
SPECIAL ARTICLE

The Actions of Cardiac Glycosides on Heart Muscle Cells

By ERNEST PAGE, M.D.

A RECENT editorial in this journal¹ comments on the difficulty of establishing a physiological basis for the clinical, electrocardiographic, and hemodynamic effects of cardiac glycosides on the hearts of patients with heart disease. It is now accepted that heart muscle is not a syncytium, but consists, like other organs, of discrete cells.² Accordingly, measurements of cardiac function reflect the integrated activity of multiple cellular units, and an analysis of the effects of a pharmacological agent may logically begin with a discussion of its action on the single heart muscle cell. A consideration of the effects of cardiac glycosides at the cellular level aims at a reformulation of their mechanism of action, taking into account current concepts of the ultrastructure, physiology, and enzyme chemistry of the myocardial cell membrane and contractile apparatus. Since these drugs act most strikingly on failing hearts, such a reformulation necessarily bears on the cellular basis of heart muscle failure. This article describes in nontechnical terms the effects of cardiac glycosides on the transport of ions through the cell membrane, and considers the mechanisms by which these drugs may affect the contractile function of the myocardial cell. Although these agents have important physiological effects on other tissues, this presentation is restricted to heart muscle.

As a framework for discussion, it is con-

venient to consider the actions of cardiac glycosides in terms of three cellular systems that must function sequentially before a contraction of the muscle cell can result. These systems are, in order, (1) the cell membrane, which regulates the chemical and ionic composition of the solution inside the cell. This membrane undergoes depolarization and repolarization, the cyclic changes in electrical potential that determine the propagated action potential of single heart muscle cells. The action potentials of many single cells, in turn, summate in time and space to produce the QRST complexes of the electrocardiogram of the whole heart, recordable from the body surface. (2) An intermediate system by which the electrical events at the cell surface are transmitted to the contractile elements located in the cell interior. (3) The contractile elements or myofibrils that contain the contractile proteins, whose configurational changes are the immediate basis of contraction and relaxation. The cellular locus for the primary reactions involved in the action of cardiac glycosides on failing hearts has not yet been identified. It is therefore useful to consider separately the experimental effects of these agents on each of the three cellular systems, and to evaluate to what extent these experimental observations explain the pharmacological phenomena of interest to clinical cardiac physiologists.

Effects of Cardiac Glycosides on the Cell Membrane

Active Transport of Sodium Ions

The cell membrane of heart muscle cells

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may be regarded as a porous partition, with interstices containing an aqueous salt solution. Through these interstices ions like sodium (Na) and potassium (K) are continuously leaking into and out of the cell. An ion tends to leak in a "downhill" direction, that is, in the direction determined by the forces acting on it. Two forces predominate in the movement of ions across cell membranes. The first of these, called the concentration gradient, causes ions to migrate spontaneously from regions where their concentration is high to regions where it is low. This force tends to bring about a redistribution (equilibrium) in which the concentration of each ionic species is the same everywhere. The second force, called the gradient of electrical potential, causes electrically charged particles like ions to be attracted to regions having a charge opposite to their own, and to be repelled by regions having the same electrical charge as that on the ion. As illustrated in figure 1A, the solution inside the heart muscle cell contains a high concentration of K and a low concentration of Na, while the solution bathing the outside of the cell is characterized by the reverse relationship, a high concentration of Na and a low

concentration of K. The concentration gradient therefore favors the diffusion of Na into the cell and the diffusion of K out of the cell. As explained in detail in a previous review,³ K ions diffusing out of the resting heart muscle cell in the form of KCl interact with charged molecules built into the walls of the interstices in the membrane. This interaction results in the setting up of an electrical potential difference across the membrane, the resting membrane potential. Because of the electrical potential difference, the intracellular solution is electrically negative with respect to the solution outside the cell (fig. 1A). Positively charged particles like the Na and K ions are therefore attracted to the cell interior by an electrical force. It is apparent from figure 1B that both forces, the concentration gradient and the gradient of electrical potential, will cause extracellular Na ions to migrate in the same direction, the result being a net movement of Na into the cell. This net movement is impeded by the cell membrane, which, in the resting state, is relatively impermeable to Na ions. Impermeability to Na is not, however, absolute. Over a sufficiently long period of time appreciable amounts of positively charged Na ions would

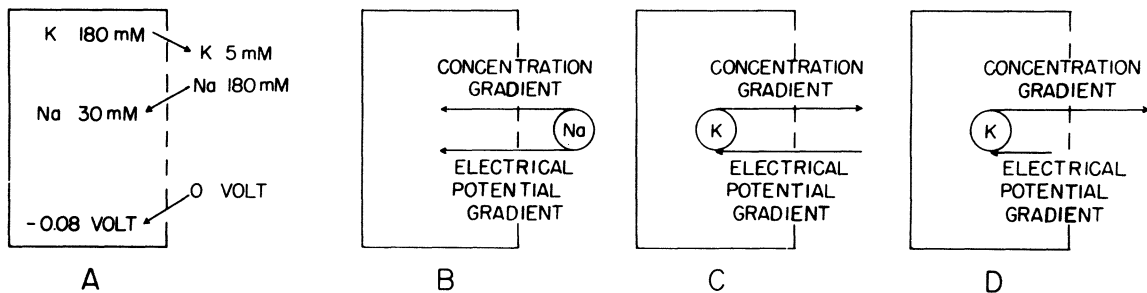


Figure 1

Forces acting on Na and K ions in resting cat heart muscle cells. A. Ion concentrations and electrical potential of the intracellular and extracellular solutions. B. Both forces act on extracellular Na ions to cause them to move into the cell, and on intracellular Na ions to retain them within the cell. C. The two forces on K ions are equal and opposite: K ion is in electrochemical equilibrium. D. Inhibition of active Na transport results in cellular uptake of Na and makes the electrical potential of the intracellular solution less negative relative to the potential of the extracellular solution. The concentration gradient now exceeds the gradient of electrical potential, and K ions diffuse out of the cell until the two opposing forces are again equal. (Intracellular concentrations are calculated with use of inulin to measure the extracellular compartment⁵⁰ and therefore differ somewhat from those in a previous review³ which were derived using mannitol.⁵¹)

leak into the cell. A net inward movement of Na would eventually equalize the concentrations of Na in the intracellular and extracellular solutions and make the electrical potential of the cellular solution less negative with respect to the outside of the cell.

A cellular accumulation of Na and an abolition of the differences in ionic concentration and electrical potential between the cytoplasmic and extracellular solutions would have profoundly deleterious effects on excitation, contraction, and cell volume. Such an accumulation does not occur because of the existence of special mechanisms located in the substance (matrix) of the cell membrane that expel Na from the cell in an "uphill" direction as rapidly as this ion diffuses inward ("downhill") through the solution-filled pores in the membrane. The expulsion of Na from the cell (fig. 1B) must take place against both the gradient of concentration and the gradient of electrical potential. Extrusion of Na is therefore an energy-consuming process and is referred to as "active transport," in contrast to passive transport, in which ions move "downhill" according to the electrochemical forces acting on them. Since experimental studies have shown the cardiac glycosides to exert marked and highly specific effects on active transport of Na, it will be of interest to consider this mechanism in detail.

Before doing so, it is necessary to examine more closely the forces concerned in the transport of K ions and the distribution of these ions resulting from these forces (fig. 1C). Like Na, the positively charged K ion is attracted to the electrically negative cell interior. Simultaneously, however, the concentration gradient favors diffusion of K out of the cell from the solution of high K concentration within the cell to that of low K concentration bathing the cell exterior. In other words, the forces due to the gradients of concentration and electrical potential, both of which act in concert to draw Na into the cell, tend to move the K ion in opposite directions. When the electrical force pulling K ions into the cell is exactly equal to the oppositely directed force set up by the gradient of

concentration, K ions are said to be in electrochemical equilibrium. In this equilibrium state there is no net diffusion of K into or out of the cell; although K ions are continuously passing through the membrane in both directions, those lost by the cell are exchanged for an equal number of K ions taken up by the cell. In spite of the unequal concentrations of K inside and outside the cell, the force set up by this inequality of concentration is counterbalanced by the electrical potential gradient, i.e., by an inequality of electrical potential. It seems probable that in this way K ion is maintained at a higher concentration inside the cell without the necessity of a second active transport mechanism for K ions.*

If Na ions are allowed to diffuse into the cell, the delicate balance between the forces acting on the K ion is disturbed (fig. 1D) by the accumulation of positively charged particles within the cell. To restore this balance, K ions must leave the cell until the opposing gradients of electrical potential and concentration are again equal. For this reason, inhibition of active transport, which prevents extrusion of Na from the cell, results in a redistribution of K ions and a fall in the cellular K concentration. The electrical potential difference across the cell membrane of the resting heart muscle cell, V_m , is determined, to a first approximation, by the logarithm of the ratio of the intracellular K concentration, $[K]_i$, to the extracellular K concentration, $[K]_o$, ($V_m \cong -61.5 \log([K]_i/[K]_o)$ at 37 C.). The decrease in intracellular K concentration secondary to interference with active trans-

* The relation between movements of K and Na discussed above is based on the simplest possible mechanism, an active transport of Na coupled by electrostatic forces to movements of K, the K ion being passively distributed. Alternative formulations include (1) separate active transport mechanisms for K and Na coupled by electrostatic forces or through a common dependence on a chemical reaction or reaction sequence; (2) utilization by both K and Na of the same membrane transport mechanism to transport K into the cell and extrude Na from the cell (usually referred to as a "K-Na exchange pump").

port of Na out of the cell is thus associated with a fall in the resting potential difference, the intracellular solution becoming less negative relative to the extracellular solution.

During the upstroke of the action potential, the electrical potential difference across the membrane reverses sign, the potential of the intracellular solution becoming transiently positive with respect to that of the extracellular solution, as described in a previous review.³ This cyclical change in the membrane potential is associated with a short-lived increase in its passive permeability (leakiness) to Na. Consequently Na transiently diffuses into the cell and must be extruded during repolarization or electrical diastole. Since this extrusion takes place against the gradients of chemical concentration and electrical potential (the potential having reverted to intracellular negativity), the reversal of the Na movements associated with spontaneous excitation and conduction involves active transport of Na. Accordingly, agents that inhibit active transport may be expected to affect repolarization.

Inhibition of Active Transport of Na by Cardiac Glycosides

The pioneering studies of Harrison,⁴⁻⁶

which antedate the concept of active ion transport, suggested that cardiac glycosides influence the K content of the heart. The nature of this effect was clarified in 1953 by Schatzmann,⁷ who demonstrated that these pharmacological agents inhibit active transport of Na and K in human red blood cells, an effect subsequently found in many cell types throughout the animal kingdom.

Figure 2 illustrates what happens to the intracellular concentrations of K, Na, and Cl and to the cell volume when papillary muscles from the right ventricles of the cat heart⁸ are exposed for 3 hours to a physiological salt solution containing the cardiac glycoside ouabain (strophanthin-G) at a concentration of 0.01 mM. It is apparent from the figure that the effect of inhibition of active transport with ouabain is a large primary increase in the intracellular Na concentration, with a large secondary decrease in the cell K concentration and a moderate increase in the cell Cl concentration. The

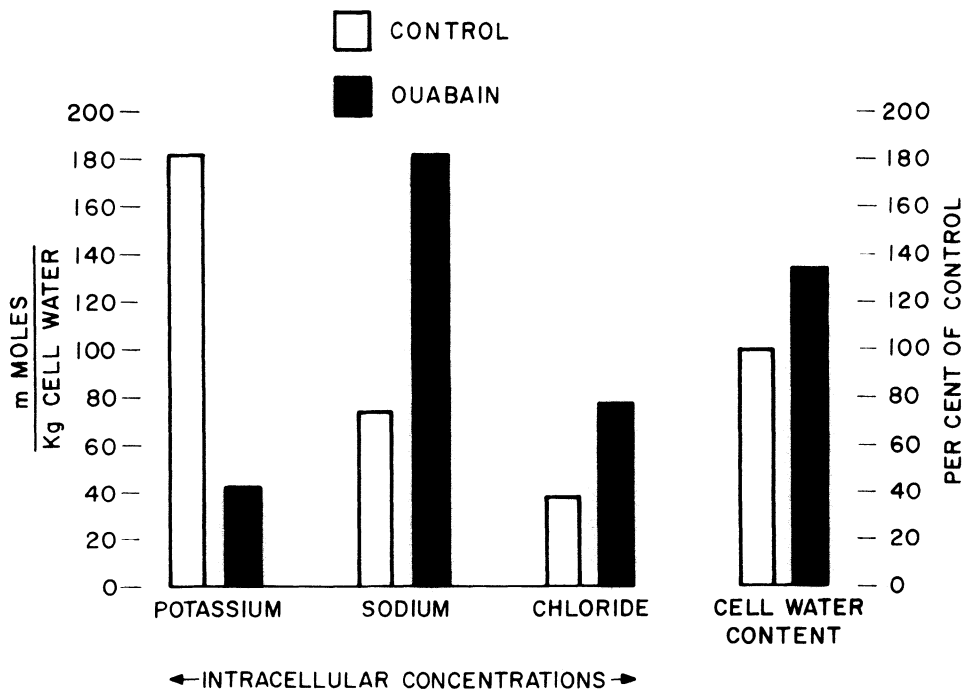


Figure 2

Changes in intracellular ion concentrations and water content of cat right ventricular papillary muscles after 3 hours in 0.01 mM ouabain. The external K concentration was 2.5 mM.

importance of active transport for the maintenance of cell volume is illustrated by the fact that these changes in intracellular ionic concentrations are associated with swelling of the cell, as reflected by an augmented cellular water content. The swollen, ouabain-poisoned cell, characterized by a low cell K concentration, low resting electrical potential difference, and high Na and Cl concentrations, is an example of what would result if ions were allowed to diffuse "downhill" toward an equilibrium state.

An important physical principle, the restriction of electroneutrality, states that for both the cellular and extracellular salt solutions the total number of positive charges must be exactly equal to the number of negative charges. This principle requires that any net entry into the cell of the negatively charged extracellular Cl ion occur in association with a positively charged ion. Since the predominant extracellular cation is Na, Cl enters the ouabain-poisoned cell as NaCl. Electroneutrality further requires that K ions leave the cell either accompanied by a permeant anion like chloride or in exchange for an extracellular cation. Under the conditions of cardiac glycoside inhibition there is no net outward movement of Cl, and the loss of cell K should reflect an exchange with extracellular Na. It therefore becomes possible to analyze the total gain in cell Na in the cardiac glycoside-poisoned cell into Na that enters as NaCl and Na that enters in exchange for K lost by the cell. An experimental comparison of the amount of Na exchanged for K with the total cellular loss of K has indeed shown that the loss of cell K occurs very nearly as a one-for-one exchange with extracellular Na.⁸

The characteristics of active transport in heart muscle are further illustrated by the dependence of the effects of cardiac glycosides on the K concentration of the solution bathing the outside of the heart muscle cell, as shown in figure 3A. The experiments described by the figure were designed to test the effect of raising the extracellular K concentration 10-fold, from 2.5 to 25 mM. At the same time the K concentration inside the cell before the addition of ouabain was kept at about 180 mM by a method previously described.³ The experiment on the left in figure 3A shows the marked cellular loss of K and uptake of Na in 0.01 mM ouabain at low

(2.5 mM) external K concentration. At this high ouabain concentration, a 10-fold increase in external K concentration to 25 mM results in a somewhat smaller ouabain effect, although the net loss of K and gain of Na are still very large. If, as in the experiment on the right side of figure 3A, the cardiac glycoside concentration is reduced by a factor of ten to 0.001 mM, the inhibition of active Na transport at low (2.5 mM) external K concentration is still marked. However, at this lower ouabain concentration, a 10-fold increase in the external K concentration to 25 mM virtually eliminates the loss of cell K and uptake of Na which are such prominent features of the ouabain effect at the lower (2.5 mM) external K concentration. These results are consistent with a competition between the K ion and ouabain for a site of combination with the active transport mechanism of the heart muscle cell membrane. According to this view, the left half of figure 3A shows the cardiac glycoside to have a high affinity for the site of attachment. Because of this high affinity, a 10-fold increase in external K concentration at a ouabain concentration of 0.01 mM is insufficient to displace the ouabain and cannot therefore prevent its inhibitory effect on active transport. By contrast, at the lower (0.001 mM) ouabain concentration (fig. 3A, right half), a 10-fold increase in external K concentration effectively saturates the critical sites with K to the exclusion of the cardiac glycoside. Figure 3B emphasizes that the significant difference at the start of this experiment, between the arrangement in which active Na transport is inhibited and the arrangement in which no inhibition occurs, is the 10-fold difference in K concentration bathing the outside of the cell membrane, the K concentration initially bathing the inside surface of the cell membrane (facing the cytoplasmic solution) being identical in both cases.

If the cardiac glycosides inhibit active transport of Na by displacing K ions from a critical site at the outward-facing surface of the cell membrane, it should be possible to

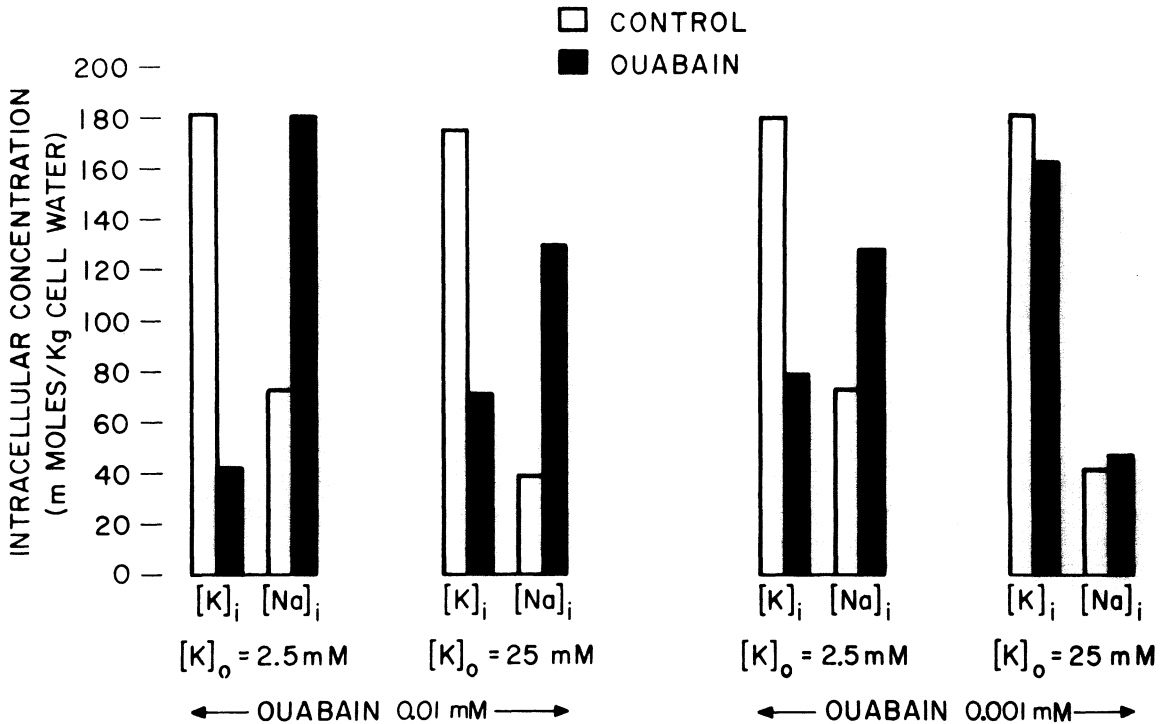


Figure 3A

Effect of a 10-fold increase in external K concentration ($[K]_o$) in 0.01 mM ouabain (left) and 0.001 mM ouabain (right).

simulate their effect by omitting K ion from the solution bathing the exterior of the cell. Such a simulation can indeed be demonstrated: Exposure to K-free solutions inhibits active transport of Na and thus brings about a net cellular uptake of Na and loss of K that resemble the net ion movements produced by ouabain.⁸ The evidence therefore suggests that K ion is necessary for active transport of Na, that it must be available at a locus situated at the external face of the cell membrane, and that this critical site is capable of combining with cardiac glycosides in competition with K ions. In the human red-blood-cell membrane, active transport of Na is similarly dependent on the K concentration of the external, but not of the intracellular solution.⁹ In addition, active transport in the erythrocyte is stimulated by intracellular, but not extracellular Na. Although the corresponding observation has not yet been made on heart muscle, it seems likely that the active trans-

port mechanism of the cardiac cell membrane may likewise function optimally only if the Na concentration bathing the internal (cytoplasmic) face of the membrane is maintained above a certain minimum value.

Phenomena like inhibition of ion transport showing specificity for a particular ion, competition between different molecular and ionic species for a site of attachment at a

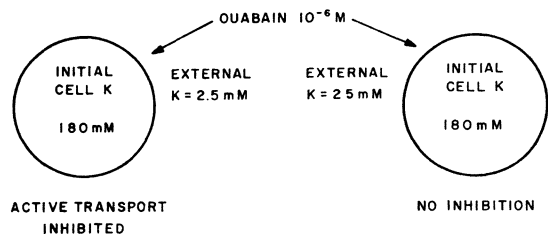


Figure 3B

Diagram illustrating that inhibition of active transport depends on the external and not on the intracellular K concentration.

surface, and differences in the ion affinity and specificity of the two spatially separate faces of the membrane correspond to the phenomena of substrate specificity, competitive inhibition, and stereospecificity in classical enzyme chemistry. It is therefore not surprising that investigators in the field of active ion transport have examined the cell membrane for enzymes involved in active transport (usually referred to as "carrier" molecules¹⁰). These attempts have had to overcome major difficulties in isolation and chemical characterization. The difficulties result from the fact that the components of the membrane responsible for active ion transport, unlike the water-soluble enzymes of classical biochemistry, are water-insoluble particles. Such particles or membrane fragments are complex combinations of protein and lipid whose enzymatic functions depend critically on the integrity of the membrane structure. In spite of these obstacles, the biochemical reactions implicated in active transport of Na are beginning to be identified. The initial observation was made by the Danish physician, J. C. Skou,^{11, 12} who discovered that a particulate preparation of crab nerve, subsequently shown to consist of membrane fragments, possesses the ability to split off (hydrolyze) the terminal, "energy-rich" phosphate bond of adenosinetriphosphate (ATP). Provided the reaction is carried out in the presence of Na and Mg ions, this enzymatic splitting of ATP (usually referred to as "ATPase activity") is greatly accelerated by K ions and specifically inhibited by the cardiac glycoside ouabain. Subsequently, similar membrane fractions from many tissues,¹³ including mammalian heart muscle¹³⁻¹⁵ have been found to possess ATPase activity. The reaction catalyzed by these preparations and the active transport mechanism of the intact heart muscle cell thus share a common dependence on the K concentration and are both inhibited by ouabain.

Since active ion transport is an energy-consuming process, it was of particular interest to find an apparent link between this process and a biochemical reaction that liberates the

energy inherent in the terminal "energy-rich" phosphate bond of ATP. This chemical bond is the form * in which a significant fraction of the energy derived from oxidation of nutritive substrates is conserved by the cell rather than being dissipated as heat. The energy so conserved can be used to perform work, whether this be work of active transport, of muscular contraction, or of chemical synthesis of cellular constituents. The enzymes which synthesize ATP* during the oxidation of fatty acids, lactate, and other substances to carbon dioxide and water are located predominantly in the membranes of numerous intracellular organelles (mitochondria). A smaller but significant fraction of ATP is synthesized during the breakdown of glucose to lactic acid by enzymes dissolved in the cytoplasmic solution. If cellular oxidation is arrested by depriving the cell of nutrients or oxygen, or if energy conservation is interfered with by uncoupling oxidation from phosphorylation, cellular depletion of energy-rich phosphate compounds will result. The active transport mechanism is deprived of its energy supply, and the myocardial cell will take up Na, lose K, and swell, in the same way as when active transport is inhibited with cardiac glycosides.⁸ It has not, however, been convincingly demonstrated that cardiac glycosides in intact muscle affect either mitochondrial and cytoplasmic oxidative reactions or the reactions by which oxidative energy is conserved as ATP (oxidative phosphorylation). Instead, current hypotheses of the mechanism of transport inhibition by ouabain center on an interference with the process by which the energy from ATP is made available to the transporting enzyme complex (carrier) in the membrane.¹⁶

The experimentally observed effects of car-

* It is by no means certain that in the intact cell the critical compound for active transport and muscular contraction is ATP, instead of some closely related intermediate compound or series of intermediate compounds. To simplify the present discussion, the term ATP is used with the understanding that other "energy-rich" intermediates may assume the functions here ascribed to ATP.

diac glycosides on ion transport in intact heart-muscle cells and on the enzymatic activity of isolated membrane fragments do not explain the nature of the therapeutic effects of these agents on the failing heart. Inhibition of active Na transport brings about changes in cell volume and cellular ionic concentrations that can hardly fail to be detrimental to cellular function. It is therefore pertinent to ask to what extent the experimental observations are relevant to the effects of cardiac glycosides as encountered in classical physiology and clinical medicine. In the opinion of the author, the answer to this question may have to await the development of a preparation of heart muscle simulating the changes that occur in the hearts of patients with failure. It remains a matter for conjecture whether, in such a preparation, conditions might be found in which cardiac glycosides stimulate active transport instead of inhibiting it. The effect of this group of drugs on movements of calcium (Ca) ion, which inhibits membrane ATPase activity,¹⁷ is of critical importance to contraction and excitation-contraction coupling (see below), and is apparently actively transported out of the heart muscle cell,^{18, 19} also needs more conclusive study than it has so far received. In assessing the relevance of the inhibition of active ion transport to other physiological effects, it is necessary to consider the possibility that tissue concentrations of cardiac glycoside that interfere with ion transport may be higher than those required for effects on excitability, impulse generation, and contractility. Moreover, the sensitivity to a given concentration may well differ for the sinoatrial node, atrioventricular node, atrial and ventricular myocardium, and Purkinje system. In addition, subtle effects of cardiac glycosides on the passive membrane permeability ("leakiness" of the membrane and related properties) remain to be excluded.

Effects of Cardiac Glycosides on Contraction and Excitation-Contraction Coupling

It can be stated at the outset that the effect of cardiac glycosides on contraction of

the heart is not understood. This uncertainty is a consequence of deficiencies in the available information about many of the steps in the sequence of cellular events leading to contraction and relaxation of the myocardial cell. Present concepts of these processes attempt to integrate relevant features of the ultrastructure, the results of physiological experiments on isolated heart muscle preparations, and the known biochemical reactions of proteins and other substances extracted from broken cells. The positive inotropic (contraction-promoting) effect of cardiac glycosides, whatever their site of action, must be mediated through the existing cellular contractile apparatus. It is therefore appropriate to outline what is known of this apparatus, before considering at what points in the contractile sequence the cardiac glycosides might intervene.

Contraction of the Heart Muscle Cell

The ultimate contractile event takes place in the myofibrils, the intracellular structures that give the banded appearance (striation) to heart muscle under the microscope. A schematic representation of the myofibril (fig. 4) shows it to be made up of longitudinally repeating units called sarcomeres, the most prominent features of which are the anisotropic or A-band, the isotropic or I-band, and the Z-band. When the myofibril is stretched, the A-band contains predominantly the thick filaments of the contractile protein, myosin, while the I-band is composed mainly of thin filaments of the complementary contractile protein, actin, anchored in a complex way²⁰ to the Z-band. The currently most widely accepted model of myofibrillar contraction is one in which the actin filaments of the I-band slide between the myosin filaments of the A-band;²¹ in this model certain points of contact between the two filaments in the region where they overlap are believed to be the sites of the fundamental chemical reaction of muscular contraction. In this reaction actin and myosin combine in the presence of ATP to produce the compound protein, actomyosin. The energy source for this combination

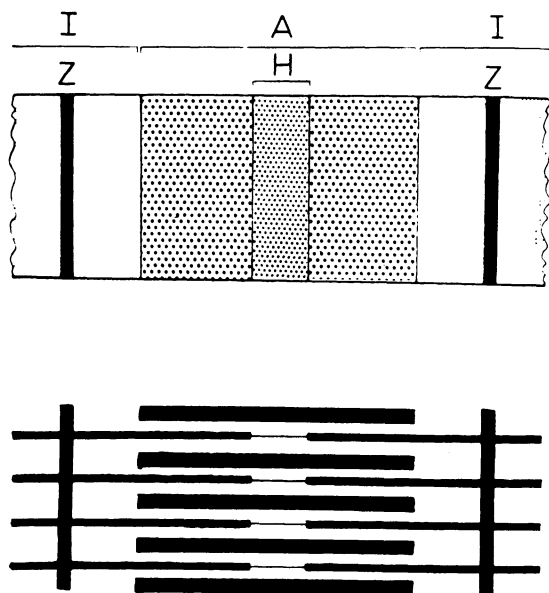


Figure 4

Diagrammatic representation of the striation bands of the myofibril (top) and the corresponding configuration of the sliding filament model (bottom). During contraction and relaxation the thin filaments, composed of the protein actin, slide between the thick filaments, composed of the protein myosin. ATP is split during an energy-consuming reaction between these two proteins, at certain points in the region of overlap. Reproduced, by permission, from Huxley and Hanson.⁵²

is the "energy-rich" terminal phosphate bond of ATP, which is split in the process. Relaxation, which likewise requires the presence of ATP, corresponds to the dissociation of the actomyosin complex into its component actin and myosin molecules. These reactions depend critically on the prevailing concentrations of Ca and Mg ions.

The interval between the initial electrical events at the cell membrane (depolarization) and the subsequent shortening of the myofibrils located in the interior of the cell is very brief. Calculations by A. V. Hill^{22, 23} based on stained sections of skeletal muscle viewed with the light microscope, suggest that myofibrillar shortening follows membrane excitation with a very short delay (latency), and that both processes are complete long before any molecule or ion could traverse the distance between membrane and myo-

fibril by ordinary diffusion. Accordingly, Hill proposed a search for an intermediate structure involved in excitation-contraction coupling. This intermediate structure may have been found in the transverse tubular system, first shown electron microscopically in heart muscle by Lindner²⁴ and later described in more detail by Simpson and Oertel²⁵ and Nelson and Benson.²⁶ Appearing in sections as a series of infoldings of the cell membrane opposite the Z-band of the myofibrillar striation pattern (fig. 5), this system of transverse tubules presumably allows the extracellular solution to penetrate deep into the cell interior to the vicinity of the myofibrils. Evidence for such penetration may also be obtained by recently developed biophysical methods,²⁷ which permit partitioning of the extracellular space of heart muscle. It is important to note, however, that the extracellular solution is separated from the cytoplasm at all points by a thick-walled membrane. A. F. Huxley assumed that electrical conduction along the membrane of such a transverse tubule proceeds as in a leaky cable. On the basis of this assumption, he was able to calculate that such a system could conduct the electrical impulse from the cell surface to the myofibril rapidly enough to account for the short interval between excitation and myofibrillar contraction observed in frog skeletal muscle. Huxley and Taylor,²⁸ using a microtechnic for applying depolarizing potassium solutions to sharply localized areas of the cell membrane, showed that contraction of the underlying myofibril resulted only when the depolarizing solution was deposited at certain sensitive points on the cell surface, corresponding to the surface terminations of the transverse tubular system. Although similar crucial experiments have not yet proved feasible in heart muscle, it seems likely that the surface openings of the cell membrane invaginations shown in figure 5 would correspond to Huxley and Taylor's potassium-sensitive loci.

Neither the discovery of a membrane structure implicated in excitation-contraction coupling nor the theoretical feasibility of a

cable-like transmission of the electrical impulse toward the myofibril explains the immediate process by which the contractile proteins within the myofibril are induced to undergo the characteristic configurational changes of contraction and relaxation. This process has been most intensively studied by measuring the rate and magnitude of tension development in isolated frog²⁹⁻³¹ and mammalian³² heart muscle preparations.

Such measurements have been carried out both during transient physiological contractions following electrically induced propagated depolarization, and during contractures in which tension is developed without a propagated action potential when the cell membrane is depolarized for more prolonged periods by increasing the K concentration of the bathing solution. When the cell membrane is kept in a depolarizing K solution,

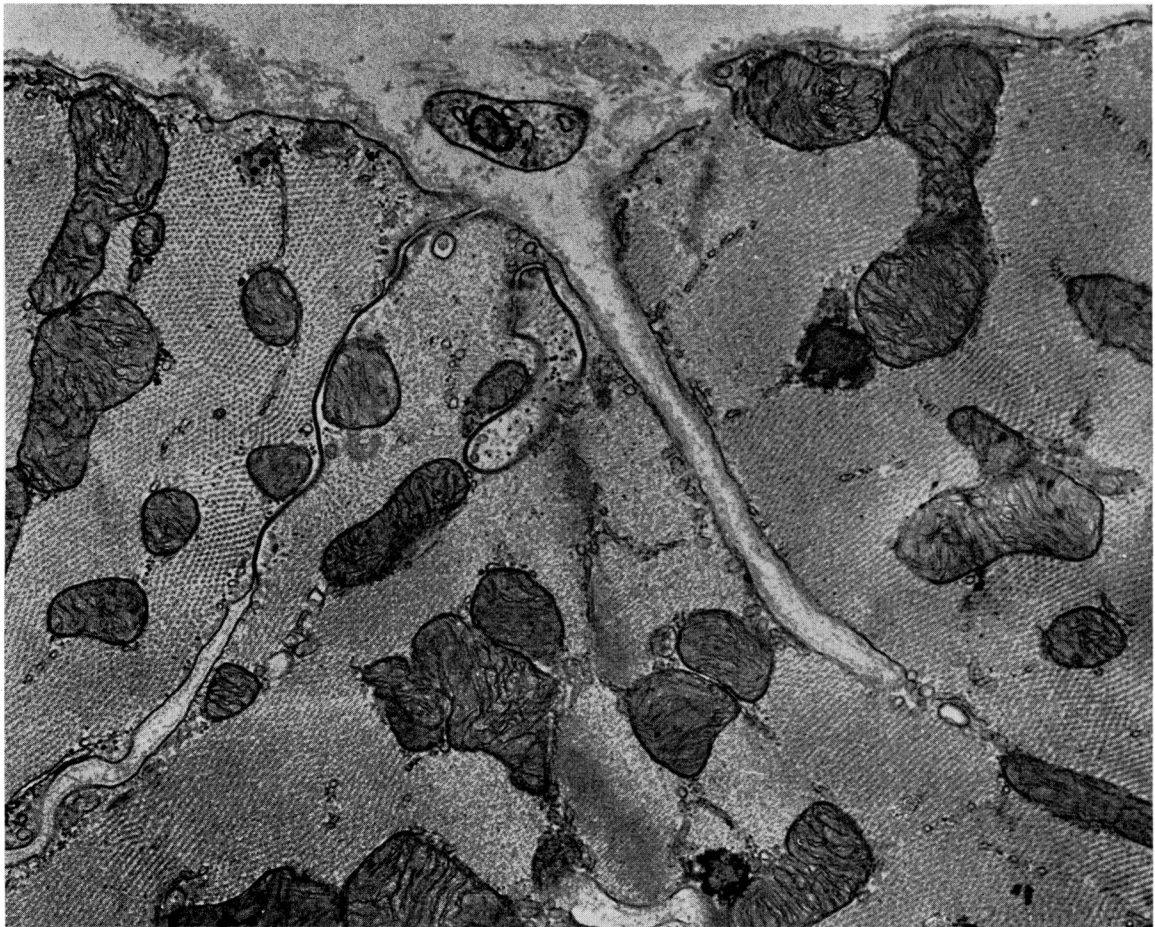


Figure 5

Electron micrograph illustrating the transverse tubular system believed to function in excitation-contraction coupling. At upper center a transverse tubule, the membranous lining of which is continuous with the cell membrane, runs obliquely downward and to the right. In vivo it is filled with a solution having the ionic composition of extracellular fluid. Contractile elements (myofibrils), cut in cross-section, may be seen in the adjacent cytoplasm, giving it a cross-hatched appearance. The large membrane-lined dark bodies are mitochondria. Transverse section of cat papillary muscle, magnification 35,000 X. This electron micrograph was prepared and made available through the courtesy of Dr. D. W. Fawcett, Harvard Medical School.

propagated action potentials, and therefore also electrical excitation, cannot take place. Any changes in developed tension during potassium-induced contracture must consequently result from effects localized nearer the contractile mechanism than the initial depolarization of the cell membrane, i.e., either at the link between excitation and contraction or at the contractile process itself.

Effects of Calcium Ion

Physiological studies on excitation-contraction coupling have drawn attention to the importance of the Ca ion, beginning with the observation by Ringer³³ that contractions of the frog heart can be reversibly stopped by omitting Ca from the bathing solution. In the same preparation, a specific effect on contraction was suggested by Mines,³⁴ who found that the electrical events at the cell membrane (action potential) persist in solutions in which the Ca concentration is sufficiently low to abolish contractions altogether. Wilbrandt and Koller³⁵ found that tension, developed by beating frog hearts, was determined by the ratio of the external Ca concentration to the square of the external Na concentration ($[Ca]/[Na]^2$), i.e., that tension could be increased either by raising the Ca concentration or by lowering the Na concentration of the medium. Lüttgau and Niedgerke,³¹ studying both contractures and contractions of frog ventricle strips, have demonstrated that the tension developed during the twitch or contracture depends, at a given magnitude of membrane depolarization, on the $[Ca]/[Na]^2$ ratio. The dependence of tension on the $[Ca]/[Na]^2$ ratio can be shown under conditions that do not simultaneously alter the resting or action potential. Niedgerke observed a significant increase in the rate of transfer of Ca⁴⁵ across the resting cell membrane when the heart was induced to develop tension either by electrical stimulation¹⁹ or by application of KCl solutions.¹⁸ Enhancement of contractile tension when the heart is stimulated to contract at an increased rate (the "staircase effect")^{29, 32, 36} or after a prolonged pause (post-extrasystolic potentiation)

are also Ca-sensitive phenomena that may be implicated in the process linking excitation to contraction.

The sensitivity to Ca of the myofibrillar reactions between the contractile proteins and ATP, corresponding to contraction and relaxation of the myofibril, has already been mentioned. The Ca concentration in the immediate environment of the myofibril appears to be subject to regulation by an intricate, membrane-limited system of tubules and vesicles, the sarcoplasmic reticulum,³⁷ which has extensive ramifications throughout the heart muscle cell. It can be shown that membrane fragments derived from the sarcoplasmic reticulum have a striking capacity to take up Ca ions.^{38, 39} This property may serve to lower the Ca concentration in the immediate environment of the myofibril, thereby allowing the contracted myofibril to undergo relaxation.³⁹ Like the reactions between the contractile proteins, the uptake of Ca by the reticulum depends on the splitting of the "energy-rich" phosphate bond of ATP.

Although both Ca and cardiac glycosides enhance cardiac contractility, physiologists have long appreciated that the mechanisms of action of these two agents are different (see review by Hajdu and Leonard).⁴⁰ Attempts to reconcile this difference by the suggestion that cardiac glycosides lower the intracellular Ca concentration have not to date been experimentally verified. In fact, concentrations of K-strophanthoside which inhibit active transport of Na appear to interfere with the extrusion of Ca from frog heart muscle cells.⁴¹ Klaus⁴² has proposed that therapeutic, as opposed to toxic concentrations of cardiac glycosides increase intracellular ionized Ca at the expense of bound Ca, thereby producing a positive inotropic effect. This ingenious hypothesis likewise needs to be supported by more direct methods.

Direct Effects on Myofibrillar Contractile Proteins

Although multiple direct effects of cardiac glycosides on solutions of extracted contractile proteins or on myofibrils isolated in rela-

tively intact form from cells have been described,⁴⁰ there is no convincing evidence that these actions are related to the physiological effects on contractility of the failing heart. Moreover, in spite of attempts with radioactively labeled drugs to show entry of these agents into heart muscle cells,^{43, 44} the presence within the cell of an intact cardiac glycoside or of a pharmacologically active compound derived from it remains to be demonstrated. By inhibiting active transport of Na, the cardiac glycosides can alter intracellular concentrations or content of K and Na. It has consequently been proposed^{40, 45} that these drugs act by their effect on the K and Na concentration inside the myofibril. Since the reactions of the isolated myofibrillar proteins do not discriminate critically between K and Na, it is difficult to account in this way for the effect on contraction.

Effects of Certain Steroids

A potentially important aspect of cardiac glycoside action is the apparent antagonism of their effects on both contractility⁴⁵ and membrane transport⁴⁶ by certain steroid hormones. Edelman et al.⁴⁷ have recently shown that the primary action of the adrenal steroid hormone aldosterone on a peripheral tissue is mediated via the cell nucleus. In the light of these studies, the possibility of direct or indirect effects of the cardiac glycosides (which contain a steroid group) on nuclear reactions and on protein synthesis needs to be critically examined, especially in chronically failing hearts.

Other Effects of Cardiac Glycosides

A critical discussion of the cellular basis of spontaneous rhythmicity, impulse generation, and conduction in heart muscle is beyond the scope of this article. For a recent appraisal of the effects of cardiac glycosides on these cellular functions the reader is referred to the review by Trautwein.⁴⁸

Approaches for Future Research

It is apparent from the foregoing discussion that the contraction cycle of heart muscle cells involves multiple steps, beginning with

depolarization of the cell membrane and ending with relaxation of the myofibril. The site of cellular action of the cardiac glycosides and the cellular functional alterations in heart failure are logically approached by identifying the changes produced at each stage between the membrane and the myofibril. At present this approach is hindered by the as yet incomplete identification of the various steps and of the structures corresponding to these steps, by the lack of a convenient preparation for the experimental simulation of heart failure, and by the relative insensitivity of available methods for measuring membrane properties and contractility in heart muscle. The information now available emphasizes phenomena involving K, Na, Ca, and ATP largely because these substances are relatively easy to measure and not because their behavior is necessarily more interesting or fundamental than that of other, less readily accessible substances or phenomena.

In approaching the related problems of cardiac muscle cell contraction, failure, and cardiac glycoside effect, many questions remain to be investigated. It is, for example, necessary to determine how the energy inherent in the chemical bonds of cellular "energy-rich" phosphate compounds is made available for work of contraction, transport, and synthesis of cellular components (the problem of energy transduction), how the relative energy expenditure for each of these forms of work is distributed under various conditions, and whether an excessive energy expenditure for one function (e.g., contraction) may compromise the supply of "energy-rich" intermediates required for the other functions. Information is needed on the synthesis as well as on the physicochemical, electron-microscopic, and x-ray crystallographic properties of myofibrillar and other intracellular proteins in normal, hypertrophied, and failing hearts both in the presence and the absence of cardiac glycosides. Under these same conditions the experiments of Huxley and Peachey⁴⁹ on skeletal muscle cells, suggesting that contractility depends on the degree of overlap between actin and myosin

filaments (fig. 4) and is critically related to the degree of stretch, must be repeated in heart muscle. Heat production and absorption in association with contraction and relaxation, as well as the mechanical properties of heart muscle, must be studied with more quantitative methods³² than heretofore to obtain more precise definitions of the effects of hypertrophy, failure, and cardiac glycoside action. The scope of the problem calls for a concerted and systematic effort by scientists from multiple disciplines of basic biological science. Only by isolating the pertinent cellular systems and structures can the relevant chemical reactions be identified and studied by the methods of enzyme, organic, and physical chemistry, which will ultimately explain heart failure and the actions of cardiac glycosides on it.

Summary

In experimental heart muscle preparations cardiac glycosides specifically inhibit the active transport of Na ions out of heart muscle cells. By this action they bring about a net cellular accumulation of Na and, secondarily, a net cellular loss of K and uptake of Cl, cell swelling, and a fall in the electrical potential difference across the resting cell membrane. The site of inhibition appears to be the external surface of the cell membrane and to involve a displacement of K ions from a critical site at this surface. Inhibition of active transport by cardiac glycosides in intact cells has a counterpart in cell membrane fragments, in which the drugs inhibit the Na- and K-activated enzymatic splitting of adenosine-triphosphate.

The contraction-promoting effect of these drugs is not understood, principally because the sequence of events in cellular contraction and its alterations in heart failure have been only incompletely identified. Cellular contraction has been divided for descriptive purposes into three stages, including depolarization, excitation-contraction coupling, and shortening and relaxation of the myofibrils containing the contractile proteins. Neither the inhibition of active ion transport nor the ex-

perimental observations on the three stages of the contractile sequence explain the therapeutic effects of cardiac glycosides. Experimental approaches to the question of cardiac glycoside action at the cellular level and to the related problem of heart muscle cell failure are suggested.

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Marcello Malpighi and the Spiral Bundles of the Heart

Malpighi gave considerable thought to the movements of the heart. Although Harvey had described these fairly exactly, it would still have been interesting to know the position of the various muscles and bundles of fibers. If the fresh heart, tough and elastic, was pulled and stretched, it eventually tore as a result of such treatment and the muscle fibers were broken. Thus it was impossible to get an idea of the construction of the whole heart's muscle fibers.

Malpighi boiled the heart until it became quite soft. The fibers then separated at the least touch, they could be pulled apart with the fingers, as if untwisting thread. By this simple means he made an interesting discovery. Although the muscle of the heart appeared to be composed of three kinds of fibers, vertical, horizontal and oblique, these were all connected to each other, and it was their spiral arrangement which caused the error in identification.—TIBOR DOBY, M.D. *Discoverers of Blood Circulation. From Aristotle to the Times of Da Vinci and Harvey*. New York, Abelard-Schuman, 1963, p. 225.