DRUGS AND PROPERTIES OF HEART MUSCLE[1]

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The study of the function of the heart muscle and the mode of action of cardioactive drugs is progressing along many different lines and is creating a rapidly increasing amount of literature. During the last few years, several review articles covering the recent progress within heart physiology and pharmacology (6, 8, 12, 14, 82, 94, 99, 107, 141) have appeared. The aim of this article is to elucidate some physiological mechanisms of the heart muscle function and the influence of drugs; it will mainly deal with experimental work performed on isolated heart muscle, and the greatest emphasis will be put on the fundamental dynamic properties of the cardiac muscle. Since these aspects of heart muscle function have not been treated previously in any detail in the *Annual Review of Pharmacology*, the discussion will also include papers that have appeared prior to 1963. Another branch of particular interest, in connection with a discussion of cardiodynamics, viz. the excitation-contraction coupling, will also be reviewed. The metabolic studies, on the other hand, have been largely omitted to allow a more detailed treatment of the other topics, within the space allocated.

The author has not attempted to produce a complete list of references but has tried to select a sufficient number of contributions to elucidate the various problems discussed.

Dynamics of Heart Muscle

Our current concepts of the mechanical events underlying the contraction of the vertebrate muscle cell are largely based on the work of A. V. Hill and collaborators (e.g. 151, for previous references). Because the frog or toad sartorius muscles have been determined to be the most suitable objects for this kind of research, the original work on the dynamics of muscular contraction has largely been carried out on these particular muscles. Although the results obtained in studies of the skeletal muscle have been considered as also largely applicable to the heart, it is only recently that an experimental approach, similar to that used on skeletal muscle, has been introduced in the study of heart muscle function (1, 32, 135). The study of the myocardial contraction is much more complicated, however, because of the specialized architecture and geometry of the heart and because of certain functional properties of the heart muscle cell.

According to Hill (50), the contractile machinery may, with respect to its functional behaviour, be represented by an active, contractile unit in series with a passive, undamped elastic element, both of these components being

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coupled in parallel with another elastic element. The complications introduced by the parallel elastic element, assumed to be located mainly in the supporting connective tissue (3, 70), can be ignored if the resting tension is small. In an isotonic contraction, the series elastic element is kept at a constant length, determined by the existing load, whilst in the isometric contraction, the shortening of the active unit produces an elongation of the series elastic element with corresponding increase in tension between the ends of the muscle.

General aspects (active state).—The mechanical output of the muscle in a single isometric twitch does not give a true picture of the degree of activity of the contractile component during the contraction course. The shortening of the active unit takes time; and the external manifestation of the activity, being dependent on the stretching of the series elastic element, is, therefore, substantially delayed. The degree of activity of the contractile unit, the "active state," according to the terminology of Hill (51), may be expressed as the maximal isometric tension that the contractile unit is able to develop, or just bear without lengthening, at a given moment. To be able to measure this tension, the recording must consequently be arranged in such a way that no movement of the series elastic element occurs. It has been demonstrated, in amphibian and tortoise muscles at 0° C, that the onset of activity actually begins in the middle of the latency period and has nearly reached its full intensity at the start of the spontaneous tension development (52, 53, 54). Activity is maximal for only a relatively short time [about 50 msec at 0° C and 10 msec at 20° C in the frog sartorius (93, 151)], and at the moment when the isometric twitch curve reaches its maximum, the active state has been reduced to about three fourths of its maximum (plateau) value.

Thus, the external manifestation of activity in a single twitch only reaches a fraction of the maximal tension of which the contractile unit is capable. On the other hand, when the muscle is tetanized at an appropriate stimulus frequency, the external tension eventually reaches the same value as that corresponding to the plateau of activity. In fact, the most convenient way of determining the maximal intensity of the active state in skeletal muscle is to record the tetanic tension. The concepts of active state have been critically discussed by Pringle (116). As was pointed out by him, the determination of activity has to rely on the assumption that the rapid length changes which are imposed on the muscle in the measurements do not, by themselves, cause any virtual change of the state of activity.

The study of active state in the cardiac muscle is considerably more complicated than in the skeletal muscle. The heart muscle is ordinarily unable to produce a fused tetanic contraction, a feature which makes the determination of the maximal intensity of the active state less accurate in the heart than in the skeletal muscle. By adapting the "quick-release" method of Jewell & Wilkie (70), it has been possible, however, to record almost the entire time course of the active state in isolated papillary muscles of guinea pigs and rabbits, as performed by Edman & Nilsson (32) with a very sensitive recording device. This procedure allows measurements to be made of the shortening

velocity of the contractile unit at various moments after stimulation, without interference from the series elastic element. The speed of shortening at any given moment is proportional to the ability of the contractile unit to develop tension (71). Consequently, a change of the duration of the active state can be properly determined by this approach (53, 71). In order to make sure whether or not the maximal intensity of the active state has been altered, the measurements have to be supplemented by recordings of the force-velocity relation (see below).

As expected from the different time courses of the isometric contractions, the active state is much longer in the heart than in the skeletal muscle. In a rabbit papillary muscle (contracting at a frequency of 30 beats per min at 30° C), intensity of the active state is maximal at about 60 msec after stimulation, and activity begins to decline at 100 msec (32). The active state has diminished to approximately 80 percent of the plateau value at the moment the isometric tension is maximum.

An inotropic change of the myocardium may be achieved by an alteration of either of the two parameters of the active state. A reduction of the duration of the active state, for instance, will decrease the height of the contraction by giving the contractile unit less time to stretch the series elastic component. If all other factors are unchanged, an alteration of the duration of the active state is revealed by a parallel reduction or lengthening of the time to peak tension. A pure change in the intensity of the active state, on the other hand, will produce a symmetrical change in height of the isometric contraction curve. The effect is equivalent to a change in amplification of the tension recording, i.e. the tension output is attenuated or magnified by a certain factor, while the time course of the contraction is the same as before.

General aspects (the series elastic element).—The series elastic element is probably not a uniform entity. A fraction of it is represented, conceivably, by connective tissue, while another portion may be an integral part of the contractile system inside the cell (70). A change of the stress-strain relation of the series elastic element will naturally alter the magnitude of the myocardial contraction. Inotropic agents seem to leave the mechanical properties of the heart muscle compliances unaffected, however. It has been found, that under conditions where marked inotropic effects have been produced by norepinephrine (134), ouabain (32), and changed frequency (1), there is no substantial change of the series elastic compliance.

General aspects (force-velocity relation).—The load of the muscle determines not only the degree of shortening but also the velocity of shortening. Maximal speed of shortening occurs when the muscle is unloaded. On the other hand, shortening velocity is zero when the load just corresponds to the maximal tension of which the contractile unit is capable. The mathematical formulation most often used to describe the force-velocity relationship is the hyperbolic equation of Hill (50):

$$(P + a)(V + b) = (P_o + a)b \qquad 1.$$

or

$$(P + a)\ V = b(P_o - P), \qquad 1a.$$

in which P is the actual load; V, velocity of shortening corresponding to the load; P_o, the maximal tension output of the contractile unit (equal to the tetanic tension); and a and b constants. Hill (50) originally found that the constant a, of the above equation, determined by purely mechanical measurements was, within the experimental error, similar to the constant of the "heat of shortening" during contraction. By this finding, the force-velocity equation acquired a deeper meaning than merely showing the algebraic correlation between two mechanical parameters. According to the hypothesis advanced by Hill, the force-velocity equation may be regarded as an expression of the basic mechanism that determines the energy flux through the contractile machinery during contraction. Its significance in reflecting specific energetic events within the contractile system has been extended in several studies subsequent to Hill's original work (see 55 and 123 for further references), and the implications of various changes of the force-velocity equation in cardiac dynamics have recently been discussed (1, 114, 134, 135). Attempts have also been made to apply the force-velocity equation to the modern theories of the molecular events in muscular contraction (67, 113, 114).

It should be noted that more recent studies on frog skeletal muscle, using refined recording techniques, have shown that the formation of heat energy during contraction is a more complicated process than was originally believed (19, 20, 55). The fraction of the contraction heat designated as heat of shortening has been found by Hill (55) to be dependent not merely on the distance of shortening, as previously concluded, but also on the actual load of the muscle. On the basis of his new results, Hill has introduced a modification of the original hypothesis. Even in its new form (55), the hypothesis supplies a connection between the constant of the heat of shortening and the constant a derived from mechanical measurements of the force-velocity equation.

The force-velocity relation of heart muscle, as established by adequate recording technique (1, 32, 135), is essentially the same as that of skeletal muscle. Up to the present, however, no thermo-mechanical measurements have been carried out on the myocardium. This lack of information means that there are limited possibilities still to evaluate the thermodynamic mechanisms underlying a change of the force-velocity relation in the heart (114, 134). Even though the information about the basic processes is still incomplete, the determination of the force-velocity curve is, nevertheless, an essential step in the analysis of the various mechanical factors involved in an inotropic change. The degree of stretch of the series elastic element during a cycle of activity is, as already pointed out, dependent on the speed of shortening of the contractile unit. This implies that one possible mechanism of a change in amplitude of the myocardial contraction is a specific change of the intrinsic shortening velocity. A study of the force-velocity curve enables us to decide which property of the contractile unit is affected at an inotropic intervention, the ability to produce tension or the ability to exert motion. The following section will give a survey of recent data of relevance to evaluate the dynamics of certain contractile changes of the heart muscle.

Inotropic changes (isolated heart muscle preparations).—An analysis of the inotropic effects of g-strophanthin has been performed by Edman & Nilsson (32) by using the quick-release technique, referred to above, supplemented by measurements of the force-velocity relation. It was found that there is a marked increase in the maximal intensity of the active state of guinea pig and rabbit papillary muscles under conditions where the cardiac glycoside produces a potentiation of the amplitude of the isometric contraction. Full activity is reached earlier after the stimulation in the presence of the glycosides. However, activity also begins to decline earlier, the net result being a reduction of the plateau of full activity. The cardiac glycoside produces a parallel shift of the force-velocity curve away from the origin but does not cause any significant change of its form, i.e. the maximal shortening velocity (V_o) is increased in proportion to the increase in maximal isometric tension (P_o). The last statement has to be made with the reservation that the end points of the force-velocity curve of the heart muscle can only be established by extrapolation. No change of the series elastic compliance is produced by the glycoside.

It can be concluded from these findings that the primary mechanism, by which cardiac glycosides produce their inotropic effects on the heart muscle, is represented by the increase in the maximal intensity of the active state, i.e., in improvement of the ability of the contractile element to exert tension. It is important to note, however, that the positive effect on the myocardial contraction, so produced, is counteracted by the other action exerted by the cardiac glycoside within the contractile system, viz. the reduction of the duration of the active state. The increase in intensity of the active state predominates, however, and the net result is, therefore, a potentiation of the amplitude of the isometric contraction in parallel with a reduction of the time to peak tension and a reduction of the total contraction time. This kind of change in the isometric contraction by treatment with digitalis glycosides has been reported by others previously (e.g., 81, 102); the decrease in the duration of the contraction appears to be most pronounced in the ventricular muscle (81). A similar effect on the isometric contraction would also be expected to occur, without alteration of the intensity and duration of the active state, if the velocity of shortening of the contractile unit were specifically increased by the drug. It is clear, however, that the velocity of intrinsic shortening and the intensity of the active state are increased in parallel by the cardiac glycoside, a finding which probably means that digitalis affects a cellular function that governs both of these parameters.

The inotropic effect of digitalis in the mammalian myocardium is strikingly similar to the contractile change produced at steady state by an altered contraction frequency. The relation of the strength of contraction to the time interval between the contractions varies a great deal among different animal species (82, 84), and also from atrium to ventricle of the same heart (81, 82, 143). There seems to be a general rule in the mammalian myocardium, however, that the increase in amplitude of the contraction produced by a frequency change—as measured when the contractile response has reached a

steady state—is paralleled by a certain decrease in the time to attain peak tension and also a reduction in the duration of the contraction as a whole (1, 11, 80). As established by direct experimental approach, the underlying mechanism is an increase of the intensity (32) and a reduction of the duration of the active state (1, 32). Similar to what has been found in the analysis of the digitalis effect, full activity is reached earlier after the stimulation (32). Furthermore, the frequency-induced inotropic change does not appear to involve any alteration of the form of the force-velocity curve, only a symmetrical shift is produced (1, 32). A slightly asymmetrical change of the force-velocity curve, by increases in the rate of stimulation, has been reported by Sonnenblick (135).

As has been known for a long time, the inotropic effects of cardiac glycosides are dependent on the frequency of contraction of the myocardium. According to some authors (38, 46, 83), digitalis is able to reduce or abolish the "staircase phenomenon," i.e., bring the myocardium to exert nearly as much tension at low as at high frequencies. These findings have encouraged the search for a common cellular mechanism, by which digitalis and a frequency change may improve the myocardial contractile strength (e.g. 46, 107). The action of cardiac glycosides on the frequency-strength relationship in mammalian myocardium has recently been reinvestigated by Tuttle & Farah (143) and by Koch-Weser & Blinks (81). It is established that cardiac glycosides are able to potentiate the contraction amplitude at all stimulation frequencies investigated [intervals of 0.3 to 600 sec (81) and 0.2 to 40 sec (143)]. At relatively low concentrations, they may produce an essentially uniform increase in contractile strength over the whole range of frequencies. By increasing concentrations, however, the effects become more and more pronounced at those frequencies where the active tension is low in the absence of drug, while there is no further increase of the contractile strength at the optimal frequencies. These findings seem to hold true irrespective of whether the preparation exhibits an ascending or descending response to increasing frequency. A similar levelling of the frequency-strength curve has also been found to occur by action of epinephrine and tyramine (83).

Kruta, Bravený & Husáková (83) point out that the frequency-dependence may simply mean that digitalis and a change in frequency, by additive actions, are able to increase the contractile output of the muscle up to a given maximum, the "ceiling of contractility," beyond which no further tension can be delivered by the contractile system under the experimental conditions. This would seem to be a most conceivable interpretation if by ceiling of contractility is meant the highest intensity of the active state that can be achieved under the prevailing conditions of the muscle. There is, thus, no real reason to assume that the ability of digitalis to affect the particular cell function, by which the intensity of the active state is increased by the glycoside, is dependent on the contraction frequency (see also 101). The synergistic effects produced by cardiac glycosides and increasing frequency on the parameters of the active state and the force-velocity relation are fully in keeping with the hypothesis of a common mechanism of action of these

two inotropic interventions (see further under EXCITATION-CONTRACTION COUPLING).

Sonnenblick (134, 135) has studied the changes in the force-velocity relation produced by calcium and norepinephrine and alterations of rest length and contraction frequency. It was found that calcium and norepinephrine, similar to ouabain (32) and a frequency change (1, 32), produce an essentially parallel shift of the force-velocity curve, i.e., a proportional increase of the shortening speed over the entire range of loads tested. On the other hand, increases in rest length were found by Sonnenblick (134, 135) to improve the ability of the preparation to develop tension (P_o) without a proportionate increase in maximal shortening velocity (V_o); in fact, the extrapolated value for V_o was found to be unchanged. Thus, the results seem to reveal an interesting difference between the mechanisms of the inotropic effects produced by elongation of the muscle fibers (the Frank-Starling effect) and those induced by the cardioactive drugs. The effects on the force-velocity curve produced by the cardioactive drugs are accordant with the experience gained in skeletal muscle, that a change of the intensity of the active state, at a given rest length, is accompanied by a proportional change of the maximal speed of shortening. Thus, as demonstrated by Jewell & Wilkie (71) in frog sartorius muscle, there is a parallel shift of the force-velocity curves obtained at each of various instances during the decay of activity in the contraction cycle. This kind of effect on the force-velocity curve would seem to indicate that the contractile change is achieved by action upon a cell function that governs the maximal rate of shortening of the contractile element as well as its ability to develop tension. Further work is needed to prove whether or not the noncongruent shift of the force-velocity curve produced by increasing fiber length (134, 135), is in fact, representative for the force-velocity relation of the contractile unit of the heart muscle cell. Due allowance has to be made for the complications in the measurements of the initial shortening velocity caused by the stretch of the parallel elastic components.

Siegel & Sonnenblick (131) have correlated the changes of the force-velocity curve with alterations of the maximal rate of isometric tension development (dP/dt) and the area beneath the rising phase of the isometric contraction curve (equal to the integrated systolic isometric tension, IIT). The studies were performed on both isolated cat papillary muscles and innervated isovolumic heart preparations *in situ*. The authors use the ratio of dP/dt to IIT as an index of the contractility of the myocardium and postulate on the basis of their results that the ratio is only changed when there is an alteration of the maximal intrinsic shortening velocity (V_o). The ratio was found to be increased by strophanthin, norepinephrine, elevated calcium concentration, and increases in frequency. These are very interesting findings because they suggest that the cardioactive drugs, in addition to increasing the total mechanical energy available in a contraction, as represented by the IIT, also cause a relative increase of the rate of delivery of this mechanical energy.

A new approach in the analysis of the effects of drugs on the frequency-

strength relationship has been used by Koch-Weser & Blinks (11, 81, 82). They postulate that the strength (amplitude) of an isometric heart muscle contraction is determined by: (*a*) the strength of the "rested-state contraction," which is the same as the contractile response after a long (10 min) pause; (*b*) a negative inotropic effect produced by the previous beat(s); and (*c*) a positive inotropic effect produced by the previous beat(s). A method has been devised to estimate the time courses of the disappearance of the two kinds of inotropic effects. Thus, according to the hypothesis advanced, the strength of a heart beat is equal to the strength of the rested-state contraction plus the sum of the cumulated positive and negative inotropic effects of preceding beats. Cardiac glycosides have been found to potentiate the rested-state contraction (81). The main effect of sympathomimetic amines (79) is to increase the positive inotropic effect produced by each beat. Acetylcholine (79), on the other hand, inhibits the development of the positive inotropic effect during contraction. The hypothesis of Koch-Weser & Blinks still remains as purely descriptive, and it is not yet possible to interpret the results in terms of the active state and the force-velocity relation. The parameters designated as "positive and negative inotropic effects of activation" may represent changes in both intensity and duration of the active state.

Inotropic changes (the intact heart).—During the last few years, many attempts have been made to investigate the dynamics of the inotropic changes in the myocardium *in situ*. However, the difficulties in controlling the many variables (127) involved in studies of the intact heart limit the possibilities of drawing conclusions about the properties of individual contractile cells. For instance, alterations of heart rate, arterial pressure (i.e. the load applied to the heart muscle during contraction), and the end-diastolic intraventricular pressure or volume (i.e., the resting length of the myocardial fibers) will modify the contractile response of the individual cells, as will also reflexogenic changes of the autonomic tone of the heart. Furthermore, due to the spherical shape of the heart, the quantitative connection between intraventricular pressure and the tangential tension of the wall is complicated according to Laplace's law, unless strictly isovolumic measurements are made (100, 131, 137). Nevertheless, by choosing proper experimental conditions, it has been possible to obtain valuable information about the basic mechanical properties of the myocardial cell in experiments on the intact heart *in situ*.

The relationship between tension and velocity of shortening of the myocardium of intact, vagotomized, normal-beating, dog hearts has been investigated by Fry et al. (36, 37). Their experimental arrangement allowed estimates to be made of the average shortening velocity per unit circumference of an imaginary slice of the intact heart. By varying the aortic pressure and the end-diastolic intraventricular pressure independently, the force-velocity relation could be studied over a wide range of loads and at different rest lengths of the myocardial fibers. Increasing diastolic heart volume was found to produce a shift of the force-velocity curve away from the origin. The results did not yield any definite information, however, as to whether or not a change in the diastolic fiber length alters the form of the force-velocity curve,

i.e., whether extension of the fibers produces parallel increases of the maximum tension (P_o) and the maximum speed of intrinsic shortening (V_o). As was discussed earlier, results obtained with isolated papillary muscle preparations seem to indicate that increases of the end-diastolic fiber length, unlike certain other inotropic changes, cause a non-congruent shift of the force-velocity curve with potentiation of P_o but without any alteration of V_o.

Increases in contraction frequency, and administration of norepinephrine and cardiac glycosides reduce the duration of the total systole with shortening of both the isovolumic phase and the ejection period. This has been demonstrated by Wallace et al. (145, see also 97) on canine heart preparations, under conditions where the end-diastolic volume and the mean aortic pressure were kept constant. The findings confirm previous observations in studies of the intact heart (e.g. 18, 126) and are consonant with the conclusions reached in the experiments on isolated myocardial tissue, that these inotropic interventions are all associated with increase of the intensity of the active state, increase of the speed of intrinsic shortening, and reduction of the duration of activity (see previous section).

When there is no activity of the contractile elements, the tension of the myocardium is probably largely supported by passive tissue components which are mechanically in parallel with the active contractile units. An inotropic change involving only the properties of the active unit should consequently not be expected to alter the passive length-tension curve of the muscle, if the muscle is allowed to relax fully between the stimuli. This holds true for the inotropic effects produced by norepinephrine (135), and calcium (135), and increased contraction rate (1), as demonstrated in isolated papillary muscle preparations. Results bearing upon this problem have been obtained in experiments with isovolumically-contracting left ventricle preparations of dogs (137). Stimulation of the cardiac sympathetic nerves, although causing a substantial increase in amplitude of the isovolumic contraction, does not alter the ventricular distensibility, i.e., the pressure-volume relationship of the completely relaxed ventricle. The results are accordant with previous findings obtained in isovolumic ventricle preparations (100, 144), showing that administration of catecholamines does not change the relaxed pressure-volume relation. Lutz & Jacob (89), recording simultaneous changes of heart circumference and intraventricular pressure in open-chest dogs, did not find any alteration of the ventricular distensibility by vagus stimulation and changes in frequency. A false expression of the passive ventricular distensibility may be obtained if the heart rate is too fast to allow the myocardium to relax completely during diastole, as pointed out by Sonnenblick, Siegel & Sarnoff (137). In such a situation, reduction in the duration of the active state, as produced by sympathetic stimulation, may restore the relaxed pressure-volume relation (137).

The periodic changes in distensibility ("tonus waves") that occur in turtle atria are probably related to the presence of a thick smooth muscle layer on the endocardial surface in these hearts. The activity of this smooth muscle coat may be affected by many different drugs (for references, see 12).

There is no evidence, however, that smooth muscle activity of the cardiac wall may influence the distensibility of the heart in other species than the turtle.

The dependence of ventricular work of the intact heart on the arterial pressure has been observed in many previous investigations (e.g. 75, 100, 128) and has been further evaluated in recent studies performed on the cat heart *in situ* (136) and isolated canine heart preparations (87). The arterial pressure may, as an approximation, be considered equivalent to an afterload of the myocardium. A change in the arterial pressure may, therefore, be expected to alter the stroke work in a characteristic way determined by the force-velocity relation and the duration of the myocardial active state (114, 134). With the isolated papillary muscle, maximum work output of a single contraction is obtained when the load amounts to approximately 40 per cent of the isometric tension (135). Norepinephrine, calcium (135), and cardiac glycosides (31), as well as increases in rest-length and contraction frequency (135), improve the work performance of the muscle and shift the work maximum towards higher loads. Observations similar to these have been made in studies of the intact heart. Thus, the stroke work is augmented by increasing arterial pressure, but, within the "physiological" pressure range investigated (136), no work maximum is reached. Increases of the end-diastolic volume, i.e., of the rest-length of the myocardial fibers, and administration of norepinephrine enhance the ventricular performance, at a given arterial pressure, by increasing the stroke volume (136).

There has been no conclusive evidence in the past concerning the interesting question of whether or not the cardiac glycosides may affect the contractile strength of the nonfailing human myocardium. Previous investigations (43, 47, 120) have shown that cardiac glycosides produce no change or even diminish the cardiac output in subjects with normal heart function or in patients with cardiac disease but without congestive heart failure. However, cardiac output alone is not an adequate measure of the contractile strength of the myocardium (127), and a positive inotropic effect of digitalis may well be concealed because of concomitant changes of other hemodynamic parameters. Evidence in favour of an increase in the contractile strength of the nonfailing human myocardium, produced by cardiac glycosides, has been reported by Braunwald et al. (17). They demonstrated that cardiac glycosides increase the isometric tension produced by a segment of the myocardium *in situ* by about one-fifth of the control value. The investigations were performed during operations, and it could be argued that the segments fixed to the transducer might not be representative of the rest of the myocardium. Further data, supporting the view that cardiac glycosides may produce a positive inotropic change of the normal human heart, have recently appeared (95, 129, 150). As shown by Mason & Braunwald (95), ouabain increases the maximum rate of rise of the intraventricular pressure of nonanesthetized subjects without congestive heart failure, under conditions where influences by changes in heart rate, end-diastolic volume, and arterial diastolic pressure were considered negligible. Thus, the results seem to indicate that cardiac

glycosides do produce a change in the contractile system of the nonfailing human heart, leading to increased velocity of shortening of the contractile elements.

The Excitation-Contraction Coupling

Much work during the last few years has been directed to the problem of analyzing the process or processes that link the electrical events in the muscle cell membrane and the activity of the contractile unit inside the cell. Several articles (5, 9, 14, 61, 78, 98, 99, 119, 124, 130) reviewing recent progress in this field of research have appeared. This section will be mainly restricted to a discussion of experimental findings of relevance to clarifying the relation between the electrical activity and the mechanical response of of the heart muscle cell, with the object of elucidating the underlying mechanisms of inotropic changes of the myocardium.

Excitation.—It is generally agreed that the mechanisms of the membrane action potential of the heart muscle are essentially similar to those proved to be responsible for the electrical activity in the nerve membrane (56, 59). Briefly, according to the ionic hypothesis, depolarization of the membrane beyond a certain threshold causes a transitory increase of the permeability to sodium, with an inward flow of this ion producing the rising phase of the action potential. The repolarization is ascribed to the return of the original sodium permeability and the production of an outward flow of potassium, down its electrochemical gradient, caused by increased permeability to potassium. There is still uncertainty as to the mechanism that determines the duration of the plateau of the cardiac action potential. There is evidence in favour of a decrease in the potassium conductivity during the plateau (16, 111, 147), the slow repolarization occurring during this phase being ascribed to the remaining slow component of the sodium conductance inactivation and conceivably also to transmembrane movements of anions (22, 26). Several different hypotheses have been advanced to explain the delay of the outward potassium current. It has been proposed that the permeability to potassium increases steeply when the membrane potential reaches a critical range (16, 25, 111, 147) or, alternatively, when potassium has accumulated to a certain concentration in the narrow space around the fibers (21, 149). More speculative mechanisms have also been proposed (14, 154).

The sodium ion can be replaced by lithium as the depolarizing agent during the rising phase of the cardiac action potential, as demonstrated by Carmeliet (23) in calf Purkinje fibers and in cat papillary muscles. The lithium ion, however, causes certain changes of the action potential configuration. There is a marked reduction of the duration of the action potential, which, at least partly, may be accounted for by the increase in potassium permeability produced by the lithium ions.

There are animal species in which the sodium ion is, obviously, not the major current carrier, as described by the Hodgkin-Huxley hypothesis. As shown by McCann (96), the action potentials generated by the myocardial cells of the moth (*Telea polyphemus*) are uninfluenced by the complete re-

moval of the external sodium. So far, however, no substitute for the sodium-carrying system has been identified. It is noteworthy that the action potentials generated by this primitive heart are very similar in shape to the action potentials produced in the atrial and ventricular areas of vertebrate hearts. This is the more remarkable as the ionic compositions of the extracellular and intracellular media of the insect heart are quite different from what is found in vertebrates.

Some authors (24, 64, 138) have questioned whether the cardiac action potential is a unitary response. Churney & Ohshima (24), studying the electrical activity of urodele amphibian heart fibers, observed that the duration of the action potentials varied in proportion to the thickness of the fiber bundle in which the recording was made. The authors suggest, as one possible explanation of their findings, that the fundamental response of the heart muscle cell is a spike potential, and that the plateau-shaped action potential represents the summation of elemental responses of neighbouring cells. According to the hypothesis put forward by Sperelakis et al. (64, 138), the cardiac action potential is a composite of fast and slow potentials, the slow component being due to a chemical depolarization at the intercalated disc. The fact that the action potential may be separated into two distinct phases, an initial spike followed by a slow depolarization, as produced by acetylcholine (155), or by certain changes of the ionic *milieu* (2, 64), was used to support the hypothesis. Such a change of the action potential may, however, also be explained in terms of an altered balance between the various ionic mechanisms involved in the electrical activity, without postulating an electrically inhomogeneous cell membrane (23).

The calcium ion is an important factor in the mechanisms responsible for the electrical activity of the cell membrane, in the myocardium (62, 148) as well as in the skeletal muscle (30). As demonstrated by Edman & Grieve (30) in skeletal muscle fibers omission of the extracellular calcium produces a decline of the resting potential and a marked reduction of the action potential amplitude before complete inexcitability is attained. Both the normal resting potentials and the full isometric twitch response are maintained, however, if magnesium, manganese, strontium, nickel, or cobalt in appropriate concentrations, are added in place of the calcium (69). Calcium probably does not play an immediate part in the development of electrical activity but may instead maintain the integrity of the systems which govern the resting potential and the production of the action potential (30).

Relation between electrical and mechanical events.—Attempts to establish the correlation between the electrical and mechanical activities of the muscle cell still have to rely on indirect approaches because of the difficulties in producing selective changes of the action potential. As demonstrated in the frog myocardium (92, 112) and in isolated skeletal muscle fibers (57), the active tension (contracture), in response to stimulation with potassium, is proportional to the degree of depolarization of the cell membrane beyond the threshold potential at which mechanical activity can be induced. In the skeletal muscle-fiber maximal contractile response is attained when the membrane

is depolarized to about −20 mV; in this case, the tension output is equal to or even slightly higher than the tetanic tension. In view of the fact that the action potential probably induces contraction by virtue of depolarization of the fiber membrane (57, 121, 122, 139), it is reasonable to assume that the ability of the propagated impulse to activate the cell is proportional to the degree of depolarization in a way which is equivalent to that found in the potassium-induced contracture. Within certain limits, it may also be that the intensity and duration of activity of the contractile system are dependent on the length of time during which the membrane is kept depolarized beyond the mechanical threshold. The relevance of these various factors in the function of the action potential in the excitation-contraction coupling of the skeletal muscle has recently been discussed by several authors (30, 33, 58, 124, 125).

Considering the excitation-contraction process in the cardiac muscle, it may be concluded that no positive correlation appears to exist between the amplitude of the action potential and the inotropic effects produced by vagal stimulation (7, 66, 140) and administration of acetylcholine (7, 133), epinephrine, norepinephrine (27, 133), cardiac glycosides (74, 133), magnesium (4), and calcium (133). There are abundant data, on the other hand, supporting the view that there is a positive correlation between the size of the isometric response and the duration of the cardiac action potential. A relevant contribution to the elucidation of this problem has been presented by Antoni et al. (4), who have studied the effects of magnesium on the electrical and mechanical properties of the frog ventricular myocardium (19° to 22° C). It was demonstrated that elevation of the extracellular magnesium concentration causes a gradual decline of the twitch response strictly in parallel with reduction of the duration of the action potentials of individual surface fibers, while there is only a slight decrease in amplitude of the action potential. Restoration of the action potential duration, as achieved by lowering of the temperature or administration of epinephrine, quinidine, procaine amide, and certain other agents, produces a parallel restoration of the isometric twitch tension. Furthermore, when the contractile response to a single action potential has been almost lost in the presence of magnesium excess, it is still possible to initiate a strong contracture by depolarizing the preparation in isotonic KCl solution, or to produce a fused tetanic contraction of considerable amplitude. Taken together, these findings strongly suggest that the progressive decline of the twitch response following the administration of magnesium is due to failure of the action potential to trigger the subsequent steps of the excitation-contraction coupling. Antoni et al. also demonstrated that the gradual decline of the contraction amplitude is associated with reduction of the rate of initial tension development, the time to peak tension, and the total contraction time. Converse changes of the various contraction parameters occur when the shape of the action potential is restored following the experimental processes mentioned. These facts support the view that both the maximal intensity and the duration of the active state are positively correlated with the duration of the propagated impulse.

The results of Antoni et al. are concordant with many other observations made in studies of amphibian and turtle heart muscles, although most data only emphasize the direct relationship between action potential duration and the time to peak twitch tension. The duration of the action potential and the rise time to peak tension are changed in parallel by alterations in stimulus rate (108); they also exhibit a very similar temperature dependence, the Q_{10} being about 2.2 (frog) in the temperature range 3° to 35° C (15, 48, 155). The reduction in duration of the atrial action potential produced by vagus stimulation and administration of acetylcholine is also associated with an equivalent reduction of the time to peak tension (155). Reichel & Bleichert (117) have demonstrated that the decline of *Aktivierung* (in essence, equivalent to the active state) of atrial, and ventricular muscle of frog and turtle follows a similar time course as the repolarization of the action potential, if the time-scale of the latter is enlarged by a factor of about 1.4. They also state that changes in temperature (0° to 10° C) and stimulation rate (2.5 to 10 per min) have the same effects on both curves. There are many observations, however, mainly in studies of mammalian myocardium, which fail to demonstrate any clear correlation between the action potential duration and the mechanical response. The next few paragraphs will summarize the results obtained in recent experiments on isolated mammalian heart muscle in which simultaneous recordings of the electrical activity and the isometric contraction have been carried out.

Cardiac glycosides in moderate concentrations reduce the duration of the action potential but do not produce any substantial change of the amplitude, as demonstrated in several different mammalian heart preparations [cat papillary muscles (28), sheep ventricle (74), and guinea pig atria (133)]. According to some authors (28, 74), the action potential duration is prolonged in the initial stage of the glycoside action and later reduced. No direct relationship seems to exist between the positive inotropic effect of digitalis and the change of the propagated impulse in that, as stated by Kassebaum (74), the potentiation of the isometric twitch is found to occur irrespective of the direction or degree of change in the action potential duration. There are still no experimental data enabling a quantitative evaluation of the relationship between the duration of the action potential and the time to peak tension of the isometric contraction. As with cardiac glycosides, the augmentation of the twitch tension produced by increases in stimulation rate is also associated with shortening of the action potential duration (133, 142). According to results obtained in cat papillary muscles (142), there is a positive correlation between the decrease in duration of the propagated impulse and the reduction of the time to peak twitch tension.

Estradiol, testosterone, and progesterone all prolong the repolarization phase of the intracellular action potential, as established in isolated rat atria (41, 42). Of the three steroids, only testosterone is found to produce a positive inotropic effect, while the other two depress the contractile strength.

The effects of calcium excess on the action potential and the isometric contraction are found to be strikingly similar to those produced by cardiac

glycosides in guinea pig atria (133). Ryanodine, on the other hand, produces effects which in several respects are opposite to those of calcium and cardiac glycosides (133). The contractile strength is decreased by ryanodine, while the action potential plateau is markedly prolonged. The effects of ryanodine on both the electrical and mechanical behavior may be reversed by strophanthin and calcium, unless they have remained too long.

Acetylcholine causes a strong reduction of the isometric twitch tension of guinea pig (133) and human (132) atria. The action potential is greatly shortened and exhibits a spike-shaped appearance. There are still no quantitative data which correlate the action potential duration and the time to peak tension at various degrees of acetylcholine effect. The positive inotropic effect of epinephrine, as investigated in the atrial muscles mentioned, is associated with a slight prolongation of the action potential plateau (132, 133).

The results obtained so far seem to make clear that alterations of the action potential play a minor part in the development of the various inotropic changes studied in the mammalian myocardium. There is no reason to believe, however, that the concomitant changes in the shape of the action potential are functionally irrelevant in the excitation-contraction coupling. Many observations made in studies of skeletal muscle support the view of a direct correlation between action potential duration and the length of active state (e.g. 65, 124, 125). Results obtained in frog hearts suggest, as already pointed out, that the duration of the active state, and very likely also the maximal intensity of activity, are influenced by alterations of the action potential duration. The situation may be more complicated in mammalian heart muscle, and, at the present time, it is difficult to formulate a comprehensive generalization of the relationship between the electrical and mechanical events in the heart applicable to various animal species. A detailed knowledge of the changes of the active state associated with the various inotropic interventions will be required to solve this problem.

An important approach in elucidating the role of the electromechanical coupling in the development of an inotropic change has been used by Otsuka & Nonomura (112) in studies of the frog heart. They demonstrated that the threshold of the mechanical response to depolarization by potassium is substantially decreased by ouabain in concentrations that produce a positive inotropic effect on the twitch. There is no increase of the maximal contracture tension of the preparation, but ouabain shifts the depolarization-tension curve by about 30 mV towards the resting potential. The possibility that the action potential becomes more effective as an activator of the contractile system by a lowering of the mechanical threshold has been stressed by Hodgkin & Horowicz (58), and most recently, by Etzensperger (33) and Sandow (124). Thus, the potentiation of the twitch response of skeletal muscle fibers caused by certain ions has been attributed to reduction of the mechanical threshold. The findings of Otsuka & Nonomura emphasize that such a mechanism may also be of relevance in the development of the positive inotropic effect of cardiac glycosides.

Calcium link.—Many observations support the view that calcium is essential in the mechanisms that link the electrical events in the muscle cell membrane and the activity of the contractile system inside the cell. Recent review articles discussing this area of research have been written by Aubert (5), Huxley (68), Bohr (13), and Nayler (105). According to the most accepted theory, depolarization of the membrane initiates an increased inflow of calcium into the cell, thereby causing activation of the cŏntractile elements. Activity ceases when the concentration of "activator" -calcium in the cell has been reduced again. There are reasons to believe that the role of calcium in the excitation-contraction mechanism is similar in the myocardium, the smooth muscle cell, and the skeletal muscle fiber, although the particular processes governing the fluxes of calcium may not be identical in all types of muscles. Certain differences between heart and skeletal muscle with regard to the need for calcium have been pointed out by Lüttgau (91) and Niedergerke (109).

Both calcium uptake and release are found to be increased during heart muscle activity; in the case of the twitch, the increased calcium inflow associated with activity has been found to be completely matched by a rise in calcium efflux. Thus, stimulation of the myocardium does not cause any net gain of calcium but increases the exchangeable fraction of the cellular calcium (44, 45, 49, 77, 85, 110, 153). There is a direct relationship between the amount of calcium uptake and amplitude of the potassium-induced isometric contracture, as demonstrated in the frog heart (110). Similarly, potentiation of the twitch amplitude produced by calcium excess (44) and increases in contraction frequency (45, 152, 153) has been found to be reasonably well correlated with increase in the calcium uptake by the myocardium. According to Grossman & Furchgott (44), about one-fifth of the total muscle calcium (guinea pig atria) becomes exchangeable at maximal contraction. Probably, only a fraction of the exchangeable calcium is functionally relevant for the activation of the contractile process (34, 109), however.

The amount of calcium entering the cell ($\sim$3 μM per l), during a beat of the frog heart, only corresponds to 1 per cent or less of the total amount of exchangeable cellular calcium (110). It is difficult to imagine that such a small concentration change would suffice to cause activation of the contractile system, unless one assumes that the exchangeable calcium is present in functionally different forms. It is proposed by Niedergerke (109) that calcium is effective as an activator of the contractile system only during a brief period after its entry into the cell, after which the calcium is deactivated, possibly by being bound to some cellular structure. According to estimations presented by Niedergerke (110), prolongation of the cardiac action potential would be expected to cause an increase of the maximal concentration of activator calcium in the cell during a contraction cycle and also prolong the time during which activator calcium is present beyond a certain threshold in each cycle. This is an interesting conclusion in view of the fact (see p. 100) that prolongation of the action potential, at least in amphibian myo-

cardia, seems to be associated with increases of both the maximal intensity and the duration of the active state.

A great interest has been focused on the calcium function in electromechanical coupling as a possible locus of action of inotropic agents, in particular cardiac glycosides (35, 39, 40, 63, 72, 76, 77, 88, 90, 115, 118). It has been shown repeatedly (39, 40, 76, 77, 90) that the positive inotropic effects of cardiac glycosides are associated with an increased calcium exchange of the myocardium. Both uptake and release of calcium are increased, but the total calcium content of the tissue is unaltered. Klaus (76) has shown with guinea pig atria that there is a roughly linear relationship between the increase in calcium uptake and the potentiation of the twitch amplitude, and this correlation appears to apply to glycosides with widely differing biological potency. At glycoside concentrations high enough to produce contracture, there is a substantial accumulation of calcium in the tissue (39, 40, 76, 77, 90).

The results would seem to offer a plausible explanation of the effects of digitalis on the contractile strength in terms of the "calcium hypothesis" of the electromechanical coupling. Thus, the intensification of active state caused by digitalis may be due to an increase in the quantity of activator calcium being released into the cell at the moment of excitation. The contracture effect of digitalis may be attributed to a permanent increase of the concentration of activator calcium in the cell. This interpretation of the digitalis effects is by no means inconsistent with the view that other steps in the excitation-contraction process are affected as well, for instance, that digitalis potentiates the mechanical output of the heart muscle by a more direct action upon the contractile proteins (29, 73, 86, 146). The hypothesis of an increased calcium influx, as a cause of the positive inotropic effect of cardiac glycosides, is attractive, however, not the least because the same hypothesis may be applied to the inotropic changes, synergistic with the digitalis effects, produced by calcium excess (44) and increased contraction frequency (45, 152, 153). Changes of the calcium mobility in the myocardial cell may also be of relevance in the development of the positive inotropic effects produced by caffeine (10, 103), nicotine (104), and tetraethylammonium ions (106), and the negative inotropic effects produced by acetylcholine (60).

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