by saying that a rise of interstitial $[K^+]$ leads to an increase of K^+ permeability of the excitable membrane, though the reason for this was mysterious. Influx and efflux measurements of ^{42}K undertaken by Carmeliet in 1961[16] seemed to confirm that there is a promoting effect of extracellular $[K^+]$ on ^{42}K efflux, another instance of the independence principle being violated. However, the amount of K lost during one single action potential was and still is considered insufficient to increase cleft $[K^+]$ to the extent that would be required for triggering repolarization[74, 138].

In 1962, on the occasion of the Congress of the International Union of Physiological Sciences held at Leiden, Noble presented[101] his computer reconstruction of a Purkinje fiber action potential. He postulated (fig. 8) channels specifically permeable to Na^+ which open as a result of depolarization and rapidly but incompletely shut as a function of time. A potential-dependent drop of K channel conductance would result in a relatively weak K^+ outward current almost in balance with a weak Na^+ inward current during the plateau. K^+ conductance (g_K) would rise to bring about repolarization. This being accomplished, g_K would stay relatively high at the begin-

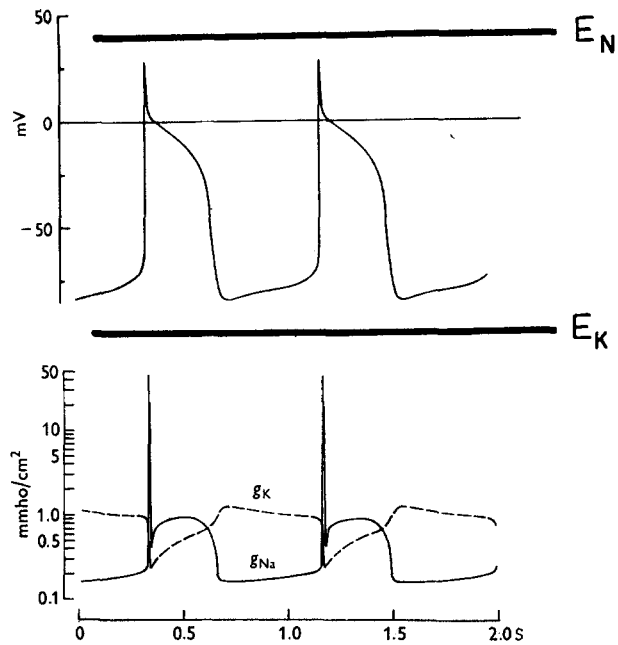

Figure 8. Upper graph: Computer reconstruction to fit the pacemaker action potentials of a Purkinje fibre[101]. Horizontal bars have been added to represent the equilibrium potentials for Na^+ and K^+, respectively. Lower graph: Time course of Na- and K conductance which would account for the potential-time course shown above.

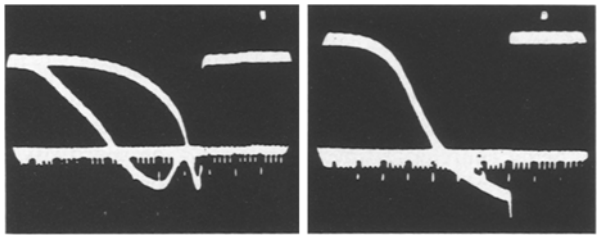

Figure 9. A normal action potential (left) of dog's ventricle becomes dissociated by procaine 0.2% into an initial part followed at times by a 'dome', the latter being associated with the generation of mechanical tension. Time marks are 100 and 20 ms; calibration voltage corresponds to 100 mV[89].

ning of diastole, to decrease slowly as a function of time, letting the membrane potential drift away from the K equilibrium potential. The time-course of the cardiac action potential was thus accounted for by a single Na channel and a single K channel, the opening of both channels depending on potential and on time.

Chloride exchange

When extracellular chloride is completely substituted for by large anions, there is no change of potential or membrane conductance at rest[16]. Some lengthening of the action potential may indicate that passive Cl^- influx normally helps to terminate the plateau. Intracellular chloride concentration is low but substantially higher than that predicted from a passive Cl^- distribution. This has been shown for sheep Purkinje fibers at rest[133], for quiescent rabbit papillary muscle[2], and for embryonic avian heart cells in tissue culture made quiescent by 30 mM KCl[107]. Astonishingly, unidirectional ^{36}Cl flux is high; higher even than ^{42}K flux[107,109]. A large portion of the transmembrane Cl flux must thus be 'electrically silent', e.g. Cl^-/Cl^- exchange[109] or Cl^-/HCO^- exchange[133].

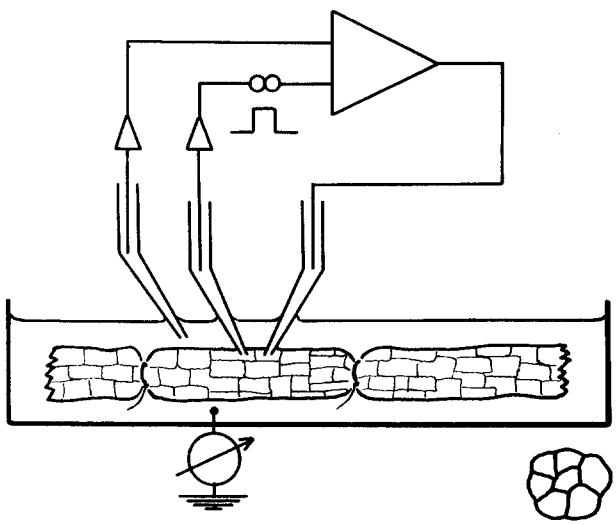

Figure 10. Diagram illustrating the voltage clamp method applied to a shortened, multicellular sheep Purkinje fibre. The amplifying system cannot distinguish between voltage changes across the surface membrane and voltage steps injected by the experimenter. The output of the system will thus impose current to produce an equal membrane potential change of opposite sign. This current is recorded as a function of time for various constant levels of de- or hyperpolarization. A cross-section through a single Purkinje fibre (lower right) shows the arrangement of electrically interconnected subunits, called Purkinje cells.

The slow inward current carried by Ca^{2+}

Towards the end of the 1950s, findings were reported by different groups which clearly did not fit the assumption of a simple Na mechanism. Action potentials could be split into the early spike and a late dome by a variety of agents[89,105] (fig. 9). Perhaps the heaviest blow was Coraboeuf and Otsuka's short communication[19] claiming that guinea pig ventricle can generate action potentials in the complete absence of sodium. This finding was confirmed by other workers but gently pushed aside for the sake of simplicity[24].

For some 15 years after the start of intracellular recording nobody had proposed that Ca ions might carry the charge for inward current during the plateau of the cardiac action potential, and, by moving in, act as a coupling agent between electrical and mechanical activity. This is so incredible because many of us must have known that Ca^{2+}, when injected by means of a micropipette, brings about a local contracture in skeletal muscle[46]. We also routinely demonstrated to the students what Ringer had described in 1883[117], namely that a frog's heart refuses to beat in a Ca-free solution. We even persuaded our students to believe that electrical activity was almost unaltered in Ca^{2+}-free solution, a finding reported in 1907 by Locke and Rosenheim[87]. In 1964 I attended a meeting of the British Physiological Society held in London. Niedergerke and Orkand demonstrated a frog's heart driven at a very low rate. The size of the action potential 'overshoot' emerging from the initial fast upstroke varied as a function of $[Ca^{2+}]_o$ as if the membrane during the crest of the action potential had the properties of a Ca electrode[99]. At the same time Reuter noticed that a rise of $[Ca^{2+}]_o$ shifted the voltage-current curve of Purkinje fibers in the direction of more inward current over a certain potential range, this component being increased in amplitude by the addition of adrenaline[113]. The situation still was not simple, for a heart could now generate action potentials in the absence of sodium as well as in the absence of calcium[60]. The conclusions drawn in 1969 by two French groups[118] seem to be well-accepted; namely, that there is a so-called 'slow inward current', with Ca^{2+} being the prevailing carrier of charge, but Na^+ competing with Ca^{2+} and taking its place if calcium is absent.

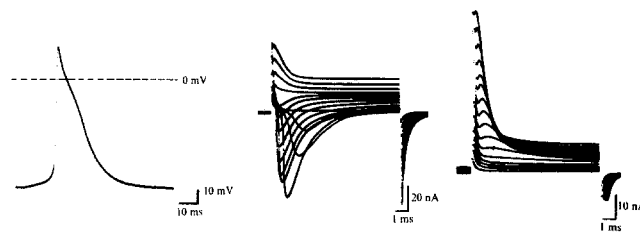

Figure 11. Clamping the rapid Na current of a single cardiac cell. Left: Action potential recorded by a suction pipette from a spontaneously active rat ventricular myocyte isolated by enzymatic treatment. Middle: Family of current-time curves recorded in 100% Na solution. The records are not corrected for capacitative or leakage currents. Upon stepping from −90 mV into the depolarizing direction a transient current appears and disappears within the first 2–6 ms. Its peak value is observed when the surface membrane is step-depolarized to the region of −20 mV (largest downward deflection, inward current). The fast deflection reverses its sign (upward, signifying outward current) for step depolarizations beyond a value assumed to be the Na^+ equilibrium potential. Right: Family of curves from the same cell in external solution free of Na. Inward transients are abolished, outward transients remain[80].

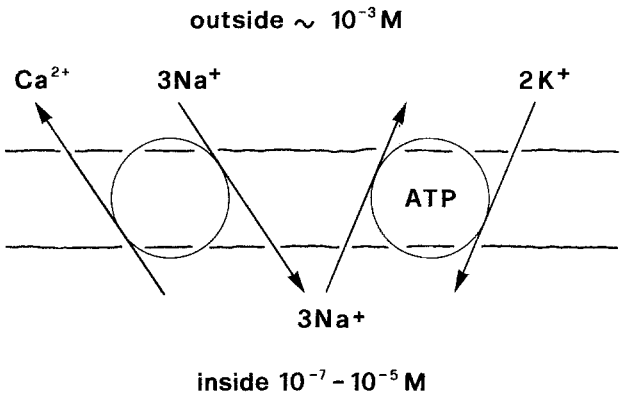

Figure 12. Active movements of ions in heart muscle. Right: The Na/K pump ejecting Na ions from the inside of the fibre. Left: The Na/Ca exchanger keeping internal Ca activity at a low level. The coupling ratio Na/Ca is assumed to be 3:1, thus producing net inward current at the level of the resting potential. Molarities refer to free calcium.

Voltage clamping

The year 1964 marks the addition of a powerful tool to record ionic currents; voltage clamping[23] (fig. 10). The experimental procedure is to impose certain potentials on the surface membrane. Having gained control over its voltage, the other variable left is time. One thus records total membrane current as a function of time. Having achieved this, one attempts to 'dissect' total current into its components, e.g. by substitution of certain ions in the bathing solution or by blocking certain 'channels' by means of pharmacological agents. For instance, the blocker of choice for the so-called 'fast sodium channel' is, as in nerve, the toxin of the Japanese puffer fish, tetrodotoxin (TTX). Successful clamps of fast inward current have now been achieved[31, 37, 78, 83]. The prototype of the unfortunately less specific blockers for the slow channels is verapamil[71]. Cardiac tissue is multicellular, and this makes it difficult to impose the same potential on the surface membrane of all cells under investigation[3, 69]. Much time and effort has been spent on identifying the minimum number of channels required to successfully compute a familiar-looking action potential, each channel being charcterized by: more or less ion specificity, a maximum conductance, a time course for opening and one for closing, both opening and closing kinetics depending on membrane voltage. For the moment it seems safe to believe in two inward current systems, 1) a fast inward current carried by Na ions and responsible for the upstroke of the action potential and 2) a slow inward current normally carried by Ca^{2+}, activating in the plateau range and inactivating as a function of time. There is a wealth of literature relating to other possible channels with more or less ion specificity; these additional channels will not be dealt with here, since the interpretation of the experimental findings may well undergo fundamental changes. While the successful computer reconstruction of an action potential does not prove anything with respect to the correctness of the underlying assumptions, the reader interested in *possible* changes of ionic conductances is referred to two theoretical models which have in the past been widely discussed: one for Purkinje fibers[90], the other for ventricular muscle[4]. To make things more complicated: the strength of a given ionic current may depend on the activities of other ions. For instance, outward current terminating the plateau may be due to increased Ca^{2+} activity within the myoplasm[1]. This would provide a satisfactory explanation for the shortening of the plateau observed under various conditions (rapid driving, hypoxia, digitalis glycosides). Other possibilities to account for a shortening of action potentials during rapid driving are K accumulation in extracellular clefts[74] (fig. 7), Ca^{2+} accumulation within a restricted compartment on the inside of the surface membrane[76, 81] or Ca^{2+} depletion within a narrow extracellular layer[51].

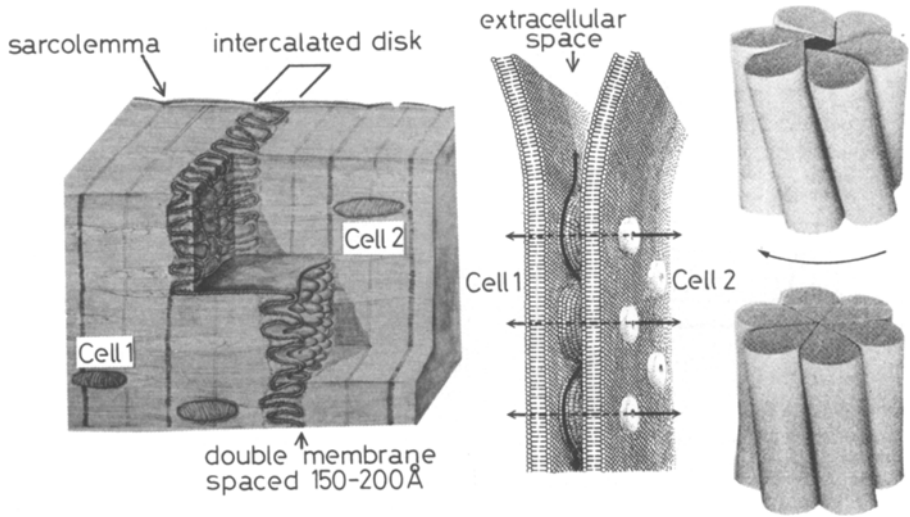

Figure 13. Intercalated disk separating two cardiac muscle cells. Left: Reconstruction of folded double membrane based on electron micrographs[108]. Middle: Nexus-type membrane thought to be the morphological counterpart of low-resistance communications between two contiguous cells[97]. Right: A possible model for a pore (black center) bordered by a twisted hexagonal structure. A clockwise rotation of the whole structure at its bottom end by 6° as indicated by the arrow would result in a transition from the open to the closed configuration[131].

Active Na/K transport

So far we have been concerned with downhill movements of ions, namely movements along the electrochemical gradient of each ion species. While the quantity of ions exchanged in the course of a single cardiac cycle is extremely small, the gradients would nevertheless run down in the absence of pump mechanisms. Cells would tend to gain Na^+ and Ca^{2+} and to lose K^+ unless metabolic energy were provided to restore the ionic gradients. The simplest assumption on which the high cellular K concentration and the low Na concentration has been explained[101] is an electroneutral pump, one Na ion out against one K ion in. This is a convenient assumption as long as one has no use for pump currents. The results of certain interventions, however, indicate that active transport is not 1:1. For instance, cooling mammalian heart below about 20 °C leads to a pronounced drop of membrane resting potential which is reversed with practically no delay when the preparation is rapidly heated to 37 °C[24].

Furthermore, depriving the extracellular solution of K ions for a few minutes seems to result in an increased Na concentration within dog Purkinje fibers, because, upon re-admission of K ions some extra charge leaves the cells, which points to the existence of electrogenic Na extrusion. The pumping rate is roughly proportional to $[Na^+]_i$ and depends on $[K^+]_o$[39].

Electrogenic Na/Ca exchange

One particularly important ionic species for heart is calcium. This ion is distributed far from electrochemical equilibrium. During the major part of the cardiac cycle the outside of the cell is electrically positive, thus tending to drive Ca^{2+} in. Yet, while the outside activity of Ca^{2+} is of the order of 10^{-5} M, the inside activity varies between 10^{-7} M at rest and 10^{-5} M during contraction. Since at least part of the Ca^{2+} necessary for contraction must have moved in from the outside, it seems unavoidable to postulate a mechanism for Ca^{2+} extrusion, operative during relaxation and diastole. A possible scheme would be an ATP-driven electrogenic Ca^{2+} pump. This is a mechanism likely to operate in red blood cells. Heart and nerve seem to get rid of their calcium in a more complicated way (fig. 12); we owe these insights to Reuter and Seitz for heart[115], to Blaustein and Hodgkin for nerve[8]. The pertinent findings are as follows: efflux of labeled Ca is markedly slowed in Na-deficient solutions, or, expressed differently, the Na ions entering the cells down their electrochemical gradient are assumed to provide the energy for

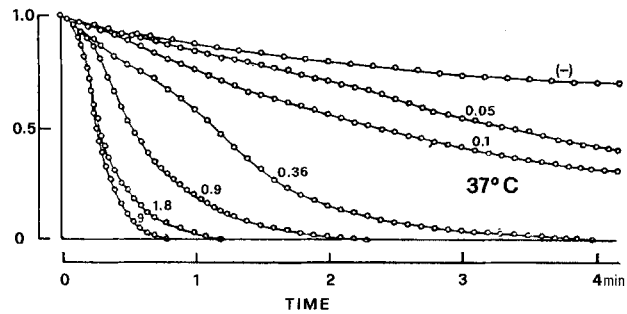

Figure 15. The time course of healing over. A guinea pig's papillary muscle is cut in solutions containing various concentrations of calcium, as indicated by the numbers (mM) near the curves. Values have been normalized, zero referring to the potential difference recorded (extracellularly) before cutting, 1.0 to the maximal potential drop observed as a result of cutting[100]. Recovery takes a time of the order of 1 min with normal calcium but is extremely slow with nominally zero calcium (–).

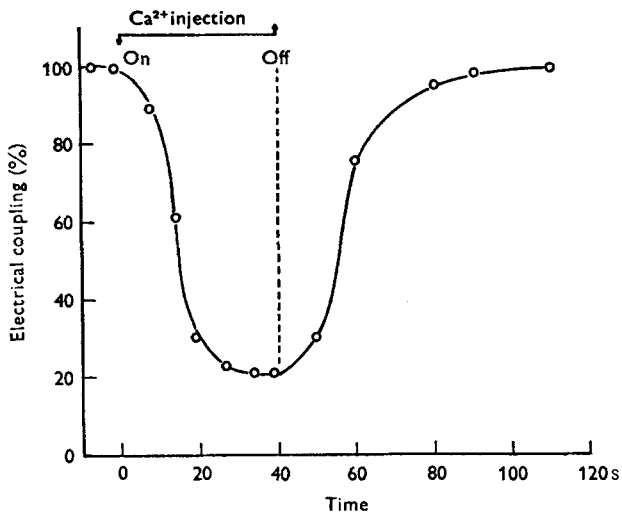

Figure 14. Electrophoretic injection of Ca^{2+} into a single cell of a dog's false tendon by means of a microelectrode. Square pulses of subthreshold direct current are made to flow into the same cell. The resulting voltage change is measured at the point of injection (V_2) and at a site of about 500 μm away from the injected cell (V_1). The coupling ratio, V_2/V_1, before Ca^{2+} injection is plotted as 100%. A decrease of coupling ratio (together with an increase of V_1, not shown) signifies an increase of cell-to-cell resistance or looser electrical coupling[26].

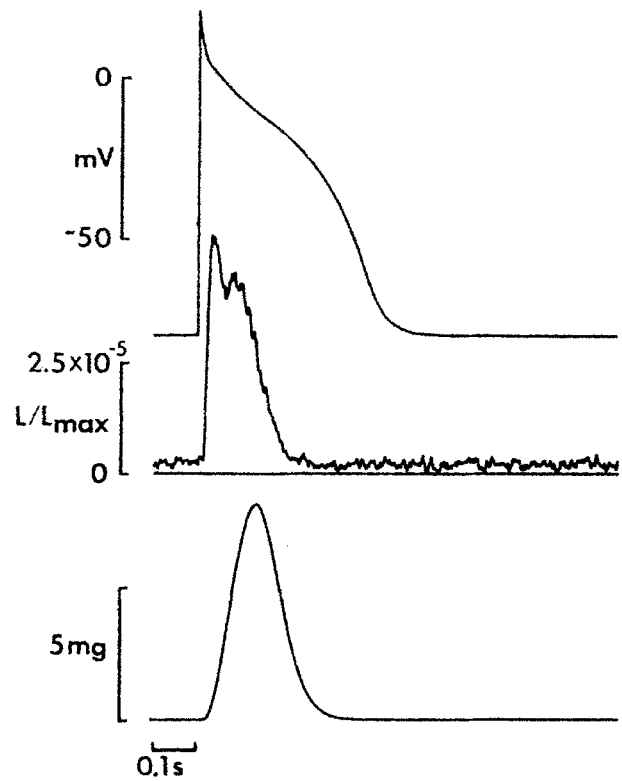

Figure 16. Simultaneous records from a dog's false tendon. Upper trace: action potential; middle trace: aequorin luminescence as a fraction of luminescence under conditions of calcium saturation (L_{max}); lower trace: force of contraction. Averaged sweeps (100) for all 3 traces[145].

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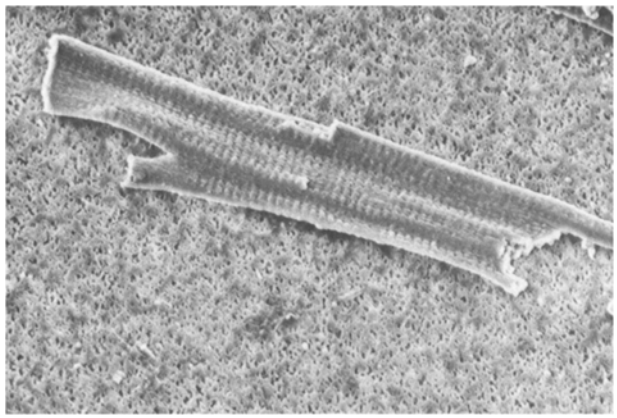

Figure 17. Single brick-like cell of a rat's ventricle obtained by enzymatic isolation[7].

extrusion of Ca^{2+} by a tightly coupled mechanism. Na^+, having entered by this 'swingdoor' would then have to be extruded by a different route. For this purpose we have the ATP driven Na^+/K^+ pump[32].

The exchange mechanism of Ca^{2+} out against Na^+ in has often been assumed to be electroneutral, one Ca^{2+} being transported against two Na^+. An attractive hypothesis is that put forward by Mullins[93]. He assumes this exchange system to be electrogenic (e.g. one Ca^{2+} against 4 Na^+). If the Na/Ca exchange system carries net charge, this current must be sensitive to membrane potential and to phasic changes of intracellular calcium in the course of the cardiac cycle[29, 103].

Plateau currents, pacemaker currents

Under this heading, as we will see, there are a great number of open questions. Membrane slope resistance during the plateau is relatively high (fig. 6) which fits the idea that there is a weak K^+ outward current almost but not quite balanced by a Ca^{2+} inward current. A time-dependent decrease of calcium inward current is part of the explanation for the final phase of repolarization[4]. A time-dependent increase of net outward current carried by K ions has been used in the first reconstruction of sheep Purkinje fiber action potentials (fig. 8). Subsequent detailed voltage clamp analyses[90, 102] have made it necessary to split the slowly increasing outward current into two components named i_{x1} and i_{x2}, respectively. The former would be activated and inactivated more rapidly and be involved in terminating the plateau. The latter would be activated and inactivated more slowly and be responsible for the shortening of the action potentials at high rates of driving. At present, the way back is reopened: to postulate a single population of channels carrying time-dependent outward current[5], or even to deny all time-dependent components of outward current[67, 68]. We seem to be back to the early days of voltage clamping[23], still finding it enormously difficult to distinguish between an increasing outward current and a decreasing inward current.

In pacemaker regions such as sinus node (fig. 17) and ventricular conducting system (figs 5 and 8) there is a slow diastolic depolarization that eventually brings the membrane potential into the threshold region. As to the current system responsible for pacemaker depolarization, the opinions also differ. A slow rise of inward current (mixed Na^+K^+) is one candidate; a slow fall of outward current switched on during the plateau (K^+) and slowly decaying in diastole is evidently the other candidate. The latter possibility seemed to be supported by overwhelming evidence until it was realized that in multicellular preparations K ions might accumulate within narrow extracellular clefts during the action potential. This would be followed by depletion of K in diastole, thus mimicking a progressive fall in membrane conductance[28]. Depending on authorship, animal species and former location of the isolated cells (nodal regions, ventricular conduction system) more or less emphasis is placed on an increasing mixed inward current[15, 95, 104] or a decreasing potassium outward current[41].

Cell-to-cell coupling: the nexus-type membrane

The question of low versus high cell-to-cell electrical resistance is now answered, in the opinion of the great majority of physiologists: low resistance[70, 142]. This can be demonstrated by the spread of electrotonic potential (fig. 19), or by the spread of tracers such as ^{42}K[139] or fluorescing dyes[27]. The morphological counterpart, namely small channels within the nexus-type membrane (fig. 13), seems to be firmly established[131]. The diameter of these (aqueous?) pores or connexons is of the order of 1.5 nm, their length of the order of 20 nm. Particles up to a

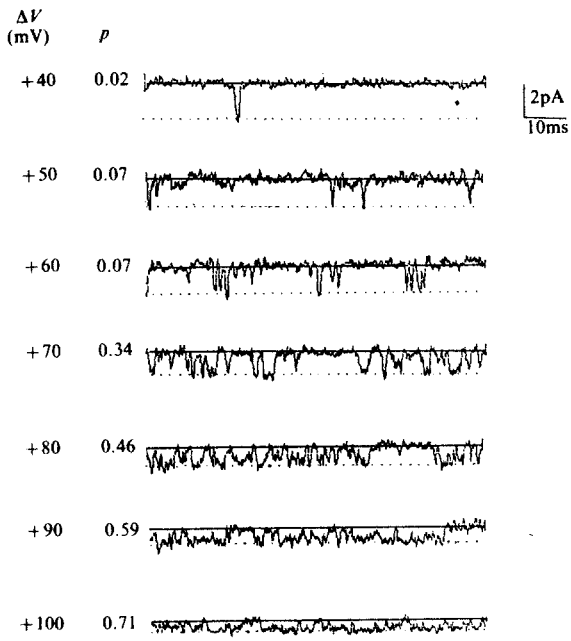

Figure 18. Tissue culture myocyte from neonatal rat heart. Cell-attached membrane patch. Inward current through a single calcium channel is lifted above the noise level by using $BaCl_2$ (96 mM) within the recording pipette. Membrane potential is step-depolarized by the indicated voltages (ΔV) from a holding potential of −70 mV. One channel opening event is seen during the recording time at −30 mV. The open probability increases with stronger depolarizations while the amplitude of inward current through the open channel decreases. The solid lines indicate the baselines after subtraction of current through the leak between the inside of the pipette and the bathing solution. The dotted lines indicate the averaged open channel currents. Besides each record is the average open probability (p). TTX added to the pipette solution; pre-incubation of the cells in 8-bromo cAMP[116].

MW of about 700 Dalton can pass with relatively little hindrance. Thus, from a metabolic and electrical point of view we have to look upon a single cardiac cell as being dependent on a large number of neighbors.

Modulation of cell coupling

The attention of investigators working in this field is now directed to finding agents that alter the resistance to current flow or tracer diffusion. We know that metabolic inhibitors uncouple to a point where propagation of the impulse becomes impossible[27, 61, 148]. A shift of pH from 7.3 to 7.0 slightly decreases electrical coupling[112]. We also know that digitalis glycosides, even in so-called therapeutic concentrations, slightly increase the intracellular longitudinal resistance[141]. On the other hand, angiotensin II, theophylline and cyclic nucleotides enhance electrical coupling[35, 48].

A decrease of conduction velocity under pathological conditions (ischemia, metabolic inhibitors) may depend on the properties of the surface membrane but may also be due to a resistance increase of the cell-to-cell pathway. A number of ions, when injected intracellularly by means of microelectrodes, increase cell-to-cell resistance: Ca^{2+}, Sr^{2+}, Na^+, H^+[26, 27]. Whether these ions all block the channels by themselves, or whether they act indirectly by setting free other ions, is at present an open question.

A phenomenon related to cellular decoupling must be postulated for 'healing over' resulting in demarcation of an intact part of the muscle from an injured region[25, 34]. When a muscle bundle is cut (fig. 15), the formation of an electrical seal requires the presence of calcium ions in the bathing solution[25]. Likewise, when surviving cardiac cells are de-coupled from their neighbors perishing in hypoxia or ischemia this process is gradual but results in a sharp boundary[61]. It seems conceivable that the gradual increase of cell calcium is responsible for allowing the metabolically more intact neighbors to shut themselves off from the damaged region.

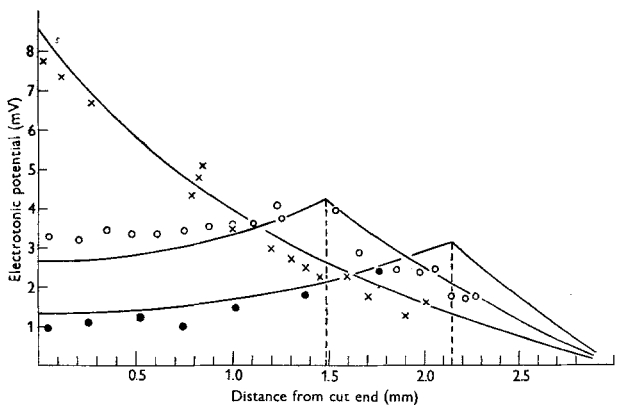

Figure 19. Spread of the electrotonic potential along a kid's single Purkinje fibre. The current leading-in microelectrode is inserted sequentially at 3 different distances from the cut (and healed-over) end. Points belonging to the 3 series of measurements have been fitted by the same space constant 2.0 mm. Considering a cell length of the order of 80 µm the result can only be explained by assuming low-resistance contacts between Purkinje cells[136].

Ion-selective intracellular electrodes; aequorin luminescence

Until about 10 years ago we measured the concentrations of ions, and for most ionic species made the tacit assumption that ionic activity – while being lower than concentration – was lower by about the same percentage inside and outside. Protons were the first ions that become accessible to measurement by so-called recessed-tip microelectrodes[129]. The construction of microelectrodes filled by ion-selective resins marked the next step, and it is today becoming common practice to measure intracellular activities of the more important ions: K^+, Na^+[33, 124, 125], H^+[112], Ca^{2+}[130, 143], and Cl^-[133]. The difference in potential recorded between one of these electrodes and a conventional intracellular KCl-filled microelectrode is a measure for ionic activity. Unfortunately, it takes many seconds or minutes to reach a steady state. At present, therefore, there is no way of using microelectrodes to follow changes of activities in the course of a single action potential.

A semi-quantitative measurement of a transient rise of $[Ca^{2+}]_i$ in connection with the cardiac action potential has been achieved by the method of aequorin luminescence[10]. Aequorin is a macromolecule isolated from a coelenterate, *Hydromedusa aequorea;* its light emission is the result of an intramolecular reaction requiring the presence of Ca^{2+} and can thus be used to detect free calcium. The method is cumbersome since several cells have to be microinjected by pressure. Also, signal averaging is necessary in most cases to lift the aequorin signal sufficiently above the noise level. The aequorin method has made it possible to state that intracellular free calcium of mammalian preparations peaks early in the cardiac cycle (fig. 16) and falls to very low levels before repolarization is complete. Strophanthidin at moderate concentrations (10^{-7} M) causes a reversible increase of free peak calcium which is paralleled by the well-known inotropic effect[145]. Caffeine often has a biphasic effect on contractile strength and this is paralleled by a similar effect on peak calcium[50].

Single channel recording with isolated cells

The year 1976 marks the introduction of yet another technique, patch-clamp recording[98]. By electrical isolation of a small portion of surface membrane (a few μm^2) current flowing through very few membrane channels, or even only one, is measured[38]. The results have fully confirmed that channels allowing electrical charge to cross the lipid membrane are highly specific for various types of charge carriers: Na^+[14], Ca^{2+}[116] K^+[106, 121] and that the strength of current per channel is a function of the driving force. They have also allowed the estimation of electrical conductance of a unit channel (expressed in picoSiemens), and the making of statements about the number of channels per μm^2 of cell membrane. The concept of a time lag in the activation of a current system[55, 56] may now be re-worded by saying e.g. that the open state of a channel becomes more probable as a function of time[116]. Inactivation of a current system may be conceived in terms of the channel's open state becoming less probable as a function of time.

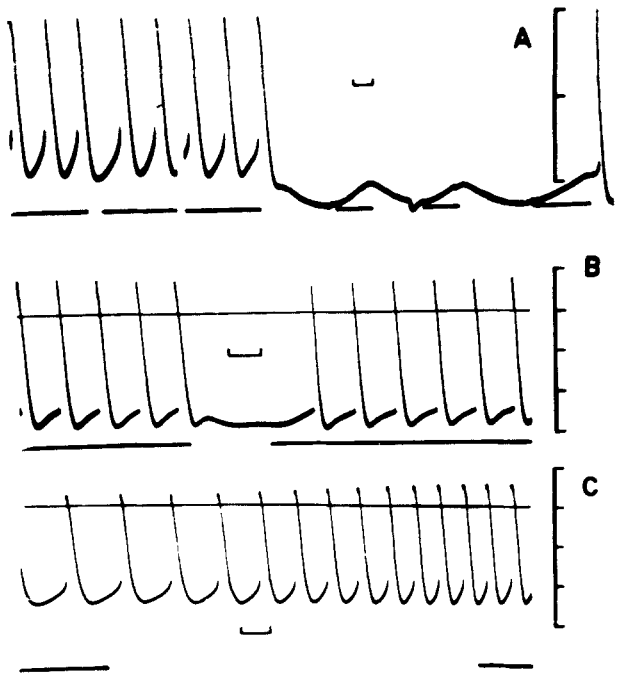

Figure 20. Intracellular action potentials from a frog's sinus node. Repetitive vagus stimulation of various durations (panels A and B, interrupted line) slows the rhythm by decreasing the slope of diastolic depolarization. Sympathetic stimulation (panel C) steepens depolarization and thereby acclerates the pacemaker. Time marks: 1 s; voltage calibaration: 20 mV[66].

The technique of patch-clamping is about to bring us insights into the mode of action of hormones and drugs. For instance, adrenaline has long been known to exert its inotropic effect by providing more slow inward current[113,114]. From the results of a patch-clamp analysis this statement may now be extended to say that adrenaline increases the open probability of single calcium channels while leaving the mean open time unaltered[116]. The possibility of gaining access to the inside of a single cell and of altering the myoplasmic composition by dialysis has added information on channel gating. While the fast Na channel seems to be opened by the electric field alone (fig. 11), the slow inward current flowing during the plateau phase depends on yet another condition; availability of metabolic energy[128].

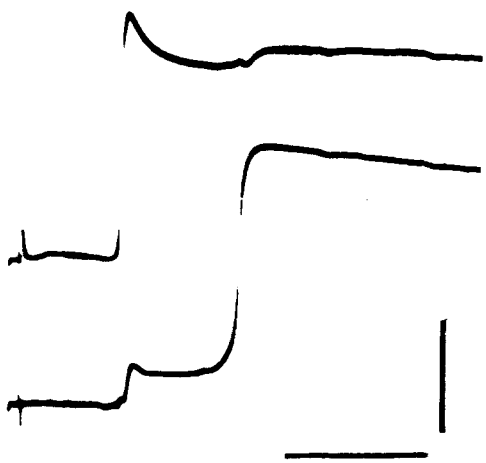

Figure 21. Interaction between a dog's terminal Purkinje fibre and a papillary muscle is associated with a transmission delay. A ventricular fibre (right) undergoes a subthreshold electrotonic depolarization when the terminal Purkinje fibre depolarizes. The Purkinje fibre (left) has a tendency to repolarize until the papillary muscle eventually responds. Calibrations: 50 mV and 10 ms[91].

Heart cells in tissue culture

Using appropriate incubation techniques and a high cell density, single cells obtained by enzymatic digestion can be induced to re-aggregate and to make low-resistance contacts. Depending on geometrical conditions, they will form cable-like strands[84], thin multicellular layers (15–40 µm) around cores of nylon threads[63,64] or spheroidal 'clusters', 50–250 µm in diameter[31,150].

Cells in tissue culture provide a preparation in which surface membranes are separated from the bathing solution by a low-resistance electrical as well as diffusional pathway. This is of importance when strong phasic membrane currents have to be resolved[31] or when the transmembrane exchange of tracers is so fast that in a thicker preparation re-uptake of tracer would diminish the measured rate of appearance in the superfusate. When tracer efflux is measured from cells in tissue culture and expressed in pmol per cm^2 of cell membrane and per second, the following figures lend themselves to a comparison: 16 pmoles $cm^{-2}s^{-1}$ for K^+[63], 30 for Cl^-[107] and 98 for Na^+[144]. In a preparation generating action potentials of 100 mV in amplitude at a rate of 2.5 s^{-1} the minimal charge displacement across a capacity of 1.5 F cm^{-2} [84] would correspond to a Na^+ inflow of 4 pmoles cm^{-2} s^{-1} and a similar K^+ outflow. The turnover of isotopes is clearly much larger. In part, this may be accounted for by a temporal overlap of inward and outward currents. In addition, however, there is good reason to postulate pumps (Na/K) and coupled ion exchange (e.g. Na/Ca[94]) which would contribute to unidirectional fluxes as measured by isotopes but would contribute to the membrane potential only in an ill-predictable way.

New concepts

The results of microelectrode recording have not provided useful tools for clinical cardiology; they are nevertheless helpful on the road to a better understanding of electrical events. A few examples will be listed below without providing full explanations.

1) Cardiac preparations have become accessible to what is called cable analysis. Injecting current into a single Purkinje fiber by means of a first microelectrode and measuring electrotonic potentials at various distances by a second microelectrode allowed a calculation of intracellular longitudinal resistance, membrane resistance and membrane capacity (fig. 19[110,136]). Small bundles of frog atrial[45] and mammalian ventricular muscle[17] as well as strands of heart cells in tissue culture[84] have been analyzed in a similar way.

2) Recording has become routinely possible from the sino-atrial pacemaker. As shown by figure 20, vagal stimulation slows while sympathetic stimulation speeds up the slow depolarization in the frog's SA node[65]. The procedure of 'mapping' the front of the spreading impulse throughout the rabbit SA node has made it possible to

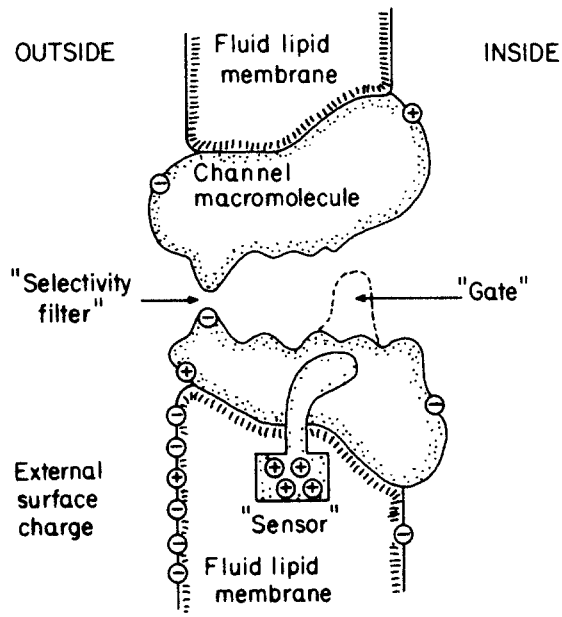

Figure 22. Fanciful diagram of an ion-specific channel. A protein macromolecule forms a pore through the lipid-bilayer membrane. The pore has a selectivity filter on the outer side and a gate near the inner side. A voltage sensor moves under the influence of the electric field and controls the opening of the gate[52]. The blocking effect of local anesthetics as well as their 'usedependent' behavior are modeled by the entrance of drug molecules through the open gate.

state that action potentials travel slowly owing to the relatively low magnitude of inward current as well as owing to the loose cell-to-cell coupling of nodal cells[9].

3) Preferential pathways with electrophysiological peculiarities have been discovered between the SA- and the AV node[53]. Their function is best appreciated by the finding that a high external K concentration may result in atrial arrest but intact sino-ventricular conduction[132].

4) A detailed description of the changing properties along the AV node was presented as early as 1958[59]: the impulse enters the AV node with a decreasing upstroke velocity, this being due to a decreasing resting potential as a function of distance. As the impulse approaches the bundle of His, there is a rise of resting potential and a gradual speeding up of conduction[105]. Groups of AV-nodal cells, when isolated from different parts of the node, behave similarly (low resting potential, low upstroke velocity) suggesting that the gradual transition from the head through the center to the tail of the node is a consequence of the degree of electrotonic coupling with atrial tissue and His bundle tissue, respectively[77].

5) An analysis of impulse conduction from terminal Purkinje fibers to ventricular tissue[91, 119] has made it clear that there is mutual electrotonic interaction in the transitional region (fig. 21). A delay of the order of 5–10 ms is plausibly accounted for by considering that a relatively small cross-sectional area of activated membrane (terminal Purkinje fibers) has to deliver local circuit current to excite a relatively large area (ventricle). The demonstration of retrograde conduction and antegrade block under conditions of high extracellular potassium (10–11 mM) removes the last doubts as to the functional continuity between the Purkinje system and ventricular muscle.

6) By showing that Purkinje fiber action potentials progressively increase in duration when travelling in a peripheral direction and shorten (electrotonically?) as they approach ventricular tissue, the concept of the 'gate' has been established[92]. This is the site of longest-lasting refractoriness, some 2–3 mm away from the junction between Purkinje fibers and ventricular muscle. The 'gate' would effectively prevent premature activity from entering the tree of the Purkinje system.

7) Some insight has been gained into the mode of action of antiarrhythmic drugs[62]. In certain myocardial tissues the process called 'recovery from inactivation' (of fast Na channels) has a considerable time lag with respect to repolarization of the membrane[44]. Drugs acting like quinidine prolong this delay of what may also be called re-availability of fast Na^+ inward current, and this effect is most prominent under conditions of rapid driving[47] ('use dependency') or slight depolarization[40]. Furthermore, the conclusion seems to be justified that antiarrhythmic drugs act from the inside of the cells rather than from the extracellular solution[42].

8) By adding KCl and thereby depressing the membrane potential into the region of −50 mV the fast Na current is inactivated. If the slow current carried by Ca ions is now enhanced by increasing extracellular calcium or applying adrenaline, it becomes possible to set up all-or-nothing action potentials propagating at speeds of the order of cm/s instead of m/s[22]. Using such solutions it was demonstrated[147] that with the additional prerequisite of a unidirectional block (fig. 23) these slowly-propagating action potentials need a very limited distance to entertain their own circus movement. This mechanism has to be looked upon as at least a possible mechanism for some of the rhythm disturbances.

9) Another class of drugs used for anti-arrhythmic treatment, the 'calcium channel blockers', limit the transmembrane inflow of Ca ions[75, 36], and possibly inhibit the prop-

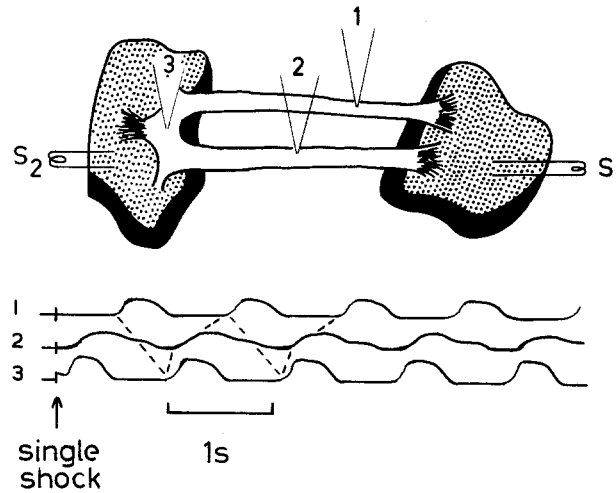

Figure 23. Circus movement of a slow response in vitro. The preparation consists of a Purkinje fibre network and two pieces of sheep ventricular muscle. The tracings below suggest that excitation spreads counterclockwise, activating in succession sites 1, 3, 2, 1, 3, 2 etc. Unidirectional block exists between sites 3 and 1. The preparation is depolarized by 15 mM KCl and its slow (plateau) inward current is increased by 5×10^{-6} adrenaline. Total pathlength 3 cm, mean conduction velocity 3 cm/s. Time mark: 1 s[147].

agation of 'slow' action potentials along partially depolarized regions of the heart[21]. Representatives of these substances are verapamil, D600, diltiazem and nitrendipine. Again, these organic molecules act more readily when applied to the inside of a cell than when added to the extracellular space[49, 82].

10) Backed by the knowledge of the ionic theory the so-called 'cardioplegic solutions' used for cardiac arrest during surgery have stepwise been improved and tested e.g. for a minimum of energy-rich phosphate consumption in the course of anoxic perfusion. While it is uniformly agreed upon that a low temperature is all-important, advantage has been taken of the following principles[12]: A low Na concentration to take the load off the Na/K pump, no calcium so as not to allow $[Ca^{2+}]_i$ to rise, increased Mg^{2+} to maintain the 'stabilizing effect' of divalent cations and a large buffer capacity (histidine/histidine HCl) to take care of the protons set free by glycolysis.

Concluding remarks

The author has closely followed developments in this field for the past 40 years[140]. It seems but natural that in his presentation he maintains a somewhat skeptical attitude. If asked to give a similar account ten years from now, the emphasis placed on the various findings would certainly be shifted and interpretations drastically revised.

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