THE CELLULAR BASIS OF CARDIAC GLYCOSIDE ACTION

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This review represents an attempt to synthesize from currently available information a picture of how the cardiac glycosides exert a positive inotropic effect on the heart muscle cell. The result is a progress report containing many uncertainties and mixed with an unavoidable minimum of authors' bias. Many aspects of the action of glycosides have not been considered, especially those of primarily clinical significance as well as effects on non-cardiac tissue. The reference list is by no means exhaustive, but should be adequate to introduce the interested reader to the various facets of the subject.

I. METABOLISM

It will be useful to begin this review with a consideration of the effects of cardiac glycosides on cellular metabolism. This subject formed the bulk of the last review on the glycosides written in this Journal in 1949 (188) and is a good starting point of orientation for the present work. In a purely formal sense, one can view what happens when a muscle is stimulated to contract and is then allowed to return to its original resting state as the operation of two cyclic processes, one being the shortening and lengthening of the contractile protein, the other being the hydrolysis and subsequent resynthesis of adenosine triphosphate (ATP).[1] The second cycle provides in some way the energy for the cyclic change in the protein, and it does not matter for the argument how the phosphate bond energy is transferred to the contractile protein. Any interference with the second cycle—either with the synthesis or hydrolysis of ATP—constitutes an interference with the supplying of energy to the contractile system [or, in Wollenberger's terminology, "energy liberation" (188)]. The amount of work obtained from the operation of the contractile protein cycle will depend on a host

[1] Whether ATP or creatine phosphate (CP) constitutes the immediate energy supply for the heart muscle is a question not settled at present (48, 49, 50, 51, 52, 127, 128). However, it seems reasonably certain that some high energy phosphate bond is the immediate source of energy for the contraction cycle.

of factors which can be summed up as the ability of the contractile protein to utilize the available energy. In other words, all the steps up to and including the transfer of energy to the contractile protein are, according to the usage employed here, part of the process called "energy liberation"; whereas what the contractile protein does with the energy transferred to it is called "energy utilization." Variations in the ability of the contractile protein to convert into useful work the energy which it draws from the ATP cycle will be reflected in the calculated mechanical efficiency of the muscle (work output/energy input).

The effect of glycosides on the failing heart, then, can be discussed by first considering whether the cause of heart failure in terms of the above frame of reference is due to impairment of energy liberation or energy utilization. In the case of the former, the defect could be in either ATP synthesis or ATP hydrolysis. If ATP synthesis were impaired, the concentration of ATP in failing heart muscle should be below normal and the therapeutic effect of the glycosides should be associated with a rise in the ATP concentration. The ATP and CP concentrations in well-oxygenated failing heart-lung preparations are not depressed (187) and do not rise on digitalization (188, p. 341). One investigator, however, found a low ATP concentration in a severely hypodynamic preparation of cat papillary muscle which returned toward normal on treatment with ouabain (66). This isolated finding has yet to be explained.[2]

If heart failure were caused by a subnormal rate of ATP hydrolysis, then the therapeutic effect of the glycosides should be associated with a parallel increase in ATP splitting (and also oxygen consumption, assuming no oxygen debt) and obtainable muscle work. Because of the parallel increase in oxygen usage and work done, the calculated efficiency of the treated muscle would not be different from that of the failing muscle. In other words, if the rate of hydrolysis of ATP were the limiting step in determining contractile activity and there were no other defects in the failing muscle, one should never see an increased efficiency following treatment with cardiac glycosides. The contrary is the case, Peters and Visscher having found that in the failing heart-lung preparation operating at constant diastolic volume the administration of cardiac glycosides caused increases of 63 to 153% in the mechanical efficiency (136). Qualitatively similar results for human heart failure have been published by Bing and coworkers (9). These experiments lead, then, to the conclusion that the defect in congestive failure is reflected in a diminished ability of the contractile protein to convert the energy which it receives into useful work. The administration of glycosides increases the capacity of the contractile system for "energy utilization," which is reflected in an increased mechanical efficiency; and any increase in oxygen

[2] Since this manuscript was submitted for publication a paper was published by Furchgott and de Gubareff on high energy phosphate compounds in isolated left atria of guinea pigs (54a). The concentrations of ATP, ADP and CP in hypodynamic atria were not significantly different from controls or from atria the contractility of which had been restored to normal with K-strophanthin. This work on isolated cardiac tissue is in agreement with the studies on failing heart-lung preparations (187, 188). Included in the paper is a discussion of the low ATP concentration found by Greiner (66) in isolated hypodynamic cat papillary muscle.

consumption associated with administration of the glycosides is secondary to an increase in mechanical work rather than to a primary action of the glycosides on the "energy liberation" cycle.

Some comment should be made about the determination of mechanical efficiency, the ratio of work output to energy input. For cardiac muscle the use of an oxygen consumption increment as a measure of the amount of energy liberated for a given work increment is probably reliable, since cardiac muscle does not incur a large oxygen debt under most circumstances (102). The conditions for measuring stroke work (the product of stroke volume and mean arterial pressure) must, however, be carefully specified in experiments for determining mechanical efficiency. It was shown, for example, by Evans (43) and confirmed many times subsequently (3, 64, 67, 100, 101, 107, 152) that for a given increment in stroke work the increment in oxygen consumption was very different depending whether stroke volume or arterial pressure was the variable increased. As emphasized recently by Katz (100), "Mechanical efficiency is *not* constant when increased work produced by increasing blood pressure is compared with that produced by increasing cardiac output. Oxygen consumption definitely increases when work is augmented by increasing blood pressure; it is slightly increased, unchanged, or even decreased when work is increased by elevating cardiac output." Therefore, in determining the effect of cardiac glycosides on mechanical efficiency an attempt must be made to keep the working conditions the same in both control and experimental situations. The experiments cited above by Peters and Visscher (136) appear to fulfill these requirements.

Certain results published in the literature might lead the casual reader to conclude that under certain experimental conditions the glycosides caused a primary increase in oxygen consumption relative to the mechanical performance of the muscle. For example, Bing and associates (9) showed that glycosides administered to normal hearts cause a decrease in left ventricular stroke work without a change in oxygen consumption and therefore a drop in calculated efficiency. However the decrease in stroke work may be due to a smaller diastolic volume and stroke volume secondary to a tone change (23) or right atrial pressure change (122) rather than to a change in the intrinsic capacity of the muscle to perform work. In fact the capacity of the normal ventricle to develop *increased* tension under the influence of cardiac glycosides has been demonstrated with a strain gauge arch attached directly to the muscle (170). Another example in which the administration of the glycosides causes an increase in oxygen consumption relative to the apparent contractile performance of the heart is provided by the work of Eismayer and Quincke (41). They reported that strophanthin causes increased oxygen consumption of the isolated frog heart without affecting twitch tension. Clearly if one is measuring tension isometrically, the calculation of efficiency is not pertinent since the muscle is prevented from doing work. However, detailed studies have shown that under such conditions the glycosides increase tension (if it is not already maximal before the drug is given), slow the rate of relaxation and finally cause contracture which can be considered the extreme limit of the slowed relaxation (70). Therefore, when a glycoside is

administered, the time over which twitch tension is sustained is increased compared to the normal. This would be expected to cause an increased oxygen uptake, since energy consumption should depend not only on tension but the time over which it is exerted.

In summary, it can be said that in the few instances when measurements were made with the precautions outlined above, it appeared that the mechanical efficiency of the failing heart is decreased and is brought back towards normal by administration of cardiac glycosides. According to the arguments presented, the defect in heart failure must, as both Wollenberger and Bing have contended, be at the level of energy utilization. The action of the glycosides would appear to be at this level rather than at the level of energy liberation.

A large number of studies have been made to determine whether cardiac glycosides have a demonstrable effect on the metabolism of tissue slices or homogenate systems. It can be stated at the outset that the glycosides have not been shown to affect the oxygen consumption of mitochondria (112) or homogenates (56, 146, 148, 186). From the accumulation of confusing and contradictory results obtained from muscle slices, it appears that the glycosides do not stimulate respiration of a fresh slice; but that following the depression of the rate of oxygen consumption which occurs during the first 1–2 hours of observation the addition of cardiac glycosides stimulates respiration, although to a level which never exceeds the rate of the freshly prepared slice (46, 86, 112, 146, 186). Some authors find that high concentrations of cardiac glycosides cause an increase and then a decrease in the rate of respiration (86, 146, 186), whereas others, using the same concentrations over the same time interval do not observe the decrease in respiration (46, 112).

It is difficult to know what relationship the above data bear to the effect of glycosides on intact muscle. That the action of these drugs on slices is not simply an artifact is suggested by the fact that effectiveness in promoting respiration of the depressed slice corresponds to the sensitivity of the whole animal to the glycosides. For example, slices from the glycoside-resistant rat were not found to be affected by the drug (85). It is also of interest that in a calcium-free medium, in which contractility of the intact muscle would be abolished, the addition of glycosides causes no increase in oxygen consumption of slices. However, it should be remembered that the respiration of the slices is profoundly depressed, due in part to the use of phosphate rather than bicarbonate buffer (29, 181). Respiration in marrow slices, for example, has been shown to be 40% higher in bicarbonate than in phosphate saline (see Warren in ref. 29). Since the oxygen consumption of the muscle slice is only a very small fraction of what it would be in the case of whole working muscle, it is possible that a change in the chemical composition of the slice mediated by the same action of the glycoside as occurs in the intact animal could cause an alteration in the depressed respiration of the tissue which would have little relationship to the *in vivo* conditions.

Interest in the effect of cardiac glycosides on metabolism has been focused recently on associated changes in cellular organic phosphate compounds, since certain of these are thought to provide the immediate source of energy for muscular contraction. The glycosides have been found to cause no change (188,

page 341), a fall (78, 138) or a rise (66) in the cellular concentration of various organic phosphates, the results depending on the experimental design and the preparation examined. Since turnover rate, not concentration, is what should normally reflect metabolic activity, the incorporation of radioactive phosphate into the muscle phosphate fractions has also been studied. Thus, Harvey (78) showed that administration of digitoxin to intact guinea pigs resulted in a fall in the rate of incorporation of P^{32} into all heart phosphate fractions measured (ATP, CP, and ester phosphate). If the administered P^{32} were readily taken up by the cell and became distributed uniformly throughout the whole intracellular pool of inorganic phosphate, and the exchange with cellular organic phosphates likewise involved the whole cellular pool of any given compound, then it might be possible to draw conclusions about the turnover rate of any phosphate fraction. But if, on the other hand, only a small portion of, say, intracellular ATP exchanges with P^{32}, it will be impossible to conclude from the specific activity of the phosphorus of the total ATP what the turnover rate was for the bulk of the cellular ATP. Apparently this is the case. There is evidence, for example, that over the period of the first 1 to 2 hours of exposure to radioactive inorganic phosphate most of the tracer accumulates in a layer at the surface of the cell rather than being incorporated into the cell interior (21). The radioactivity of, say, the total ATP isolated from the whole cell may thus be determined by a tiny fraction of cell surface ATP, the bulk of the ATP being intracellular and unlabelled. The data of Fleckenstein and Janke (49) can probably best be explained in these terms. They found that the rate of incorporation of inorganic P^{32} into the cellular organic phosphate fractions of resting striated frog muscle was only about 0.25% of the total phosphorus exchange rate according to calculations based on the oxygen consumption of the muscle. In other words, for every 400 phosphate ions incorporated into organic phosphate fractions only one came from the extracellular pool. From these data and the fact that the rate of incorporation was not influenced by stimulation of the muscle, it is clear that the uptake of tracer phosphate does not provide any measure of the metabolic activity related to muscular contraction. Evidence along the same lines was provided earlier by the work of Bollman and Flock (11) who found that the phosphocreatine concentration in rat skeletal muscle fell to one-fifth the resting value during prolonged stimulation and was rapidly resynthesized during rest. The specific activity of phosphocreatine phosphorus after four such stimulation-rest cycles over the period of 1 hour in phosphate labelled saline was not significantly greater than that for a resting muscle soaked for the same period of time. In this work also, therefore, the rate of incorporation of tracer phosphorus does not reflect the rate of turnover of total cellular phosphocreatine phosphorus.

Because of the difficulties in interpretation noted above, neither homogenate, slice, nor tracer studies have added substantially to the understanding of the effect of glycosides on cellular metabolic processes.

II. MUSCLE PROTEINS AND MODELS

With the isolation of the contractile proteins of skeletal and heart muscle and the use of an increasing variety of methods for studying the properties of these

proteins, many investigators began to study the effects of the cardiac glycosides on both separated fractions and on protein models of the contractile system.

One of the first results in the field had to do with the polymerization of G-actin to form a long chain called F-actin, which occurs when the ionic strength of the solution is increased by the addition of potassium chloride. It was first found by Horvath and coworkers (93) that the rate of polymerization of heart muscle G-actin was increased in the presence of 2 to 4 μg/ml of g-strophanthin or digitoxin. Up to 100 μg/ml had no effect on the polymerization of actin prepared from skeletal muscle. These basic observations were confirmed by Snellman and Gelotte (161), Cowle and Thorp (27) and Wollenberger (189). Snellman and Gelotte believed that their solutions of actin contained a deaminase which partially inhibited the polymerization reaction, and that the glycosides prevented the action of the deaminase. However, Wollenberger, working in the same laboratory, showed that glycosides enhanced polymerization of actin solutions in which no deaminase activity could be detected. He also found that actin extracted promptly from the hearts of freshly killed animals polymerized at a rapid rate comparable to that of skeletal muscle actin, and, like the latter, was not affected by the addition of cardiac glycosides. The action of the glycosides, then, was confined to cardiac actin solutions prepared from hearts which had remained in the sacrificed animal for some time before extraction. Working with such solutions, Wollenberger made an important contribution to the problem by comparing the effects of pairs of glycosides which were structurally similar but one of which had no pharmacological activity in whole animals. Thus he compared Scillaren A and hexahydroscillaren A (inactive); also emicymarin and alloemicymarin (inactive). All 4 of these glycosides increased the rate of polymerization to the same extent, showing that there was no correlation between *in vivo* pharmacological activity and the effect on the isolated protein.

Wollenberger's approach provides an excellent first test for evaluating the physiological significance of actions of glycosides observed in isolated systems. Throughout this section, it will be seen that glycosides have effects on various properties of the contractile proteins, but if there is no correlation between those effects and the *in vivo* activity of the compound tested, then it cannot be assumed that the observed effect on the protein is related to the pharmacological action of the glycosides.

One of the characteristic qualities of actomyosin solutions is a high anomalous[3] viscosity typical of highly asymmetric molecules. The addition of ATP causes dissociation of the protein complex into actin and myosin, which is reflected in a marked decrease in viscosity. After the ATP is hydrolyzed by the myosin ATP-ase recombination of the protein components occurs and the viscosity consequently increases. It is thus possible to measure the original viscosity, the drop on addition of ATP, and the time interval before the viscosity begins its return to the original value [a measure of ATP-ase activity, (31)]. All the studies reviewed below were made on skeletal muscle actomyosin. Waser *et al.* found

[3] A decrease in relative viscosity with increasing flow rate is referred to as anomalous.

that the addition of glycosides to actomyosin solutions caused a very small decrease in viscosity, the magnitude of any one glycoside depending on the concentration and the degree of binding to the protein (172, 175). The effect was abolished by small concentrations of ethanol or methanol (10^{-5} to 10^{-4} M) and there was no correlation between the action on viscosity and the *in vivo* pharmacological activity, inactive glycosides having the same effect as active ones. The drop in viscosity induced by ATP is not affected by cardiac glycosides (11, 39, 175). ATP-ase activity is either increased or decreased by ouabain depending on the purity of the preparation (39, 84).

The only investigator who has made a correlation between *in vivo* pharmacological activity and any effect on isolated contractile proteins has been Waser, who demonstrated glycoside action on both the thixotropy and the potassium binding of "synthetic" actomyosin (5 g myosin + 2 g actin). Actomyosin solutions (protein concentration about 3 mg/ml) exhibit thixotropy; that is, a tendency to form a gel on standing. If stirred at a constant speed, the gel tends to break up slowly, as evidenced by a progressive drop in viscosity over a period of about 15 minutes. By measuring the resistance of the solution to stirring, it is possible to estimate the original strength of the gel. Using a large number of glycosides, including 4 active-inactive pairs, Waser found that pharmacologically active glycosides decreased to a small extent the thixotropy of actomyosin, whereas the inactive glycosides increased it. He also found a correlation between potency for decreasing thixotropy and the degree of pharmacological activity in the intact animal (172, 173).

In another study (174) he measured the influence of glycosides on the potassium binding of actomyosin. This was done by placing equal volumes of 0.6 N KCl on opposite sides of a dialysis membrane, one side containing actomyosin. Glycosides were added to one or the other side, the final concentration being 2 or 4 μg/ml; and after equilibration for 3 to 4 days the K^+ in the protein-free side was measured. It was found that pharmacologically active glycosides caused a decrease in the K^+ of the protein-free side from 600 mEq to as low as 560 mEq/l. Since this was shown not to be due to binding by the dialysis membrane, it was concluded that the drug caused an increased potassium binding by the protein. Pharmacologically inactive glycosides caused no increased binding.

At a higher level of structural complexity, studies have been made on threads and bands prepared from actomyosin solutions. Mallov and Robb (123) first showed that the ATP-induced shortening of threads of skeletal muscle actomyosin was increased by the addition of a cardiac glycoside. These findings were confirmed by Bowen (13). The same results were later obtained by Robb and Mallov (142), using threads prepared by compression of surface films of beef heart actomyosin.

The effect of glycosides on actomyosin bands was determined as part of a series of studies from Bing's laboratory which was designed to find out whether actomyosin prepared from heart muscle of patients in congestive failure was different from normal heart muscle actomyosin. Dettli and Bing (35) developed a refinement of the Hayashi (81) method by compressing the surface film of protein less com-

pletely to form a band rather than a thread, and measuring shortening at constant tension without ever removing the band from the surface of the liquid. The amount of actomyosin extracted per g of wet heart and the load-% shortening relationships were the same whether actomyosin was extracted from a freshly killed dog heart or whether the heart was allowed to remain in the animal at room temperature for 1 hour before extraction (36). This study was considered to be a necessary preliminary to comparing the difference between normal and failing hearts from human autopsy material. Kako and Bing found that the contractility (% shortening *vs.* load) of actomyosin bands prepared from hearts of patients with congestive failure was significantly lower than that from normal hearts (98). The contractility of both groups was unaffected by digoxin, but in the presence of digoxin + 10^{-3} M Ca^{++} the contractility of the congestive failure protein increased to the normal range. It would have been of interest if this careful study had included the effects of pharmacologically inactive glycosides.

Although actomyosin threads or bands are macroscopic aggregates of muscle protein which have contractility, the model which probably reflects most closely the original structural arrangement of the protein is the glycerol extracted muscle fiber (164). The effects of glycosides on this preparation have been studied by several workers (40, 109, 162). One author found that the contractility of glycerol extracted psoas muscle fibers was increased in the presence of critically narrow concentration ranges of digitoxin and calcium ions (40). No glycoside effect was shown in the other two studies, one on heart (162) and the other on striated muscle (109).

III. THE SITE OF GLYCOSIDE ACTION

If the cardiac glycosides were to exert their pharmacological effect by a direct action on the contractile proteins, then it must follow that they penetrate into the interior of the cell, and (considering the small number of molecules which are effective) bind to the contractile protein. It is clear from a number of studies that glycosides bind to certain proteins including actomyosin (168, 171) and serum albumin (68, 145), but not serum globulin (68, 145). There is no certainty, however, that the glycosides enter the cell, as consideration of the following studies will show. A paper on the distribution of digitoxin among various particulate cell fractions of rat heart separated according to the basic techniques of Hogeboom *et al.* (91) was published by St. George *et al.* (147). Digitoxin was administered intravenously, the rats were killed 5 minutes later, and the hearts were fractionated by differential centrifugation into 4 fractions. The authors found that 89% of the digitoxin in the tissue was in their fourth fraction, *i.e.*, the particle-free supernatant remaining after spinning at 22,000 × *g* in a Spinco horizontal-head ultracentrifuge. They assumed that the actomyosin was in this fraction and concluded, therefore, that the glycoside entered the cell and became bound to the actomyosin. The conclusion can be questioned, since in order for the actomyosin to be present as soluble protein in the fourth fraction, it must have been extracted by the 0.85% sucrose used as the suspending medium in the preparation of the cell fractions. This would be surprising, in view of the high

ionic strength of solutions required for the extraction of contractile proteins from minced muscle. Furthermore, although glycosides disappear rapidly from the circulation, at the time of sacrifice only 5 minutes after injection of the drug a large fraction of the glycoside should still be present in the interstitial fluid, which of course would also be found in the fourth fraction. One cannot conclude, therefore, that the bulk of the digitoxin recovered was bound to actomyosin.

The other study pertinent to this question was done by Harvey and Pieper (80) who perfused isolated guinea pig hearts with a Krebs bicarbonate solution containing C^{14} digitoxin for 2 hours at 37°C and then, after flushing out the vascular bed with non-radioactive material, fractionated the heart by differential centrifugation. In contrast to the results of the workers cited above who found most of the digitoxin in the final supernatant, about 90 % of the radioactivity was recovered in the cellular particulate fractions. (The large difference in the results of the two groups is due to the fact that the digitoxin in the rat heart study was probably mostly in the interstitial fluid because of the short equilibration and lack of vascular space washout.) Harvey and Pieper tried to determine whether the observed distribution of digitoxin among the isolated fractions reflected the *in vivo* cellular localization of the drug, or whether the *in vivo* distribution might have been altered during the homogenization and centrifugation of the tissue fragments. Since they found that prior alteration in the environment of the surviving heart (changing Ca^+, K^+ or temperature) altered the distribution of radioactivity in the isolated fractions, they concluded that their findings were not simply a fractionation artifact. Despite this, their studies present two major difficulties in interpretation. The first is whether a mammalian heart perfused with a balanced salt solution is normal with respect to, for example, oxygenation and membrane permeability to various substances. The second is whether the cellular components to which digitoxin might bind, notably the actomyosin and the cell membrane, are adequately separated into different fractions by the differential centrifugation technique. Since the fractions in which most of the digitoxin was found probably contain both cell membrane fragments and actomyosin, it would appear that these data cannot be used to decide between the two most probable loci of action of the cardiac glycosides.

IV. IONS IN NORMAL MUSCLE

Since a description of the effects of cardiac glycosides on the concentration and movement of intracellular ions constitutes a large part of this review, certain facts and theories which bear on conditions that obtain in the normal muscle cell at rest and during the contraction cycle will be presented briefly here.

Intracellular ionic composition. It is well known that the main intracellular cation of muscle is potassium, sodium being to a large extent excluded. The concentration of Na^+ in rat heart muscle, for example, is probably not more than 13 mM/kg of fiber water (120). Among the divalent ions calcium is of interest, but information about intracellular calcium ion concentrations is meager. The available data for mammalian tissues [rabbit blastocytes (106), mammalian striated muscle (45)] suggest a value around 1 mM/kg of cell water; and measure-

ment of calcium diffusion rates in squid axoplasm indicates that at least in this tissue the calcium is present in a bound form (45). The question whether the monovalent ion potassium is also bound has been considered by numerous workers. Lewis and Saroff (116), using an anion impermeable membrane, found that at pH 6.4 in 0.15 M KCl, potassium was bound to myosin A to the extent of about 12 ions per mole of myosin. Actin and albumin exhibited no potassium binding capacity. K^+ binding by myosin A would amount to not more than 5% of the total cell K^+, however, and even this value might be less in the presence of cellular divalent cations. Experiments with intact cells, either on the basis of electrical conductivity or exchange rates of radioactive potassium, have also been interpreted (not without reservations) to show that little or none of the intracellular potassium exists in a bound form.

The membrane potential. The asymmetry of ion concentrations across the cell membrane gives rise to a membrane potential, the interior of the heart muscle cell being 70 to 90 mV negative with respect to the outside (28). This resting membrane potential has been shown to vary with changes in the extracellular potassium concentration, following approximately the relationship

$$E_m = \frac{RT}{F} \ln \frac{K_i}{K_o}$$

in which K_i and K_o are the intracellular and extracellular potassium concentrations, respectively (1, 35). Such a relationship was predicted by Boyle and Conway on the assumption that Donnan equilibrium obtained, that the resting membrane was permeable to potassium and chloride but impermeable to sodium, and that the cell was in a "balanced state," *i.e.*, no net transfer of any ion across the membrane occurred. It is now believed that sodium can permeate the resting membrane to some degree, but that the cell is capable of extruding any sodium which enters so that a low intracellular concentration is maintained (35). The Boyle-Conway theory requires that the cell be in a "balanced state," whereas it is likely, at least in the case of heart muscle, that net fluxes of ions across the cell membrane occur even during the interval between contractions. Equations for the resting membrane potential, which incorporate the assumption that net fluxes of ions do occur in the resting cell, have been derived (63, 89). They reduce finally to the form of the above equation, so that experimental agreement with that equation does not provide for a choice between the basic assumptions.

It remains now to describe the changes thought to occur during the contraction cycle. It has been known for many years that the contraction cycle is associated with an increased exchange of both sodium and potassium (44). The temporal relations of these ion movements have been analyzed with the help of electrophysiological data (88) and it is thought that the rapid rising phase of the action potential spike is caused by a rapid influx of sodium due to a sudden increase of cell permeability to this ion. (See ref. 28, page 61, for a summary of contrary evidence in certain experiments with cardiac tissue.) The action potential plateau and subsequent return to resting potential levels are not fully understood, but are probably associated with a re-establishment of cell impermeability to sodium,

and also with an outward movement of potassium caused either by the change in membrane potential (178) and/or an increased cell permeability to potassium (28). In any case, by the end of the action potential it is reasonably certain that the cell has gained sodium and lost potassium, a state of affairs which tends to be reversed during the period of rest before another stimulus occurs.

V. MEMBRANE POTENTIAL

With the development of techniques for recording heart muscle cell transmembrane potentials with intracellular microelectrodes (194), it has become possible to determine resting membrane potentials and monophasic action potentials of heart muscle. The subject has been reviewed recently by Cranefield and Hoffman (28). The action potential of ventricular muscle differs from that of nerve or striated muscle in its long duration, 0.15 to as long as 6 seconds depending on conditions; and unlike striated muscle contraction begins much before repolarization is completed. The action potential is so long that observers have divided it into several phases (28, 193), the initial spike being followed by a rapid but small drop (phase 1), a plateau (phase 2), and a third phase in which there occurs a return to resting potential values at a rate intermediate between that of phases 1 and 2.

The effects of digitalis on the electrical events of the cardiac cycle appear to be as follows. Initially an increase in the magnitude of the spike is sometimes observed (193). This change is transient, and is followed by a decrease in spike amplitude. Thus, normally the spike describes a change in potential from -90 mV to a positive value of about 30 mV, whereas in the presence of toxic concentrations of glycosides the spike decreases to the point where the "positive overshoot" disappears and even the zero potential level is no longer reached (26, 193). Dudel and Trautwein have found that the glycosides cause a diminution in the rate of rise of the action potential spike (37). The most marked effects of the glycosides, however, are on the repolarization phase. Following a transient increase in the total duration of the action potential there is marked shortening, due mostly to a decrease in the time taken for repolarization (37, 193).

The meaning of these findings in terms of cellular ion movements is not known at this time, but it is perhaps worth considering them in the light of a hypothesis outlined by Cranefield and Hoffman (28). As a result of the depolarization of the cell membrane, the outflow of potassium would be expected to increase since the ion is no longer held back by a large intracellular electronegativity. However, the membrane does not allow completely free diffusion of K^+, and so efflux is still comparatively slow during the plateau (phase 2). The outflow is sufficient, however, to cause the accumulation of some K^+ at the extracellular surface of the membrane and this by some mechanism increases permeability to potassium (P_K) which starts the rapid K^+ outflow of phase 3 and the return of the membrane potential to normal. Thus, any factor which tends to raise K^+ concentration at the membrane should increase P_K and diminish the time needed for repolarization; whereas a low external K^+ concentration should lengthen this time. Consistent with the theory are the findings that 1) in a K-free medium repolarization

is greatly prolonged (113); 2) close intraarterial injection of K^+ shortens the action potential (177); and finally 3), interventions which cause accumulation of K^+ at the outer surface of the membrane, either by inhibiting the rate of re-entry (digitalis) or diminishing the time available for re-entry between contractions (high frequency) also are associated with a shortened action potential.

Dudel and Trautwein (37) have reported that cardiac glycosides cause an increase in the membrane resistance of the Purkinje fibers of the cat during both rest and activity. This finding suggests that in addition to an effect on active transport the glycosides alter the permeability of the cell membrane. The data of Glynn on red cells are consistent with such a conclusion (59).

VI. STIMULATION FREQUENCY AND CONTRACTILE FORCE

The twitch tension developed by cardiac muscle is affected in large measure by variations in the frequency and regularity of stimulation. Comparable effects have been observed in smooth (32, 114) and striated muscle (141), being much less obvious in the latter tissue. The action of cardiac glycosides cannot be properly evaluated without some understanding of the relationships between stimulation frequency and contractile force. In the case of heart muscle most of the observations are probably pure examples or mixtures of two basic phenomena, the so-called Bowditch staircase and the reverse staircase. Post-extrasystolic potentiation may represent yet a third mechanism.

The first phenomenon was reported by Bowditch in 1871 (12). Working with the isolated frog heart, he noted that following a prolonged period of asystole, the amplitude of contractions which occurred when the heart was subsequently stimulated at a regular frequency began at a low level and increased progressively to a maximum which was then maintained at a plateau (Fig. 1A). Later workers confirmed this observation, not only for amphibian but also for some mammalian hearts, and also generalized the Bowditch findings in the terms that for any regular stimulation rate a characteristic level of twitch tension was achieved (33, 74), being low at low frequencies and higher at increased frequencies over a certain range (Fig. 1B). It was clear, however, that an opposing phenomenon frequently operated simultaneously. This was first described by Woodworth (195) in experiments on dog heart muscle. He stimulated the muscle at a regular fast frequency until a steady twitch tension was obtained. When he shifted to a slow frequency the first one or two twitches were greater than the previous ones [the "poststimulation potentiation" of later workers (144)] and then progressively declined as expected to a level characteristic of the slow rate (Fig. 1C). The increased amplitude of the first twitches at the slow rate led him to believe that the twitch tension was the result of two effects, "the stimulating effect of a rapid succession of contractions and the recuperative effect of a long pause." In certain animals, such as the rat, the opposing phenomenon is the dominant one (5, 105), so that the twitch tension *decreases* with increasing frequency (Fig. 1D). A similar relationship is seen in rabbit atrium treated with fluoroacetate (104). Of the names used to refer to this opposing phenomenon, "reverse staircase" will be employed in this review. The relative contributions of these two phenomena for

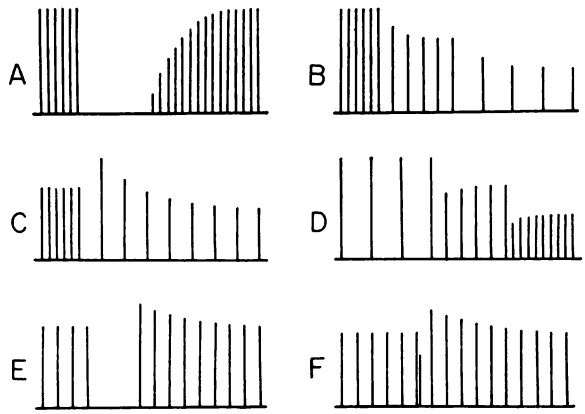

FIG. 1. Schematic diagrams of frequency-tension relationships. Continuous tracings of contractions. See text for details.

A. Bowditch staircase. Following a rest period the first contractile response is small, but rises progressively with each stimulus until a plateau is reached. B. Bowditch staircase. The higher the stimulus frequency, the higher the tension. C. Post-stimulation potentiation, a mixture of Bowditch and reverse staircase. The first contraction after shifting from a high to low frequency is greater than before (reverse staircase). Then there is progressive drop to a new plateau characteristic of the slow stimulus frequency (Bowditch staircase). D. Reverse staircase. The higher the stimulus frequency the lower the tension. E. Rest contraction, an example of reverse staircase. Stimulation rate before and after the rest is the same. The first few contractions after the rest are greater than before. F. Post-extrasystolic potentiation. The first few contractions after the extrasystole are greater than before.

any one species may change over the observed frequency range. For example Kruta (110) has found a multiphasic amplitude-frequency relationship in guinea pig heart, the reverse staircase being dominant at low frequencies, the Bowditch staircase having the main effect at higher rates. The same findings have also been reported for rabbit atrium (104). The so-called "rest contraction" (144) is another example of the reverse staircase phenomenon (Fig. 1E), since the interval of rest can be regarded as a temporary shift to a lower frequency which causes an increase in twitch tension. To this already long list of phenomena must be added still another one, first observed by Langendorff in 1885 (see 195), namely, the effect of an extrasystole in causing an increased amplitude of the following contraction (Fig. 1F). Woodworth, enlarging on the earlier findings, found that 2 or more extrasystoles had a greater stimulating effect than 1, that the effect persisted on the average for about 8 subsequent beats, and that the earlier in the contraction cycle the extrasystole occurred the greater was the potentiating effect. These observations were later confirmed by others (19, 55, 90).

Recently investigators have attempted to elucidate the mechanisms underlying the above phenomena. The Bowditch staircase in the frog heart has been studied by Hajdu (70) who found that there was a net loss of potassium from the heart into the bathing solution when a previously non-beating heart was stimulated electrically. The developed tension was higher at higher stimulus frequencies, according to the Bowditch staircase (Fig. 1B) and the net K^+ loss

was likewise greater the higher the stimulation rate. It was also found that in a low potassium Ringer a given tension could be achieved at a lower stimulus frequency than in a normal potassium Ringer. In view of the fact that potassium leaves the cell during contraction and must be transported back into the cell during recovery (see page 183), the hypothesis was advanced that as the stimulus frequency is increased the time available for the re-entry of potassium between contractions is inadequate for the complete replacement of the ion lost during the previous contraction, with the result that a lower steady state level of potassium within the cell is finally reached. Over a certain range, then, there is a cellular potassium level characteristic for each stimulus frequency; and either this level or some other cellular parameter which changes as the K^+ changes determines the magnitude of the twitch tension, lower K^+ being associated with higher tension and *vice versa*. Vick and Kahn produced evidence confirming part of the above study by showing that a net loss of potassium from guinea pig heart occurred when the stimulation rate was increased (169).

None of the published studies on the reverse staircase provides any clue about the cause of this phenomenon. Speculations have ranged from the idea that during rest time is provided for certain metabolic reactions to achieve cellular recovery (103, 104, 195) to the postulation of a potentiating substance (144).

Post-extrasystolic potentiation has been thought by many (19, 20, 135, 153, 195) to be an example of the Bowditch staircase. An analysis of the problem can be facilitated by reference to an experiment on cat papillary muscle published by Hoffman (90), which is similar to an earlier one of Garb and Penna (55). Fig. 2 is a schematic diagram to show the essential part of the experiment. It represents a continuous tracing of contractions induced by stimulation at an *average* frequency of 1 per second. Whereas the stimulation was regular in the first and last part of the tracing, in the section bounded by the two arrows the average frequency was maintained by stimulating at a regular rate of ½ per second and following each regular contraction with an extrasystole. It can be seen that

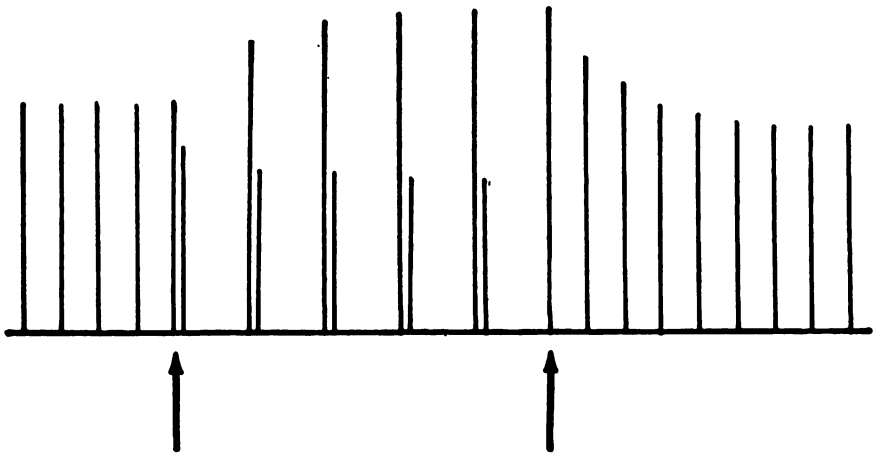

FIG. 2

when the shift was made to the irregular frequency the tension of the post-extrasystolic contractions rose progressively to a plateau, and dropped back when regular stimulation was resumed. This rise in tension is partly due to reverse staircase, since the interval between any extrasystole and the following contraction is almost 2 seconds, rather than 1 second as in the control period, and the effect of the reverse staircase would be to cause a greater tension after the longer rest period. However, the rise in tension is progressive for several beats, and the reverse staircase, in the experience of the reviewers, appears to take effect rapidly in a few contractions. It seems likely, therefore, that an additional factor contributes to the rise in tension, either the postulated potassium shift of the Bowditch staircase or a completely different phenomenon. In the former case the progressive rise in the tension of the post-extrasystolic contractions would be associated with a *pari passu* drop in cellular potassium until a new steady state level is reached. Since the average frequency remains the same during the experiment, the development of a lower steady state level of cell potassium could, for example, occur if potassium loss during an extrasystole following close on a normal beat is greater than during a regularly spaced contraction at the same average frequency. No information on this aspect of the Bowditch staircase is currently available, and so the choice between an unknown or a potassium mechanism cannot be made at this time.

Hoffman believes that post-extrasystolic potentiation is not associated with potassium shifts, in part because he could find no change in resting membrane potential during the potentiation period, and he believes that a net efflux of potassium from the cell should be associated with a temporary rise in the K^+ immediately surrounding the fiber large enough to cause a drop in the membrane potential. Even if post-extrasystolic potentiation were caused by potassium shifts, the question remains whether sufficient potassium loss would occur to cause a measurable change in resting potential, and therefore this negative evidence is inconclusive.[4]

[4] A criticism should also be made of the argument (55, 135, 144) that post-extrasystolic potentiation could not be due to K^+ loss because the potentiation effect may persist for several minutes. For example, it has been stated, "if stimulation is suspended after the extra interpolated contraction, the augmentation effect persists for periods up to 300 seconds. Surely this interval is more than ample for any lost potassium to reenter the muscle since presumably lost potassium is recovered completely within a period of 2 seconds when a series of contractions show equal force at a rate of 30 per minute. This suggests that Hajdu's theory, based on experiments with the frog heart, is not a suitable explanation for the staircase or augmentation phenomenon in mammalian heart muscle" (135). The point which seems to be missed in this argument is that once the muscle reaches a steady state after a period of stimulation at a given frequency, then the potassium lost during contraction is replaced during the interval between contractions *no matter what the length of that interval*, be it 1 second or 60 seconds. The question is how long it takes to reach a *new* steady state when the frequency is changed, in the course of which there will be a progressive net potassium loss or gain by the fiber. Judging by the length of time required to reach a new tension plateau after a change in frequency, several minutes may elapse before the new steady state potassium level is reached, and likewise, if extrasystoles cause an increased potassium loss a prolonged interval could be required to achieve complete restoration of the original steady state.

Changes in the elasticity of striated muscle during the appearance of the staircase phenomenon or post-tetanic potentiation have been studied recently by Ritchie and Wilkie (141). Their work was derived from the observation of Hill (87) that when a muscle is stimulated a sudden increase in elastic modulus occurs (called by Hill the development of the "active state") much before any observable contraction begins, so that by the end of the latent period the muscle is capable of bearing a load equivalent to the maximum tension set up in an isometric tetanus. During a single twitch the muscle may not reach this tension, since the elastic modulus begins to decline before the contractile tension, which develops gradually, has reached a peak. It follows, then, that any factor which increases either the intensity of the active state or its duration should result in an increased single twitch tension. Using a quick release method for determining the intensity of the active state in frog sartorius, Ritchie and Wilkie found that previous stimulation of a frog sartorius caused an increase in the duration of the active state, and they believe that this accounts for the staircase phenomenon and the post-tetanic potentiation which can be seen in frog skeletal muscle. Such biophysical measurements have not yet been made on cardiac muscle.

VII. POTASSIUM

Calhoun and Harrison (18), after finding a low potassium concentration in the hearts of patients who died of congestive failure (17), set out to find whether digitalis had any effect on the concentration of cardiac potassium and their results published in 1931 were the beginning of a long series of papers in this field. They administered digitalis to dogs, dividing their animals into 4 experimental groups according to glycoside dosage: control, therapeutic, toxic (characterized by vomiting) and fatal. They found no significant difference in the dry weights of the hearts of the 4 groups. The potassium concentration was markedly diminished in the toxic and fatal groups (average for left ventricle of 56.0 mEq/kg wet tissue as against 79.3 for the controls), whereas the difference between the normal and therapeutic group was slight (a diminution in the latter of 3%). To quote their conclusions, "from these data, it seems clear that digitalis in toxic dosage causes a diminution in the potassium content of the cardiac muscle of dogs but not of their skeletal muscles, while in amounts corresponding to therapeutic doses in man this effect is either absent or very slight." They reasoned, then, that the doses used in man were not adequate to account for the observed potassium decrease in their patients who died of congestive heart failure, but they did not rule out the possibility that the slight diminution in cardiac potassium of the dogs caused by therapeutic doses might be significant and even considered it conceivable that digitalis "may act in such a way as to readjust the ionic balance" of the failing heart.

During the ensuing years many workers tried to decide whether presumably therapeutic concentrations of cardiac glycosides did indeed cause a loss of potassium from the heart (2, 15, 24, 57, 65, 69, 70, 75, 83, 92, 99, 134, 137, 156, 169, 176, 191, 192). Since a small loss of potassium would cause only a very small percentage difference in the tissue concentrations of groups of normal and

digitalized hearts, Wood and Moe (191) used another approach which was designed to avoid the statistical problem raised by direct tissue analysis. They tried to detect a net change in cardiac potassium by measuring the increase in potassium concentration in the blood perfusing dog heart-lung preparations before and during the administration of a cardiac glycoside. Their results showed that potassium was lost slowly from both the heart and lungs of their preparations during the control period, and that the rate of potassium loss was increased significantly not only by toxic but also by therapeutic doses of lanatoside. Furthermore, there was a positive correlation between the increase in mechanical efficiency caused by the glycoside and the rate of increase of potassium concentration in the venous blood. They concluded "the concept that potassium loss from cardiac muscle is purely a toxic manifestation of digitalis action is not supported by these studies" (191). A net tissue potassium loss under the influence of therapeutic (non-contracture-causing) doses of glycosides has since been demonstrated for isolated frog (70, 99) and guinea pig (169) hearts by measuring the resultant increase in potassium concentration in the perfusion solution. Experiments along the same lines have also been done with intact animals, since development of techniques for coronary sinus catheterization have enabled investigators to measure coronary arteriovenous potassium concentration differences before and during the administration of cardiac glycosides. None of the groups of workers has measured coronary blood flow in these studies, their estimates about cardiac net potassium changes being based on the coronary arteriovenous concentration difference alone. The abstract of Harris and coworkers (75) indicates that following the administration of K-strophanthoside to intact dogs, an elevation occurred in both arterial and coronary sinus blood potassium concentration; in some but not all cases the potassium increase in coronary sinus blood was said to be significantly greater than in the arterial blood. The detection of an increase in the coronary A-V potassium concentration difference depends, of course, on the rate of liberation of potassium from the heart and on the rate of coronary blood flow. Gonlubol and associates (65) point to these factors as possible reasons for their failure to detect a significant cardiac arteriovenous potassium difference after the administration of Cedilanid to human subjects. Regan *et al.* (139) tried to establish conditions in which the rate of potassium liberation might be expected to be maximal by administering rapidly to dogs the fast-acting acetylstrophanthidin which caused a peak effect on ion movements at about 6 minutes. They consistently found a potassium loss from the heart under these conditions, and Hellems *et al.* (83) from the same laboratory reported similar findings in 7 patients with cardiac failure.

The results obtained by tissue analysis were not nearly as consistent as those mentioned above in which measurements were made of the potassium lost to the surrounding fluid. In some studies the digitalized hearts appeared to have a lower potassium concentration than the controls, but the statistical significance of the difference remained in question (2, 18, 176, 192). In other studies no change or even an increase in potassium concentrations was reported after very small or presumably therapeutic amounts of cardiac glycoside (15, 57, 69, 92,

176). Various criticisms can be made of these studies. For example, Hagen (69) found potassium loss with toxic but not with therapeutic doses, but the large difference between the two dosages (3.6 to 4.6 as against 24 μg/g wet rabbit heart) suggests that the therapeutic dose was ineffective. Gertler's results (57) may also be due to inadequate dosage [0.1 mg/kg Digitaline Nativelle (digitoxin) daily to rabbits], considering the rapidity with which the rabbit is said to excrete cardiac glycosides (61). However, in another study of rabbits on the same dosage schedule digitalis-induced potassium loss from certain body tissues was reflected in a decreased size of the exchangeable potassium pool, with values returning to normal levels a week after the drug was discontinued (2). Technical considerations cause difficulty in interpreting the findings of Holland *et al.* (92) who studied hearts in a Locke solution containing only 0.002 M $NaHCO_3$ bubbled with a gas containing 5% CO_2; and those of Wedd (176) whose glycoside-treated ventricular strips were frequently unresponsive to electrical stimulation so that they can hardly be compared with the stimulated controls. Likewise, the results of Sherrod (158), who gave glycosides to dogs in intravenous doses at 5-minute intervals until death (9–13 doses) cannot be attributed necessarily to the effects of the drug in view of the agonal state of the animals. None of the above criticisms would seem to apply, however, to the study of Boyer and Poindexter (15), who administered glycosides to 11 cats, either in single dosage or over a period of several days, so that there was electrocardiographic change without signs of toxicity. Their apparently careful tissue analysis of the left ventricles of these animals showed no significant change in water content, but an *increase* in the concentration of potassium (both in mEq/kg wet heart or as the calculated intracellular concentration based on chloride space measurements). Their control potassium concentrations are surprisingly low; there is still some possibility that they gave less than the dose needed to produce a positive inotropic effect (despite the presence of EKG changes), and if there was a great slowing of the heart rate potassium concentration might shift upwards; but the correct explanation for their divergent results remains in doubt.

Despite the difficulties mentioned in the foregoing paragraph, it would seem that the weight of evidence, especially that which derives from analysis of perfusion fluid changes and is based on a variety of experimental material ranging from the isolated amphibian heart to the intact human, leads to the conclusion that administration of cardiac glycosides in therapeutic dosage is associated with a net loss of potassium from the heart. A number of investigators have tried to determine whether this loss is caused by an increased potassium efflux from the cell or by a diminished rate at which potassium is transported into the cell. Based on a comparison of the action of digitalis and veratrum on the isolated frog heart, Hajdu suggested that the glycosides decreased the rate of potassium re-entry during the recovery phase of the contraction cycle (70, 165, page 92). Similar conclusions were reached by Vick and Kahn in studies of the potassium release from guinea pig hearts during alternating periods of rapid and slow beating in the presence of ouabain or veratridine (169). The direct measurement of potassium efflux and influx has since been achieved with the use of radioactive

potassium, a decrease in the influx with no change in outflux being reported for both frog ventricle (156) and guinea pig auricle (137). Much of the influx data from the study on guinea pigs, however, was obtained on hearts that had ceased to beat, reflecting a toxic effect of the drug. Schreiber (156) analyzed frog ventricle potassium influx curves into rapidly and slowly exchanging components, and concluded that the curves reflect uptake of potassium into two anatomically distinct intracellular pools. Ouabain inhibited uptake of only the slowly exchanging potassium component, and he believes that if the drug acted at the membrane an inhibition of both rapidly and slowly exchanging components would be observed. However, it would not appear that the data speak for the existence of two intracellular pools as distinct from two pathways for the transmembrane uptake of potassium, and as long as the latter possibility exists ouabain could act at the membrane on only one pathway, the other component of the uptake curve remaining unaffected by the drug. The studies just cited, in which influx is found to be slowed while efflux remains unchanged, were of course done in the unsteady state when a net loss of potassium from the cell must have been occurring as a result of the action of the glycosides. Conn, using a similar approach, has obtained transfer rates of potassium across the cell membrane in the heart of an intact animal, not during digitalization but after a presumed new steady state has been reached as a result of continuing glycoside action when influx and outflux should be equal (24). He administered to dogs 0.2 to 0.4 mg digitoxin daily for 10 to 14 days. No toxicity at this dosage is mentioned. At the end of this time an infusion of K^{42} was given, the rate being adjusted to maintain a steady arterial specific activity, and the time curve of appearance of radioactivity in the coronary sinus blood was determined. From the analysis of such curves he obtained transfer rates between cell and interstitial fluid of 4.25 ± 0.25 (SE_M) mEq K/kg per min for controls and 3.74 ± 0.15 for the digitalized group. The combined results of the isotope studies, then, show that potassium influx is diminished as a result of digitalis administration, and that after new steady state conditions are reached the transmembrane rate of potassium movement continues to be depressed.

Similarly, Schatzmann (154) has shown that cardiac glycosides inhibit the uptake of potassium and extrusion of sodium of human red cells previously stored in the cold. Inhibition of red cell potassium influx by glycosides has been confirmed by others (58, 59, 95, 97, 163). A correlation between potency of this effect and *in vivo* pharmacological activity has been shown by Kahn (96).

The question which follows naturally from the results of all the studies reviewed above is whether the positive inotropic action of the glycosides is a direct result of the change in tissue composition associated with the loss of potassium from the cell. The study by Hajdu referred to earlier on the mechanism of staircase in the isolated frog heart is applicable to this problem (70). In this work a correlation was made between tension changes and alterations of cation concentrations in both tissue and perfusion fluid. Determination of extracellular space with inulin permitted calculation of intracellular changes. Associated with the positive inotropic effect of digitalis he observed a decrease in the net weight of

the whole tissue, a slight increase in the intracellular concentration of sodium and a moderate decrease in that of potassium from 114 ± 0.9 (SE_M) mEq/l of fiber water for the control group to 108 ± 1.7 for the digitalized group. For each heart the increase in the potassium concentration of the perfusion fluid during the course of digitalization was also measured, the results indicating that a loss of 13.7 ± 0.8 milliequivalents of potassium per liter of fiber water had occurred. Since the decrease in the fiber water concentration of potassium was only 6 mEq/l (114 − 108) instead of 13.7, one could account for a loss of 13.7 mEq/l only by assuming that the cells had lost water along with the potassium. The sum of the intracellular *concentrations* of sodium and potassium (mEq/l of fiber water) was not significantly altered by the glycosides, due to the concomitant loss of water; but the actual amount of sodium plus potassium expressed as mEq/kg dry weight of the cells had clearly decreased. In addition to the above findings, certain other results described in that paper may be summarized. 1) When, after a period of rest, the heart is stimulated at a specified frequency until a steady state twitch tension is reached, a net loss of potassium from the muscle occurs; and the higher the frequency over a certain range the higher the tension and the greater the net loss of potassium. 2) At any given frequency the twitch tension characteristic for that frequency is increased if the heart is perfused in a K-deficient medium. 3) A similar increase is observed if the perfusion medium is sodium-deficient, tissue analysis revealing a decreased intracellular sodium concentration while the potassium concentration remains unchanged. In other words, the contractile force of the heart is increased when either sodium *or* potassium is lost from the muscle cell. This suggests the hypothesis that what may perhaps influence the contractile protein is the *sum* of the sodium and potassium concentrations, a decrease caused by loss of either ion producing a greater twitch tension. However, in the case of the cardiac glycosides, the sum of these monovalent cation concentrations is not changed because of the concomitant water loss; and only the concentration relative to that of the contractile protein is decreased, (*i.e.*, protein concentration increases because of water loss, total monovalent cation concentration remaining the same). If, then, it is the change in the cellular ionic pattern which accounts for the positive inotropic effect of the glycosides, a unifying hypothesis to account for all the observed facts would be that the contractile protein is influenced by the ratio of protein concentration to the sum of the monovalent cation concentrations (*i.e.*, M of protein per M of monovalent cation). The ratio is increased by glycosides by virtue of a decrease in cellular water and potassium. It can be increased primarily by a decrease in ion without a water shift by raising stimulation frequency, or by lowering extracellular sodium or potassium concentrations. When, on the other hand, a change in the monovalent cation concentration is caused by water loss alone (due to immersion of the heart in a hypertonic perfusion medium, for example), there is no change in the protein/monovalent cation ratio and no significant change in contractility.

It has become generally accepted that potassium ions inhibit the action of digitalis, and it was suggested by Loewi in 1917 that intravenous administration of potassium salts might abolish glycoside toxicity (118). This approach has been

used in clinical medicine by later investigators (42, 149). Sampson, for example, showed that the ectopic beats caused by toxic doses of digitalis in humans were abolished by the oral administration of enough potassium acetate to cause a 10 to 30% rise in serum potassium concentration usually within an hour (149). Conversely, it was shown that with depletion of body potassium (secondary to gastrointestinal loss, mercurial diuretic administration, or malnutrition) signs of digitalis poisoning occurred at a lower drug dosage than that characteristic of the potassium-repleted state (119). Increasing extracellular potassium concentration is associated with an increase in the minimal lethal dose of glycosides in guinea pigs (82), with an inhibition of the effect of glycosides on the rhythm of the embryonic duck heart (54), with a diminution in toxicity for isolated rabbit heart (4), and with a decreased glycoside effectiveness in the heart-lung preparation(16). In many of these studies potassium is not said to inhibit the action of glycosides on muscle contractility, but is said, rather, to prevent effects on the rhythmicity of the heart (42, 54, 149) as well as reduce toxicity for other tissues [gastrointestinal tract (4, 119)]. The investigators who do report an inhibition of glycoside action on contractile force do not control the heart rate, a factor which influences heart muscle potassium balance (70) and also the effectiveness of the glycosides (179). The question, which cannot be answered at present, is whether potassium inhibits glycoside action by altering rhythmicity in some unknown way or by preventing potassium loss from the muscle cell. There is some evidence (71) which suggests that the intracellular potassium concentration of frog heart muscle is not altered by moderate increases in perfusion fluid potassium above about 1.5 mM/l. Below this level the intracellular potassium concentration falls. One might postulate on the basis of this information that the passive diffusion of potassium out of the cell during contraction is determined mainly by the time available before membrane repolarization occurs rather than by the concentration gradient at least over the range of experimental changes in external K^+ (0 to 10 mM/l); and that the transport mechanism for potassium re-entry is saturated at concentrations of outside potassium above approximately 1.5 mM/l in the case of the frog, so that only below this concentration would the rate of re-entry be diminished. If this is correct, the effect of potassium in inhibiting the toxicity of glycosides in human patients, for example, where the external potassium concentration change is rather small, may not occur by increase of potassium in the heart muscle cells.

An observation which at first seems paradoxical in the light of the work showing inhibition of glycoside effects by increasing external potassium is that in experiments with isolated hearts it is possible to observe disappearance of ouabain-induced contracture when the normal perfusion medium is replaced by a potassium-*free* solution (153). This is assumed to be due to a large increase in membrane potential (70) which follows the profound reduction in external potassium.

VIII. CALCIUM

In contrast to the large store of information about relationships between potassium and muscle contractility, detailed knowledge concerning calcium in muscle

is fragmentary, due in part to the difficulties in measuring the concentration of this ion. Despite the lack of quantitative results, the importance of calcium in muscular contraction was appreciated very early. Ringer (140), in 1883, observed that the contractions of the isolated frog heart disappeared in a calcium-free salt solution and were restored by the addition of calcium. He also noted that a further increase in the calcium concentration "rounds the top, and also broadens the trace of the beat." The effect of calcium on contraction bore some resemblance to the effect of cardiac glycosides, and great interest arose in the combined actions of these two substances. Werschinin, in 1910, for example, found that systolic arrest of a perfused frog heart following the addition of strophanthin was more complete and appeared earlier when the calcium concentration of the perfusion fluid was twice normal (180). A large number of workers after this maintained that the activity of the glycosides as reflected by toxicity in intact animals was enhanced by high serum calcium concentrations (14, 38, 60, 62, 117, 160). [The only exception to these results (129) was later criticized by Gold and Kwit (61) on the basis of inadequate glycoside levels due to the rapid excretion of the drug by rabbits.]

A relationship between calcium and the cellular mechanism of glycoside action was first suggested by Konschegg in Loewi's laboratory (108). After finding that frog heart contractility which had been abolished in a calcium-free medium was restored after the addition of strophanthin, he suggested that the cardiac glycosides could act in the absence of calcium and could perhaps serve as a substitute for calcium. Loewi pursued Konschegg's experimental approach by using sodium oxalate in the medium in order to be more certain of the absence of calcium from the perfusion medium. Under these conditions the heart exhibited no twitch tension, and the addition of strophanthin caused contracture without restoring the twitch (118). He believed that strophanthin acts by increasing the sensitivity of the heart to calcium, a small calcium ion concentration existing in the medium as a result of the dissociation of the calcium oxalate. Without entering into the controversy which centered around Loewi's contention, it might be helpful to state the problem in terms of two possibilities. One is that glycosides act by altering the intracellular concentration of calcium, thereby increasing contractility. The other is that glycosides increase contractility through a mechanism which does not involve a change in intracellular calcium. A choice between the possibilities can be made, then, according to whether or not intracellular calcium concentration is changed by cardiac glycosides. For a positive inotropic effect the expected intracellular change would be an increase in calcium. In at least three papers published several years after Loewi's work it was claimed that the glycosides could act in the absence of external calcium, which could be adduced as indirect evidence that these drugs do not exert their effect by increasing the concentration of intracellular calcium. This view was first stated by Mandelstamm (124) who recognized that although calcium was needed for optimal contractility, in a calcium-free medium the glycoside effect was still detectable, *i.e.*, at a very low level of twitch tension characteristic of the calcium-free medium. The glycosides caused an increase in contractile force, a plateau

of tension, a prolongation of the total duration of contraction and even contracture. The difference in speed of onset of calcium and digitalis actions on contractility (47, 133) has also helped investigators separate the effects of the two substances. Nyiri and DuBois (133) express this as follows. "The digitalis action, therefore, takes place in the regular way and at the regular speed in the presence or absence of calcium. In the absence of this ion, however, we do not see the effect because the heart muscle has lost its inotropic property. At any time that we reestablish the contractility of the muscle fiber in the course of the digitalis poisoning, the effect of the corresponding time appears." In other words, since it appears that the steady progression of glycoside action can occur in the absence of external calcium, the mechanism of action cannot be to cause directly a net gain of intracellular calcium. Of course this conclusion can be open to question since in all these early experiments there still may be traces of calcium in the perfusion medium due to contaminating amounts in other salts or in the glycoside preparations (133). When care is taken to exclude ionic calcium from the perfusion fluid and the glycoside preparation by addition of EDTA, toxic concentrations of glycosides have no detectable effect (72) but addition of calcium causes immediate contracture (73). This indicates that the primary action of glycosides on the muscle cell, which normally is cumulative during the first 10 minutes of exposure, occurs in the virtual absence of external calcium ion, the positive inotropic action becoming apparent imediately on the addition of calcium. The action of glycosides on another tissue, to inhibit the potassium reentry into previously cold stored red blood cells, is likewise not affected by the absence of calcium (121). The evidence reviewed up to this point does not rule out the possibility that the primary action of the glycosides on muscle is to cause some change within the fiber as a result of which calcium accumulates. Such a change would develop slowly in the presence or absence of calcium, but a capacity for the glycoside-treated cell to take up calcium rapidly would have to be postulated in view of the immediate effects noted on shifting from a calcium-free to a normal perfusion medium (73, 133).

Recently, the availability of Ca^{45} has provided an opportunity for measuring the effect of glycosides on the efflux and influx of this ion. Wilbrandt and Caviezel (183) have found that Ca^{45} efflux from frog hearts, previously loaded with tracer either by soaking or by *in vivo* injection 24 hours before sacrifice, is partially inhibited by 2.5 μg/ml of K-strophanthoside. Reports from two other laboratories indicate that the influx of Ca^{45} is not altered by non-toxic doses of cardiac glycosides (79, 166). These results together suggest that the glycosides should cause a net gain of calcium, at least when the concentration of calcium in the medium is normal. The fact that such an increase has not been found (79) can always be attributed to the possibility that the change expected on the basis of sensitive flux measurements is small enough not to be detectable as a gross concentration difference. There is another category of evidence which should be considered before an attempt is made to reach any conclusions. Weizsäcker (179) many years ago showed in a group of carefully designed experiments that the time of onset of contracture after strophanthin was added to the medium

perfusing a frog heart depended not on the duration of action of the drug but on the number of contractions which occurred in the presence of the drug. The heart was pumped artificially at a constant rate to effect adequate mixing and oxygenation, and at the same time was stimulated electrically at different frequencies. At a frequency of 15/min it required 30 minutes and 490 contractions for systolic arrest, whereas at a frequency of 35/min toxicity was apparent in only 14 minutes after approximately the same number of contractions. Similar conclusions have been reached by later workers (151, 182). On the other hand, Niedergerke found that the Ca^{45} efflux from frog heart, measured over a period of 90 minutes, was not altered by the interpolation of a 10-minute interval of rapid stiumlation (130). Now if the inotropic effect of digitalis is achieved because of a diminution in Ca efflux, but Ca efflux is unaffected by stimulation, then one would not expect the time of onset of glycoside toxicity to depend on stimulation frequency as Weizsäcker and others have shown. This argument when considered in addition to the experiments in calcium-free media suggests that the positive inotropic effect of the cardiac glycosides is not caused by a postulated increase in the concentration of intracellular calcium. However, Thomas found that calcium influx was increased when contracture of frog heart was induced by ouabain or a potassium-free solution (166). This is in contrast to the unchanged calcium influx measured in the presence of non-toxic doses of cardiac glycosides (79, 166), and suggests the possibility that a great loss of potassium caused by high concentrations of cardiac glycosides or a potassium-free medium may be associated with a rise in calcium influx.

The interpretation of efflux data is made difficult by the rapidity of change in contractility following a shift in the calcium concentration of the perfusion fluid. For example, Niedergerke published a table for frog ventricle strips (130) which shows that 3 minutes after the external calcium has been changed from 2 mM to 1 mM per liter there is already a 40% loss in tension but only an 18% loss in Ca^{45}. If frog ventricle is washed with a completely calcium-free medium, the twitch tension drops to zero within a few seconds (72). Strophanthidin-induced contracture also disappears within 1 to 3 minutes after replacing with a strophanthidin containing calcium-free solution (73). A similarly rapid change is observed in isolated frog nerve, where excitability disappears within the one-minute period required for shifting from a normal to a calcium-free solution (53). It is likely, then, that much of the calcium important not only for the normal twitch but also for contracture is very rapidly exchangeable, and the efflux curves published for frog ventricle (130, 183) reflect the movement of a more slowly exchanging calcium which is not involved in rapid changes of contractility.

The following hypothetical frame of reference may be useful in thinking about the role of calcium in muscle function. There are three probable sites where calcium is important for a normal twitch. The first is at the cell membrane which, though it cannot function in the complete absence of calcium, is very resistant to calcium deprivation. In the case of frog nerve, for example, the calcium concentration must be reduced below 10 μM/l before the excitability of the membrane is affected (53), and persistence of the action potential of frog

heart muscle has likewise been observed at very low concentrations of calcium (126, 167). Two more loci for the action of calcium need to be postulated because of the differences in calcium sensitivity for twitch and contracture. The magnitude of the twitch tension is very sensitive to changes in external calcium concentration, and rapidly drops to zero when perfusion fluid calcium is removed. It seems probable that this is due to depletion of a calcium-sensitive mechanism responsible for propagation of excitation from the membrane to the contractile protein rather than to failure of the contractile protein itself, since under such conditions of calcium depletion the contractile protein can be made to go into contracture without restoration of a normal twitch. These non-propagated responses (contractures) can be achieved with cardiac glycosides in a medium deficient enough in calcium to eliminate twitch tension (118) and also by the recently described cardiotonic protein system which can cause contracture in the complete absence of outside calcium ions (72). In other words, if one imagines a sequence of processes leading to the normal twitch, the first site where calcium is needed has to do with membrane excitability, the second calcium-sensitive locus has to do with propagation of excitation from the membrane inwards, and there is a third site beyond this, possibly at the contractile protein, which can be influenced to cause contracture. There is reason to believe that this third site also requires calcium, since the contractures mentioned above are induced either by cardiac glycosides which fail to act in the complete absence of external calcium or by the cardiotonic protein system which is thought to act by transporting its protein-bound calcium into a particular locus in the muscle cell.

This discussion cannot be concluded without considering the opposing actions of calcium and monovalent cations on contractility. Ringer found that the effects of high calcium in the perfusion medium could be abolished by the addition of potassium (140). It was later shown that the contractile response in a sodium-deficient medium was similar to that caused by high calcium or strophanthin and it was stated that "lack of potassium or sodium and excess of calcium all produce increase of systolic tone in the heart" (34). These results have been confirmed and extended by Wilbrandt and Koller (184), and by Niedergerke and Lüttgau (132) who showed that variations in peak tension with shifts in the concentration of sodium and calcium of the perfusion medium could be expressed as a single curve if tension peak were plotted against the ratio $[Ca^{++}]/[Na^{+}]^2$. If the results of these experiments are reflections of changes in intracellular ion content, then they are at least qualitatively in agreement with conclusions derived from material reviewed above which can be summarized as follows. The capacity of cardiac contractile protein to develop tension appears to be enhanced 1) by a decrease in the total intracellular monovalent cation (*i.e.*, the sum of sodium and potassium), and 2) by an increase in some moiety of intracellular calcium. One or both of these sets of protein-cation relationships may be influenced by alterations in stimulus frequency or external ion concentration and by addition of cardiac glycosides or the cardiotonic protein system, as discussed in the foregoing sections.

Niedergerke and Harris have reported that Ca^{45} efflux is diminished if sodium

or potassium ions are eliminated from the perfusion medium (133). These results, like those of Thomas *et al.* (166), suggest the possibility that a part of the antagonistic effects of sodium and potassium versus calcium is due to alterations in transmembrane fluxes. The experiments reported, however, relate so far only to extreme changes in perfusion medium.

IX. CONGESTIVE HEART FAILURE

A review on the cellular action of digitalis would be incomplete without some consideration of the human disease for which its use is frequently so efficacious. Digitalis is most effective in the varieties of heart failure characterized by an impairment in energy utilization, and it is this group which will be under discussion below. Certain other types of cardiac failure will not be included in the present treatment, such as those due to 1) known defect in energy liberation, *e.g.*, beri-beri heart disease; 2) infarction of a large portion of previously normal ventricular muscle; 3) mechanical impairment of normal venous inflow, as in constrictive pericarditis; 4) sudden change in circulatory dynamics caused, for example, by rupture of chordae tendineae or acute formation of an arteriovenous fistula. The most common examples of the group under consideration are chronic congestive heart failure secondary either to an increased work load (as in hypertension or certain types of valvular disease) or to so-called arteriosclerotic heart disease. In all these cases cardiac hypertrophy occurs first, without clinical signs of myocardial insufficiency, but sooner or later progressive dilatation of the heart and the development of frank congestive failure supervene. The essence of this development of failure appears to be a progressive decline in contractility defined as the capacity per gram of muscle to develop tension at a specified fiber length. A roughly analogous viewpoint has appeared many times in the literature. For example, "... in both the precongestive and congestive phase, a failing ventricle is one which does not respond to a given venous pressure with an output as great as that of the normal ventricle. The pressure-output curve is displaced downwards" (196). The venous pressure-output curve is not, of course, strictly speaking, a measure of contractility. The best approximation in the intact human is a curve of left ventricular stroke work plotted against pulmonary capillary pressure (115); (with the availability of left heart catheterization techniques, left ventricle end diastolic pressure can now be measured). This still leaves to be conjectured the weight of the muscle mass and the relationship between diastolic pressure and fiber length before an estimate of myocardial contractility can be made.

Moderately rigorous definitions should be applied in considering certain questions such as, for example, whether contractility of the ventricular muscle is diminished in the case of hypertrophy before development of frank congestive failure. The statement that contractility is decreased is based on the fact that for a given rise in venous pressure induced by intravenous infusions the increase in cardiac output is less than in normals. But, if the elastic modulus of the hypertrophied heart remained unchanged the fiber length at any given venous pressure

would be less than for the normal heart,[5] so that the fiber length increments for a given venous pressure rise would not be comparable. And even if the fiber lengths were known, any difference observed in stroke work would have to be interpreted in the light of the relationship between the contractile force exerted by the muscular wall of a spherical organ and the resulting hydrostatic pressure produced, which varies markedly with the radius of the spherical structure. The statement quoted in the previous paragraph therefore does not constitute evidence that contractility of the ventricle in the "pre-congestive" phase is necessarily diminished.

Having defined impaired contractility as an essential feature of cardiac failure, one is faced with the question of what causes the decline in contractile capacity. The onset of frank congestive failure frequently occurs without any apparent precipitating event. The chronic hypertensive with cardiac hypertrophy who has exhibited no signs of decompensation for many years frequently at some point begins to develop myocardial insufficiency in the absence of infarction, or further increase in blood pressure. The reason for the decline in contractility would not seem to be due to impaired oxygenation secondary to the hypertrophy as suggested by Harrison (76), since hypertrophy may have existed many years before obvious impairment in contractility begins. Nor does it appear that cardiac failure is due to a diminished availability of any other link in the chain of energy supply needed for contraction (188). The remaining alternatives are that a primary change in the contractile protein occurs, or that a change in the cellular environment of this protein alters its contractility.

Evidence that congestive heart failure is associated with a change in the contractile protein has been presented from two laboratories up to the present time. The more recent work, already referred to in the section on the effect of glycosides on muscle proteins, is that of Kako and Bing (98), who reported a diminished contractility of actomyosin bands prepared from the hearts of patients with congestive failure. The experimental material consisted of 10 to 20 g samples of heart muscle from 12 patients removed from 1 to 6 hours after death in congestive failure secondary to arteriosclerotic or hypertensive heart disease. The authors maintain that contractility of actomyosin bands was unaffected by death-to-autopsy intervals of up to 6 hours. It is not specified whether the muscle was taken from the right or left ventricle.

The earlier report by Benson (6) was based on heart muscle removed from dogs with experimental heart failure caused by the surgically induced lesions of pulmonary stenosis and tricuspid insufficiency. It should be noted at this

[5] If for simplicity one expressed the elasticity of a cardiac muscle bundle in terms of Young's modulus, in which changes in length but not changes in cross-sectional area are considered, the expression for the elastic modulus can be written $Y = \frac{Fl_0}{A\Delta l}$ in which F is the force applied (corresponding to end-diastolic pressure), A is the cross-sectional area, l_0 is the unstretched length and Δl is the change which occurs when the force is applied. If Y is unchanged in the hypertrophied heart, since A is increased F must also be increased to obtain the same fractional change in fiber length as occurs in the normal.

point that the sequence of cardiac hypertrophy followed some time later by myocardial insufficiency has not been reported for experimental animals. Generally, the methods employed to induce failure cause either death within a short period of time following surgery, or myocardial hypertrophy without insufficiency (which might develop if the animals were followed many months or years). Benson's dogs had edema, ascites, and elevated right atrial pressures, but unlike chronic congestive failure in human patients there was no cardiac hypertrophy. When compared with the controls, it was found that both right and left ventricular muscle samples contained a diminished concentration of actomyosin, measured as a percentage of total tissue protein to avoid the effects of changes in water content of the muscle. Actomyosin was determined according to the weight of the protein precipitate or by an estimate based on the viscosity drop when ATP is added to the solution. At least part of the apparent diminution in actomyosin was thought to be due to dissociation of actin and myosin, since control actomyosin preparations revealed a single peak in the ultracentrifuge, whereas the experimental group showed a second peak, possibly uncombined myosin. A recent report indicates that the contractility of glycerol-extracted muscle fibers prepared from hearts of the experimental group is lower than normal (8).

If one accepts as correct the results of these technically difficult experiments, the question remaining is whether the alterations in the actomyosin caused the cardiac failure or were a result of it. It has frequently been noted in the case of Benson's paper that the muscle protein changes were found in both ventricles despite the fact that the increased work load was limited to the right. Although there are many exceptions, in general one would expect the development of chronic congestive failure in a human with hemodynamically comparable lesions to be predominantly right-sided.[6] It is possible, therefore, that the protein changes noted are secondary to the heart failure, associated as it is with progressive emaciation and probably impaired function of many organs. Since Kako and Bing do not analyze right and left ventricular muscle separately, their human autopsy material casts no additional light on this problem. As will be seen below, the evaluation of tissue electrolyte changes in cardiac failure also centers around the question whether the observed changes have anything to do with causing impaired contractility or whether they are secondary to the chronic hypoxia and other abnormalities which are the result of the myocardial insufficiency. The problem regarding both electrolyte and protein changes could perhaps be resolved by examining cases of less advanced cardiac failure who die suddenly of other causes.

Benson suggests that the actomyosin changes which he found might be secondary to the dilatation of the heart chambers, the heart failure thus being due to a dissociation of the actomyosin brought about by the excessive stretching caused

[6] "Likewise, persons with a high degree of mitral stenosis in whom during life the signs of striking mitral insufficiency are absent are found at necropsy to have enlargement consisting of both hypertrophy and dilatation of the right ventricle, and the left ventricle in such instances may even be atrophic" (76, p. 89).

by the increased work load. Such an explanation might apply to his experimental animals, but would not account for the slow development of myocardial insufficiency in, for example, the chronically hypertensive human which may begin only after several years of a permanently increased work load. In this situation, since there is no new increase in work load at the time failure and dilatation begin, some change in the quality of the actomyosin must precede and be the cause of any alteration in the size of the chambers at that stage of the disease.

The alternative explanation for the diminished contractility characteristic of congestive heart failure is that instead of a primary change in the actomyosin itself observable in the isolated protein, there is some alteration in the intracellular environment which results in a diminished contractility of the protein. Such an alteration might occur because of an intrinsic change in a cellular regulatory function, or because of a change in a hypothetical circulating cardiotonic system which could function normally to affect contractility of cardiac muscle. The measurement of changes in the intracellular ionic environment of cardiac muscle in congestive heart failure has been a favorite subject of investigation since the early work of Harrison and others (17, 22, 77, 94, 124, 185). It has generally been found that the heart muscle of patients who die of congestive heart failure is low in potasium and high in sodium, expressed as mEq/100 g of dried heart to eliminate differences due to variations in water content (17, 22, 77, 94, 125, 185). No significant alterations in the sum of tissue sodium and potassium are apparent, on the basis of published measurements. Although not specified in the earlier work (17, 77, 125, 185), it is likely that many of the patients in congestive failure were digitalized at the time of death. From the eleven patients in the series of Clarke *et al.* (22) who were not digitalized, however, it is clear that the fall in potassium and rise in sodium may occur in the absence of digitalis. (In fact, these authors believe that the administration of digitalis causes an *increase* in cellular potassium and a decrease in sodium, thus shifting the ionic pattern back towards normal.)

The most reasonable interpretation of these findings is that the observed increase in tissue sodium and decrease in potassium do not cause myocardial insufficiency, but are the result of it. Similar alterations have been seen in the skeletal muscle of patients with non-cardiac edema (77), and are known to occur whenever tissue metabolism is impaired (30, 157, 159). Digitalis is thought to improve myocardial contractility not by *increasing* cellular potassium as Clarke *et al.* believe, but by some other mechanism, after which as a result of improved tissue metabolism a shift in the Na/K ratio towards normal may occur. It may be argued that the cardiotonic action of digitalis could hardly be expected to occur by decreasing intracellular potassium, since the potassium concentration in the muscle of hearts in congestive failure is already low. However, the total intracellular sodium plus potassium is probably not low; and in fact in the case of isolated tissues impairment of normal metabolism is associated with a greater gain of sodium than loss of potassium (30, 157). Thus it is possible, according to the view presented on page 192, that the glycosides may increase contractility in the failing heart by causing a further decrease in potassium without a corre-

sponding sodium gain and therefore a decrease in the sum of sodium plus potassium. The shift in the Na/K ratio towards normal then follows the improvement in contractility. It should be noted in passing that the decrease in intracellular potassium induced by glycosides (which can occur without a corresponding gain in sodium) is very small compared to that which occurs during hypoxia (and is accompanied by at least an equivalent gain in sodium). The former is associated with increased contractility, the latter with its deterioration.

Whatever the mode of action of the cardiac glycosides, the improvement effected by the continued administration of these drugs is temporary, and sooner or later the progressive diminution in contractility characteristic of chronic congestive heart failure becomes apparent. [The transient effect of digitalis in spontaneously failing isolated heart preparations is comparable, even though the mechanism of failure may be different (150)]. It does not appear that the deterioration is due to development of tolerance to the glycosides, since at least the toxic effects continue to become evident at approximately the same dosage level as originally. In other words, the glycosides do not appear to arrest permanently the downhill course and therapy is not specific, as for example, thyroid hormone is for myxedema. It would seem, therefore, that the cause of the failure and the mechanism of improvement by glycosides are different; and if the latter is by a primary effect on the monovalent ions, the former must occur in some other way.

Since it is difficult to implicate shifts in intracellular monovalent cations as the cause of congestive heart failure, the calcium ion should be considered because of its well-known effect on muscle contractility. The problem is hard to approach by measurement of total muscle calcium, not only because of the difficulty in determining calcium but also because there may be several calcium pools and only a part of the total may be related to contractility. It is further complicated by the fact that not only ionized calcium but also certain protein-bound calcium in the serum may have cardiotonic activity (72). Therefore, additional research will be needed to determine whether alterations in cellular calcium may be one of the causes of congestive heart failure in man.

X. CONCLUSIONS

In this final section an attempt will be made to justify the title of this review, by recapitulating the main evidence from which some picture of the cellular basis of glycoside action can be drawn. It was argued in the sections on metabolism and congestive heart failure that most cardiac failure was secondary to an impairment in energy utilization and that the salutary action of the glycosides was related to an increase in the capacity of the contractile protein to convert the available chemical energy into mechanical work. The question of how the glycosides effect such a change has currently been resolved into two alternatives: 1) alteration of the intracellular ionic environment; and 2) direct action on the muscle protein. The evidence that cardiac glycosides, in therapeutic as well as toxic concentrations, cause a loss of potassium from cardiac muscle is now widely substantiated. The reviewers believe that this potassium loss is causally related to the increase in contractility, since positive inotropic effects can also be produced in other ways which lead to a diminution in intracellular potassium (high

stimulation frequency, low-potassium perfusion medium). The fact that a diminution in intracellular sodium also enhances contractility leads to the idea that the muscle protein is affected by the sum of intracellular sodium and potassium, so that it is the total monovalent cation relative to the amount of protein which is important, a decrease causing increased contractility and *vice versa*. It does not appear that the glycosides have any direct effect on cellular calcium, although because of the importance of this ion for normal muscle contractility it has long been studied in relation to cardiotonic drug action.

The possibility that the glycosides exert an additional effect directly on the contractile protein cannot be ruled out, especially in view of the interesting observation that a correlation can be made between the ability of the glycosides to increase the potassium binding of isolated actomyosin and the pharmacological activity of the drugs *in vivo*. Further work along these lines is needed, since at this point it has not been established whether the glycosides enter the cell interior and, if so, whether the effects on potassium binding cause an increased contractility of the muscle protein.

It has been beyond the scope of this review to describe the studies dealing with the action of cardiac glycosides on ion movements in other tissues. Suffice it to say that these drugs appear to influence ion fluxes in kidney, red blood cells, blood vessels, stomach, large intestine and thyroid; and the ion affected is not always potassium, as it appears to be in the case of cardiac muscle. This comparison suggests the interesting possibility that the cellular mechanism for transporting different ions consists of a part which is common to many cells and an additional part which confers ionic specificity to the system. The cardiac glycosides, then, would appear to act on the non-specific part of the system, a postulation which can perhaps be tested by future research.

REFERENCES

1. Adrian, R. H.: The effect of internal and external potassium concentration on the membrane potential of frog muscle. J. Physiol. 133: 631–658, 1956.
2. Aikawa, J. K., and Rhoades, E. L.: Effects of digitoxin on exchangeable and tissue potassium contents. Proc. Soc. exp. Biol., N.Y. 90: 332–335, 1955.
3. Alella, A., Williams, F. L., Bolene-Williams, C. and Katz, L. N.: Role of oxygen and exogenous glucose and lactic acid in the performance of the heart. Amer. J. Physiol. 185: 487–494, 1956.
4. Baker, J. B. E.: The influence of calcium and potassium ions on the toxicity of ouabain. Brit. J. Pharmacol. 2: 259–267, 1947.
5. Benforado, J. M.: Frequency-dependent pharmacological and physiological effects on the rat ventricle strip. J. Pharmacol. 122: 86–100, 1958.
6. Benson, E. S.: Composition and state of protein in heart muscle of normal dogs and dogs with experimental myocardial failure. Circulation Res. 3: 221–228, 1955.
7. Benson, E. S., Hallaway, B. E. and Freier, E. F.: Extraction and quantitative characteristics of actomyosin in canine heart muscle. Circulation Res. 3: 215–220, 1955.
8. Benson, E. S., Hallaway, B. E. and Turbak, G. E.: Contractile properties of glycerol-extracted muscle bundles from the chronically failing canine heart. Circulation Res. 6: 122–128, 1958.
9. Bing, R. J., Maraist, F. M., Dammann, J. F., Jr., Draper, A., Jr., Heimbecker, R., Daley, R., Gerard, R. and Calazel, P.: Effect of strophanthus on coronary blood flow and cardiac oxygen consumption of normal and failing human hearts. Circulation 2: 513–516, 1950.
10. Blain, J. M., Eddleman, E. E., Siegel, A. and Bing, R. J.: Studies on myocardial metabolism: V. The effects of lanatoside-C on the metabolism of the human heart. J. clin. Invest. 35: 314–321, 1956.
11. Bollman, J. L. and Flock, E. V.: Phosphocreatine and inorganic phosphate in working and resting muscle of rats, studied with radioactive phosphorus. J. biol. Chem. 147: 155–165, 1943.
12. Bowditch, H. P.: Über die Eigenthumlichkeiten der Reizbarkeit, welche die Muskelfasern des Herzens zeigen. Arb. a.d. Physiol. zu Leips. 6: 139–176, 1871.
13. Bowen, W. J. Effect of digoxin upon rate of shortening of myosin B threads. Fed. Proc. 11: 16, 1952.

14. Bower, J. O. and Mengle, H. A. K.: The additive effect of calcium and digitalis. J. Amer. med. Ass. 106: 1151–1153, 1936.
15. Boyer, P. K., and Poindexter, C. A.: The influence of digitalis on the electrolyte and water balance of heart muscle. Amer. Heart J. 20: 586–591, 1940.
16. Burstein, S. G., Bennett, L. L., Payne, F. E. and Hopper, J., Jr.: Effects of potassium and lanatoside-C on the failing heart in heart-lung preparations. Fed. Proc. 8: 20, 1948.
17. Calhoun, J. A., Cullen, G. E., Clarke, G. and Harrison, T. R.: Studies in congestive heart failure: vi. The effect of overwork and other factors on the potassium content of the cardiac muscle. J. clin. Invest. 9: 393–403, 1930.
18. Calhoun, J. A. and Harrison, T. R.: Studies in congestive heart failure: ix. The effect of digitalis on the potassium content of the cardiac muscle of dogs. J. clin. Invest. 10: 139–144, 1931.
19. Cattell, McK. and Gold, H.: The relation of rhythm to the force of contraction of mammalian cardiac muscle. Amer. J. Physiol. 133: 236P–237P, 1941.
20. Cattell, McK. and Gold, H.: Relation of rhythm to force of contraction of mammalian cardiac muscle. Amer. J. Physiol. 182: 307–310, 1955.
21. Causey, G. and Harris, E. J.: The uptake and loss of phosphate by frog muscle. Biochem. J. 49: 176–182, 1951.
22. Clarke, N. E. and Moscher, R. E.: The water and electrolyte content of the human heart in congestive heart failure with and without digitalization. Circulation 5: 907–914, 1952.
23. Cohn, A. E. and Stewart, H. J.: The relation between cardiac size and cardiac output per minute following the administration of digitalis in normal dogs. J. clin. Invest. 6: 53–77, 1928.
24. Conn, H. L., Jr.: Effects of digitalis and hypoxia on potassium transfer and distribution in the dog heart. Amer. J. Physiol. 184: 548–552, 1956.
25. Conway, E. J.: Nature and significance of concentration relations of potassium and sodium ions in skeletal muscle. Physiol. Rev. 37: 84–132, 1957.
26. Coraboeuf, E., De Lozé, C. and Boistel, J.: Action de la digitale sur les potentiels de membrane et d'action du tissu conducteur du coeur de chien étudiée à l'aide de microélectrodes intracellulaires. C. R. Soc. Biol., Paris, 147: 1169–1172, 1953.
27. Cowle, J. B. and Thorp, R. H.: Action of cortisone on the polymerisation of actin. Nature, Lond. 171: 1067, 1953.
28. Cranefield, P. F. and Hoffman, B. F.: Electrophysiology of single cardiac cells. Physiol. Rev. 38: 41–76, 1958.
29. Creese, R.: Bicarbonate ion and striated muscle. J. Physiol. 110: 450–457, 1949.
30. Creese, R.: Measurement of cation fluxes in rat diaphragm. Proc. roy. Soc., ser. B 142: 497–513, 1954.
31. Csapo, A. I. Studies on the adenosine-triphosphatase activity of the uterine muscle. Acta physiol. scand. 19: 100–114, 1949.
32. Csapo, A. I. and Corner, G. W.: The antagonistic effects of estrogen and progesterone on the staircase phenomenon in uterine muscle. Endocrinology 51: 378–385, 1952.
33. Dale, A. S.: The staircase phenomenon in ventricular muscle. J. Physiol. 75: 1–16, 1932.
34. Daly, I. DeB. and Clark, A. J.: The action of ions upon the frog's heart. J. Physiol. 54: 367–383, 1920.
35. Dettli, L. and Bing, R. J.: Contractile properties of actomyosin threads and bands prepared from dog heart. Amer. J. Physiol. 187: 145–150, 1956.
36. Dettli, L. and Bing, R. J.: Contractility and extractibility of heart actomyosin after death. Circulation Res. 4: 519–522, 1956.
37. Dudel, J. and Trautwein, W.: Electrophysiologische Messungen zur Strophanthinwirkung am Herzmuskel. Arch. exp. Path. Pharmak. 232: 393–407, 1958.
38. Edens, E. and Huber, J. E.: Über Digitalis-bigeminie. Dtsch. Arch. klin. Med., Leips. 118: 476–494, 1916.
39. Edman, K. A. P.: Wirkung des Ouabains auf das Aktomyosin der Herzmuskulatur. Experientia 7: 71–72, 1951.
40. Edman, K. A. P.: Action of cardiac glycosides on the ATP-induced contraction of glycerinated muscle fibers. Acta physiol. scand. 30: 69–79, 1953.
41. Eismayer, G. and Quincke, H.: Stoffwechseluntersuchungen am Kaltblüterherzen: V. Mitteilung. Einfluss von Strophanthin und Hexeton auf Atmung und Arbeit des Herzens. Arch. exp. Path. Pharmak. 156: 308–315, 1930.
42. Enselberg, C. D., Simmons, H. G. and Mintz, A. A.: The effects of potassium upon the heart, with special reference to the possibility of treatment of toxic arrhythmias due to digitalis. Amer. Heart J. 39: 713–728, 1950.
43. Evans, C. L. and Matsuoka, Y.: The effect of various mechanical conditions on the gaseous metabolism and efficiency of the mammalian heart. J. Physiol. 49: 378–405, 1915.
44. Fenn, W. O. and Cobb, D. M.: Electrolyte changes in muscle during activity. Amer. J. Physiol. 115: 345–356, 1936.
45. Fenn, W. O., Cobb, D. M., Manery, J. F. and Bloor, W. R.: Electrolyte changes in cat muscle during stimulation. Amer. J. Physiol. 121: 595–608, 1938.
46. Finkelstein, M. and Bodansky, O.: The effect of cardiac glycosides on the respiration of cardiac muscle. J. Pharmacol. 94: 274–287, 1948.
47. Fischer, H.: Beitrag zur Frage des Synergismus zwischen Digitalis- und Calciumwirkung. Arch. exp. Path. Pharmak. 130: 194–241, 1928.
48. Fleckenstein, A. and Janke, J.: Papierchromatographische Trennung von ATP, ADP und anderen Phosphor-Verbindungen im kontrahierten und erschlafften Froschmuskel. Pflüg. Arch. ges. Physiol. 258: 177–190, 1953.
49. Fleckenstein, A. and Janke, J.: Der Austausch von radioaktivem, P^{32}-markiertem Orthophosphat mit dem P^{α}, P^{β} und P^{γ} von ATP und mit Kreatinphosphat bei Muskelruhe, Temperaturvariation und electrischer Reizung. Pflüg. Arch. ges. Physiol. 265: 237–263, 1957.

50. FLECKENSTEIN, A., JANKE, J., DAVIES, R. E. AND KREBS, H. A.: Chemistry of muscle contraction: Contraction of muscle without fission of adenosine triphosphate or creatine phosphate. Nature, Lond. 174: 1081–1083, 1954.
51. FLECKENSTEIN, A., JANKE, J., KRUSE, R. AND LECHNER, G.: Über das Verhalten von Adenosintriphosphat, Adenosindiphosphat und anderen Phosphorsäure-Estern bei der Kontraktur des Froschrectus durch Acetylcholin, Nicotin, und Succinylbischolin. Arch. exp. Path. Pharmak. 224: 465–475, 1955.
52. FLECKENSTEIN, A., JANKE, J., LECHNER, G. AND BAUER, G.: Zerfällt Adenosintriphosphat bei der Muskelkontraktion? Pflüg. Arch. ges. Physiol. 259: 246–266, 1954.
53. FRANKENHAEUSER, B.: The effect of calcium on the myelinated nerve fiber. J. Physiol. 137: 245–260, 1957.
54. FRIEDMAN, M. AND BINE, R.: Observations concerning the influence of potassium upon the action of a digitalis glycoside (lanatoside-C). Amer. J. med. Sci. 214: 633–638, 1947.
54a. FURCHGOTT, R. F. AND DE GUBAREFF, T.: The high energy phosphate content of cardiac muscle under various experimental conditions which alter contractile strength. J. Pharmacol. 124: 203–218, 1958.
55. GARB, S. AND PENNA, M.: Some quantitative aspects of the relation of rhythm to the contractile force of mammalian ventricular muscle. Amer. J. Physiol. 182: 601–606, 1955.
56. GENUIT, H. AND HAARMANN, W.: Zur Frage der Spezifität der Digitalisstoffwechselwirkungen an Herz- und Skeletmuskulatur von Ratte und Meerschweinchen. Arch. exp. Path. Pharmak. 196: 481–492, 1940.
57. GERTLER, M. M., KREAM, J., HYLIN, J. W., ROBINSON, H. AND NEIDLE, E. G.: Effect of digitoxin and quinidine on intracellular electrolytes of the rabbit heart. Proc. Soc. exp. Biol., N.Y. 92: 629–632, 1956.
58. GLYNN, I. M.: Action of cardiac glycosides on red cells. J. Physiol. 128: 56P–57P, 1955.
59. GLYNN, I. M.: The action of cardiac glycosides on sodium and potassium movements in human red cells. J. Physiol. 136: 148–173, 1957.
60. GOLD, H. AND EDWARDS, D. J.: The effects of ouabain on the heart in the presence of hypercalcemia. Amer. Heart J. 3: 45–50, 1927.
61. GOLD, H. AND KWIT, N.: Digitalis and calcium synergism. Science 86: 330–331, 1937.
62. GOLDEN, J. S. AND BRAMS, W. A.: Mechanism of the toxic effects from combined use of calcium and digitalis. Ann. intern. Med. 11: 1084–1088, 1938.
63. GOLDMAN, D. E.: Potential, impedance and rectification in membranes. J. gen. Physiol. 27: 37–60, 1943.
64. GOLLWITZER-MEIER, K. AND KRÜGER, E.: Zur Verschiedenheit der Herzenergetik und Herzdynamik bei Druck- und Volumleistung. Pflüg. Arch. ges. Physiol. 238: 279–289, 1936.
65. GONLUBOL, F., SIEGEL, A. AND BING, R. J.: Effect of a cardiac glycoside (cedilanid) on the sodium and potassium balance of the human heart. Circulation Res. 4: 298–301, 1956.
66. GREINER, T.: The relationship of force of contraction to high-energy phosphate in heart muscle. J. Pharmacol. 105: 178–195, 1952.
67. GREMELS, H.: Zur Physiologie und Pharmakologie der Energetik des Säugetierherzens. Arch. exp. Path. Pharmak. 169: 689–723, 1933.
68. HAARMANN, W., HAGEMEIER, A. AND LENDLE, L.: Über die Bindung von Digitalisglykosiden und Digitaloiden an die Eiweissstoffe des Blutserums. Arch. exp. Path. Pharmak. 194: 205–216, 1940.
69. HAGEN, P. S.: The effects of digilanid C in varying dosage upon the potassium and water content of rabbit heart muscle. J. Pharmacol. 67: 50–55, 1939.
70. HAJDU, S.: Mechanism of staircase and contracture in ventricular muscle. Amer. J. Physiol. 174: 371–380, 1953.
71. HAJDU, S.: Bioassay for cardiac active principles based on the staircase phenomenon of the frog heart. J. Pharmacol. 120: 90–98, 1957.
72. HAJDU, S. AND LEONARD, E.: A serum protein system affecting contractility of the frog heart, present in increased amounts in patients with essential hypertension. Circulation Res. 6: 740–750, 1948.
73. HAJDU, S. AND LEONARD, E.: Unpublished observation.
74. HAJDU, S. AND SZENT-GYÖRGYI, A.: Action of DOC and serum on the frog heart. Amer. J. Physiol. 168: 159–170, 1952.
75. HARRIS, A. S., FIRESTONE, J. E. AND LIPTAK, R. A.: Relation of potassium ions in extracellular fluid to ectopic ventricular arrhythmias induced by K-strophanthoside. Proceedings of the 28th Scientific Sessions of the A.M.A., 1955, p. 52, Chicago, 1955.
76. HARRISON, T. R.: Failure of the circulation, pp. 82, 89. Williams and Wilkins Co., Baltimore, 1935.
77. HARRISON, T. R., PILCHER, C. AND EWING, G.: Studies in congestive heart failure. IV. The potassium content of skeletal and cardiac muscle. J. clin. Invest. 8: 325–335, 1930.
78. HARVEY, S. C.: Radiophosphorus metabolism of the guinea pig heart and the action of digitoxin and pentobarbital. Amer. J. Physiol. 183: 559–564, 1955.
79. HARVEY, S. C. AND DANIEL, E. E.: Possible interrelationship of digitoxin and calcium studied with radiocalcium on the isolated guinea pig heart. J. Pharmacol. 106: 394, 1952.
80. HARVEY, S. C. AND PIEPER, G. R.: Intracellular distribution of digitoxin-C^{14} in the heart. J. Pharmacol. 114: 14–27, 1955.
81. HAYASHI, T.: Contractile properties of compressed monolayers of actomyosin. J. gen. Physiol. 36: 139–152, 1952.
82. HAZARD, R. AND HAZARD, J.: Variations de la sensibilité à la digitaline en fonction de la kaliémie. C. R. Soc. Biol., Paris 146: 832–834, 1952.
83. HELLEMS, H. K., REGAN, T. J., TALMERS, F. N., CHRISTENSEN, R. C. AND WADA, T.: The mode of action of acetyl strophanthidin on the failing human heart. J. clin. Invest. 35: 710, 1956.
84. HELMREICH, E. AND SIMON, K.: Zur Frage der Wirkung von Digitalisstoffen auf das Aktomyosin und die Adenosintriphosphatase des Herzmuskels. Z. Naturf. 7b: 341–344, 1952.
85. HERMANN, R. G.: Studies on the effect of ouabain on cardiac muscle respiration. Arch. int. Pharmacodyn. 81: 235–242, 1950.

86. Hermann, R. G., Flamboe, G. E. and Chen, K. K.: The effect of nine cardiac steroids and epinephrine on the respiration of heart muscle slices. J. Pharmacol. 112: 22–28, 1954.
87. Hill, A. V.: The abrupt transition from rest to activity in muscle. Proc. roy. Soc., ser. B 136: 399–420, 1949.
88. Hodgkin, A. L.: The ionic basis of electrical activity in nerve and muscle. Biol. Rev. 26: 339–409, 1951.
89. Hodgkin, A. L. and Katz, B.: The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol. 108: 37–77, 1949.
90. Hoffman, B. F., Bindler, E. and Suckling, E. E.: Postextrasystolic potentiation of contraction in cardiac muscle. Amer. J. Physiol. 185: 95–102, 1956.
91. Hogeboom, G. H., Schneider, W. C. and Pallade, G. E.: Cytochemical studies of mammalian tissues: I. Isolation of intact mitochondria from rat liver; some biochemical properties of mitochondria and submicroscopic particulate material. J. biol. Chem. 172: 619–635, 1948.
92. Holland. W. C., Greig, M. E. and Dunn, C. E.: Factors affecting the action of lanatoside C on the potassium content of isolated perfused guinea pig hearts. Amer. J. Physiol. 176: 227–231, 1954.
93. Horvath, I., Kiraly, C. and Szerb, J.: Action of cardiac glycosides on the polymerisation of actin. Nature, Lond. 164: 792, 1949.
94. Isseri, L. T., Alexander, L. C., McCaughey, R. S., Boyle, A. J. and Myers, G. B.: Water and electrolyte content of cardiac and skeletal muscle in heart failure and myocardial infarction. Amer. Heart J. 43: 215–227, 1952.
95. Joyce, C. R. B. and Weatherall, M.: Cardiac glycosides and the potassium exchange of human erythrocytes. J. Physiol. 127: 33P, 1955.
96. Kahn, J. B., Jr.: Effects of various lactones and related compounds on cation transfer in incubated cold-stored human erythrocytes. J. Pharmacol. 121: 234–251, 1957.
97. Kahn, J. B., Jr. and Acheson, G. H.: Effects of cardiac glycosides and other lactones, and of certain other compounds, on cation transfer in human erythrocytes. J. Pharmacol. 115: 305–318, 1955.
98. Kako, K. and Bing, R. J.: Contractility of actomyosin bands prepared from normal and failing human hearts. J. clin. Invest. 37: 465–470, 1958.
99. Kanda, Z., Sekiya, A., Sakai, K., Ueno, N. and Kamei, T.: Studies on the potassium content of the heart muscle receiving k-strophanthin and the influence of potassium on the respiration of heart muscle. Jap. J. Pharmacol. 6: 105–114, 1957.
100. Katz, L. N.: Analysis of the several factors regulating the performance of the heart. Physiol. Rev. 35: 91–106, 1955.
101. Katz, L. N., Jochim, K., Lindner, E. and Landowne, M.: The effect of varying resistance-load and input-load on the energetics of the surviving mammalian heart. Amer. J. Physiol. 134: 636–644, 1941.
102. Katz, L. N. and Long, C. N. H.: Lactic acid in mammalian cardiac muscle: I. The stimulation maximum. Proc. roy. Soc., ser. B 99: 8–19, 1925.
103. Katzung, B. and Farah, A.: Influence of temperature and rate on the contractility of isolated turtle myocardium. Amer. J. Physiol. 184: 557–562, 1956.
104. Katzung, B., Rosin, H. and Schneider, F.: Frequency-force relationship in the rabbit auricle and its modification by some metabolic inhibitors. J. Pharmacol. 120: 324–333, 1957.
105. Kelly, J. J. and Hoffman, B. F.: Mechanical behavior of the rat papillary muscle. Fed. Proc. 17: 84, 1958.
106. Keynes, R. D. and Lewis, P. R.: The intracellular calcium contents of some invertebrate nerves. J. Physiol. 134: 399–407, 1956.
107. Kiese, M. and Garan, R. S.: Mechanische Leistung, Grösse und Sauerstoffverbrauch des Warmblüterherzens. Arch. exp. Path. Pharmak. 188: 226–246, 1938.
108. Konschegg, A. von: Über Beziehungen zwischen Herzmittel- und physiologischer Kationenwirkung. Arch. exp. Path. Pharmak. 71: 251–260, 1913.
109. Korey, S.: Some factors influencing the contractility of a non-conducting fiber preparation. Biochim. biophys. Acta 4: 58–67, 1950.
110. Kruta, V.: Rhythmic activity of cardiac muscle. I. Variation of mechanical response as function of rhythm. Arch. int. Physiol. 45: 332–357, 1937.
111. Kuschinsky, G., Lange, G. and Turba, F.: Über die Wirkung von Digitoxin auf die Freilegung von Actomyosin und seinen Komponenten aus den Strukturen des Muskels. Arch. exp. Path. Pharmak. 215: 250–260, 1952.
112. Langemann, H., Brody, T. M. and Bain, J. A.: *In vitro* effects of ouabain on slices and mitochondrial preparations from heart and brain. J. Pharmacol. 108: 274–280, 1953.
113. Lee, J. and Woodbury, J. W.: Effect of potassium lack and digitoxin on transmembrane potentials and tension of frog ventricle. Fed. Proc. 17: 94, 1958.
114. Leonard, E.: Alteration of contractile response of artery strips by a potassium-free solution, cardiac glycosides and changes in stimulation frequency. Amer. J. Physiol. 189: 185–190, 1957.
115. Lewis, B. M., Housday, E. J., Haynes, F. W. and Dexter, L.: The dynamics of both right and left ventricles at rest and during exercise in patients with heart failure. Circulation Res. 1: 312–320, 1953.
116. Lewis, M. S. and Saroff, H. A.: The binding of ions to the muscle proteins. Measurements on the binding of potassium and sodium ions to myosin A, myosin B, and actin. J. Amer. chem. Soc. 79: 2112–2117, 1955.
117. Lieberman, A. L.: Studies on calcium: VI. Some inter-relationships of the cardiac activities of calcium gluconate and scillaren-B. J. Pharmacol. 47: 183–192, 1933.
118. Loewi, O.: Über den Zusammenhang zwischen Digitalis- und Kalziumwirkung. Arch. exp. Path. Pharmak. 82: 131–158, 1917.
119. Lown, B., Salzberg, H., Enselberg, C. D. and Weston, R. E.: Inter-relationship between potassium metabolism and digitalis toxicity in heart failure. Proc. Soc. exp. Biol., N. Y. 76: 797–801, 1951.

120. LOWRY, O. H.: Electrolytes in the cytoplasm. Biol. Symp. 10: 233-245, 1943.
121. LUNDSGAARD-HANSEN, P.: Vergleich der Wirkungen von Calcium und Digitalis auf die aktiven und passiven Kaliumbewegungen durch die Erythrocytenmembran. Arch. exp. Path. Pharmak. 231: 577-585, 1957.
122. MCMICHAEL, J. AND SHARPEY-SCHAFER, E. P.: The action of intravenous digitoxin in man. Quart. J. Med. 13: 123-135, 1944.
123. MALLOV, S. AND ROBB, J. S.: Behavior of actomyosin threads. Fed. Proc. 8: 104-105, 1949.
124. MANDELSTAMM, M.: Über den Zusammenhang swischen Digitalis- und Calciumwirkung. Z. ges. exp. Med. 51: 633-651, 1926.
125. MANGUN, G. H., REICHLE, H. S. AND MYERS, V. C.: Further studies on human cardiac and voluntary muscle. Arch. intern. Med. 67: 320-332, 1941.
126. MINES, G. R.: On functional analysis by the action of electrolytes. J. Physiol. 46: 188-235, 1913.
127. MOMMAERTS, W. F. H. M.: Is adenosine triphosphate broken down during a single muscle twitch? Nature, Lond. 174: 1083-1084, 1954.
128. MOMMAERTS, W. F. H. M.: Investigation of the presumed breakdown of adenosine-triphosphate and phosphocreatine during a single muscle twitch. Amer. J. Physiol. 182: 585-593, 1955.
129. NAHUM, L. H. AND HOFF, H. E.: Effect of calcium on the digitalized heart. Proc. Soc. exp. Biol., N.Y. 38: 860-864, 1937.
130. NIEDERGERKE, R.: The rate of action of calcium ions on the contraction of the heart. J. Physiol. 138: 506-515, 1957.
131. NIEDERGERKE, R. AND HARRIS, E. J.: Accumulation of calcium (or strontium) under conditions of increasing contractility. Nature, Lond. 179: 1068-1069, 1957.
132. NIEDERGERKE, R. AND LÜTTGAU, H. C.: Calcium and contraction of the heart: Antagonism between calcium and sodium ions. Nature, Lond. 179: 1066-1067, 1957.
133. NYIRI, W. AND DUBOIS, L.: Experimental studies on heart tonics: III. The relationships of calcium ions, hydrogen ions and digitalis. J. Pharmacol. 39: 111-128, 1930.
134. ORABONA, M. L. AND MANGANELLI, G.: Modificasioni del potassium nei tessuti di ratti sottoposti a sforso ed in ratti trattati con digitale. Boll. Soc. ital. Biol. sper. 30: 10-11, 1954.
135. PENNA, M. AND GARB, S.: Relationship of changes in ionic concentrations to the augmentation produced by extra contractions in isolated mammalian ventricular muscle. Amer. J. Physiol. 184: 572-574, 1956.
136. PETERS, H. P. AND VISSCHER, M. B.: The energy metabolism of the heart in failure and the influence of drugs upon it. Amer. Heart J. 11: 273-291, 1936.
137. RAYNER, B. AND WEATHERALL, M.: Digoxin, ouabain and potassium movements in rabbit auricles. Brit. J. Pharmacol. 12: 371-381, 1957.
138. REBAR, J., JR., REBAR, B. T. AND OMACHI, A.: Influence of digitoxin on labile and inorganic phosphates, lactate, glycogen, potassium and sodium in dog ventricle. Circulation Res. 5: 504-509, 1957.
139. REGAN, T. J., TALMERS, F. N. AND HELLEMS, H. K.: Myocardial transfer of sodium and potassium: Effect of acetyl strophanthidin in normal dogs. J. clin. Invest. 35: 1220-1228, 1956.
140. RINGER, S.: A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. J. Physiol. 4: 29-42, 1883.
141. RITCHIE, J. M. AND WILKIE, D. R.: The effect of previous stimulation on the active state of muscle. J. Physiol. 130: 488-496, 1955.
142. ROBB, J. S. AND MALLOV, S.: Effect of ouabain on actomyosin threads. J. Pharmacol. 106: 251-259, 1953.
143. ROHDE, E. AND OGAWA, S.: Gaswechsel und Tätigkeit des Herzens unter dem Einfluss von Giften und Nervenreisung. Arch. exp. Path. Pharmakol. 69: 200-238, 1912.
144. ROSIN, H. AND FARAH, A.: Post-stimulation potentiation of contractility in the isolated auricle of the rabbit. Amer. J. Physiol. 180: 75-82, 1955.
145. ROTHLIN, E. AND KALLENBERGER, A.: Über das Glykosidbindungsvermögen verschiedener Eiweissfraktionen des Blutes. Arch. int. Pharmacodyn. 81: 520-529, 1950.
146. ROTHLIN, E. AND SCHOELLY, O.: Einfluss herswirksamer Glycoside auf die Atmung des Herzens, gemessen mit der Warburg-Apparatur. Helv. physiol. acta 8: C69-70, 1950.
147. ST. GEORGE, S., FRIEDMAN, M. AND ISHIDA, T.: Intracellular distribution of digitoxin. Proc. Soc. exp. Biol., N. Y. 83: 318-320, 1953.
148. SALOMON, K. AND REISSER, O.: Zur Frage des Einflusses von Digitoxin und Strophanthin auf oxydative Vorgänge in Versuchen am Modell sowie am atmenden überlebenden Herzmuskelgewebe. Arch. exp. Path. Pharmak. 177: 450-462, 1935.
149. SAMPSON, J. J., ALBERTON, E. C. AND KONDO, B.: The effect on man of potassium administration in relation to digitalis glykosides with special reference to blood serum potassium, the electrocardiogram and ectopic beats. Amer. Heart J. 26: 164-179, 1943.
150. SANYAL, P. N. AND SAUNDERS, P. R.: Action of ouabain upon normal and hypodynamic myocardium. Proc. Soc. exp. Biol., N. Y. 95: 156-157, 1957.
151. SANYAL, P. N. AND SAUNDERS, P. R.: Relationship between cardiac rate and the positive inotropic action of ouabain. J. Pharmacol. 122: 499-503, 1958.
152. SARNOFF, S. J., BRAUNWALD, E., WELCH, G. H., JR., CASE, R. B., STAINSBY, W. N. AND MACRUZ, R.: Hemodynamic determinants of oxygen consumption of the heart with special reference to the tension-time index. Amer. J. Physiol. 192: 148-156, 1958.
153. SCARINCI, V.: Soluzioni di Ringer prive di potassio rimuovono la contrattura da ouabaina e restaurano la contrattiltà del cuore di rana. Experientia 9: 187-189, 1953.

154. SCHATZMANN, H. J.: Herzglykoside als Hemmstoffe für den aktiven Kalium- und Natriumtransport durch die Erythrocytenmembran. Helvet. physiol. acta 11: 346–354, 1953.
155. SCHEIDER, F. AND FARAH, A.: Some factors influencing potentiation of cardiac contractility following an extrasystolic beat. Proc. Soc. exp. Biol., N. Y. 91: 646–650, 1956.
156. SCHREIBER, S. S.: Potassium and sodium exchange in the working frog heart. Effects of overwork, external concentrations of potassium and ouabain. Amer. J. Physiol. 185: 337–347, 1956.
157. SHAW, F. H. AND SIMON, S. E.: The nature of the sodium and potassium balance in nerve and muscle cells. Aust. J. exp. Biol. med. Sci. 33: 153–177, 1955.
158. SHERROD, T. R.: Effects of digitalis on electrolytes of heart muscle. Proc. Soc. exp. Biol., N. Y. 65: 89–90, 1947.
159. SIMON, S. E., SHAW, F. H., BENNETT, S. AND MULLER, M.: The relationship between sodium, potassium and chloride in amphibian muscle. J. gen. Physiol. 40: 753–777, 1957.
160. SMITH, P. K., WINKLER, A. W. AND HOFF, H. E.: Calcium and digitalis synergism. Arch. intern. Med. 64: 322–329, 1939.
161. SNELLMAN, O. AND GELOTTE, B.: A reaction between a deaminase and heart actin, and inhibition of the effect with cardiac glycoside. Nature, Lond. 165: 604, 1950.
162. STUTZ, H., FEIGELSON, E., EMERSON, J. AND BING, R. J.: The effect of digitalis (cedilanid) on the mechanical and electrical activity of extracted and non-extracted heart muscle preparations. Circulation Res. 2: 555–564, 1954.
163. SULSER, F. AND WILBRANDT, W.: Die Wirkung von Corticosteroiden und Herzglykosiden auf Ionentransporte am Erythrocyten. Helv. physiol. acta 15: C37–39, 1957.
164. SZENT-GYÖRGYI, A.: Free-energy relations and contraction of actomyosin. Biol. Bull., Wood's Hole 96: 140–161, 1949.
165. SZENT-GYÖRGYI, A.: Chemical physiology of contraction in body and heart muscle, p. 92. Academic Press, New York, 1953.
166. THOMAS, L., JR., JOLLEY, W. AND GRECHMAN, R.: Effect of calcium lack and ouabain on calcium uptake in frog's heart. Fed. Proc. 17: 162, 1958.
167. TRAUTWEIN, W. AND ZINK, K.: Über Membran- und Aktionspotentiale einzelner Myokardfasern des Kalt- und Warmblüterherzens. Pflüg. Arch. ges. Physiol. 256: 68–84, 1952.
168. TURBA, F. AND SCHOLTISSEK, CH.: Untersuchungen mit C^{14}-markierten Digitalis-Stoffen. Arch. exp. Path. Pharmak. 222: 206–207, 1954.
169. VICK, R. L. AND KAHN, J. B., JR.: The effects of ouabain and veratridine on potassium movement in the isolated guinea pig heart. J. Pharmacol. 121: 389–401, 1957.
170. WALTON, R. P., LEARY, J. S. AND JONES, H. P.: Comparative increase in ventricular contractile force produced by several cardiac glycosides. J. Pharmacol. 98: 346–357, 1950.
171. WASER, P. G.: Bindung von Herzglykosiden an Actomyosin. Helv. physiol. acta 13: C37–38, 1955.
172. WASER, P. G.: Über die Wirkung von Herzglykosiden auf Actomyosin. Cardiologia 29: 214–229, 1956.
173. WASER, P. G.: Über die Wirkung von Herzglykosiden auf Actomyosin: 3. Thixotropieveränderungen. Helv. physiol. acta 15: 125–133, 1957.
174. WASER, P. G.: Verstärkte Bindung von Kaliumionen an Actomyosin durch herzwirksame Glykoside. Helv. physiol. acta 15: C42–43, 1957.
175. WASER, P. G. AND VOLKART, O.: Wirkung von Herzglykosiden auf Actomyosin. Helv. physiol. acta 12: 12–22, 1954.
176. WEDD, A. M.: The influence of digoxin on the potassium content of heart muscle. J. Pharmacol. 65: 268–274, 1939.
177. WEIDMANN, S.: Shortening of the cardiac action potential due to a brief injection of potassium chloride following the onset of activity. J. Physiol. 132: 157–163, 1956.
178. WEIDMANN, S.: Elektrophysiologie der Herzmuskelfaser. Huber, Bern, 1956.
179. WEISSÄCKER, V.: Über die Abhängigkeit der Strophantinwirkung von der Intensität der Herztätigkeit. Arch. exp. Path. Pharmak. 72: 282–304, 1913.
180. WERSCHININ, N.: Über die systolische und diastolische Herzwirkung des g-Strophanthins. Arch. exp. Path. Pharmak. 63: 386–404, 1910.
181. WHITE, W. F. AND SALTER, W. T.: The response of hypodynamic myocardium to known concentrations of cardiac glycosides. J. Pharmacol. 88: 1–9, 1946.
182. WILBRANDT, W., BRAWAND, K. AND WITT, P. N.: Die quantitative Abhängigkeit der Strophanthosidwirkung auf das Froschherz von der Tätigkeit des Herzens und von der Glykosidkonzentration. Arch. exp. Path. Pharmak. 219: 397–407, 1953.
183. WILBRANDT, W. AND CAVIEZEL, R.: Die Beeinflussung des Austritts von Calcium aus dem Herzmuskel durch Herzglykosid. Helv. physiol. acta 12: C40–42, 1954.
184. WILBRANDT, W. AND KOLLER, H.: Die Calciumwirkung am Froschherzen als Funktion des Ionengleichgewichts zwischen Zellmembran und Umgebung. Helv. physiol. acta 6: 208–221, 1948.
185. WILKINS, W. E. AND CULLEN, G. E.: Electrolytes in human tissue. III. A comparison of normal hearts with hearts showing congestive heart failure. J. clin. Invest. 12: 1063–1074, 1933.
186. WOLLENBERGER, A.: Metabolic action of cardiac glycosides: I. Influence on respiration of heart muscle and brain cortex. J. Pharmacol. 91: 39–51, 1947.
187. WOLLENBERGER, A.: On the energy-rich phosphate supply of the failing heart. Amer. J. Physiol. 150: 733–745, 1947.
188. WOLLENBERGER, A.: The energy metabolism of the failing heart and the metabolic action of the cardiac glycosides. Pharmacol. Rev. 1: 311–352, 1949.

189. WOLLENBERGER, A.: Non-specificity of the effect of cardiac glycosides on the polymerization of actin. Experientia 10: 311-312, 1954.
190. WOOD, E. H. AND MOE, G. K.: Studies on the effect of the digitalis glucosides on potassium loss from the heart of the heart lung preparation. Amer. J. Physiol. 123: 219-220, 1938.
191. WOOD, E. H. AND MOE G. K.: Correlation between serum potassium changes in the heart-lung preparation and the therapeutic and toxic effect of digitalis glucosides. Amer. J. Physiol. 129: 499-500, 1940.
192. WOOD, E. H. AND MOE, G. K.: Electrolyte and water content of the ventricular musculature of the heart-lung preparation with special reference to the effects of cardiac glycosides. Amer. J. Physiol. 136: 515-522, 1942.
193. WOODBURY, L. A. AND HECHT, H. H.: Effects of cardiac glycosides upon the electrical activity of single ventricular fibers of the frog heart, and their relation to the digitalis effect of the electrocardiogram. Circulation 6: 172-182, 1952.
194. WOODBURY, L. A., WOODBURY, J. W. AND HECHT, H. H.: Membrane resting and action potentials of single cardiac muscle fibers. Circulation 1: 264-266, 1950.
195. WOODWORTH, R. S.: Maximal contraction, "staircase" contraction, refractory period, and compensatory pause of the heart. Amer. J. Physiol. 8: 213-249, 1902.
196. YOUMANS, W. B. AND HUCKINS, A. R.: Hemodynamics in failure of the circulation, p. 37. Charles C Thomas, Springfield, Ill., 1951.