GENERATION AND CONDUCTION OF IMPULSES IN THE HEART AS AFFECTED BY DRUGS

WOLFGANG TRAUTWEIN

Department of General Physiology, University of Heidelberg, Germany

TABLE OF CONTENTS

I. INTRODUCTION

The rhythm of the heart is a rather complex phenomenon. Its basis is the rhythmic spontaneous generation of excitation in the pacemaker, which is normally located in the sinoatrial node. There are, however, other specialized structures with inherent myogenic automaticity: the atrioventricular node, the bundle of His, and the Purkinje fiber system Many efforts have been undertaken to detect specific potential changes corresponding to spontaneity in the pacemaker (70, 77, 224, 253). Using extracellular electrodes, Goldenberg and Rothberger (100) and Bozler (16) were the first to record a spontaneous depolarization during diastole at the locus of the pacemaker. This is in contrast to the constant diastolic membrane potential of the ordinary cardiac fibers. By the technique of intracellular recording (201) the membrane potential of single cardiac fibers could be measured, and this was first done by Woodbury *et al.* (349). Slow diastolic depolarization was found to be a general feature of pacemakers (17, 315, 326, 335, 336). With the development of the ionic theory an explanation of the slow diastolic depolarization in terms of changes in ionic conductances became possible. In this review a number of physiological and pharmacological influences which affect cardiac rhythm will be discussed. Electrophysiological understanding of drug effects on rhythmicity is emphasized. The author has not tried to present a complete listing of drugs which influence the rhythm of the heart. Much information related to the present topic can be found in recently published reviews and monographs (20, 53, 125, 270, 302, 331, 346).

II. SPONTANEOUS EXCITATION

A. The pacemaker potential

Earlier observations of the cyclic potential changes at the site of the pacemaker have been confirmed and enlarged in the last 10 years by using the technique of intracellular recording of membrane potentials. The action potential repolarizes to a level of about −80 mV. This is followed in pacemaker fibers by a slow diastolic depolarization, which, at about −60 mV, reaches threshold, *i.e.*, elicits the next action potential (see Fig. 1 A and C in comparison to Fig. 1 B and D). Such pacemaker potentials have been found in the sinus of the turtle (17, 145), in the sinus venosus of the frog heart (315), the sinus node of several mammalian hearts (304, 335), the atrioventricular node of the rabbit heart (128, 129), and the bundle of His and the Purkinje system of rabbit, dog, cat, sheep and kid hearts (50, 128, 129, 326). The slope of the diastolic depolarization differs considerably when excised tissues from the sinoatrial node, atrioventricular node and specialized conducting system are compared. In Purkinje fibers, rates of depolarization between 5 and 40 mV/sec may be observed, whereas in an excised

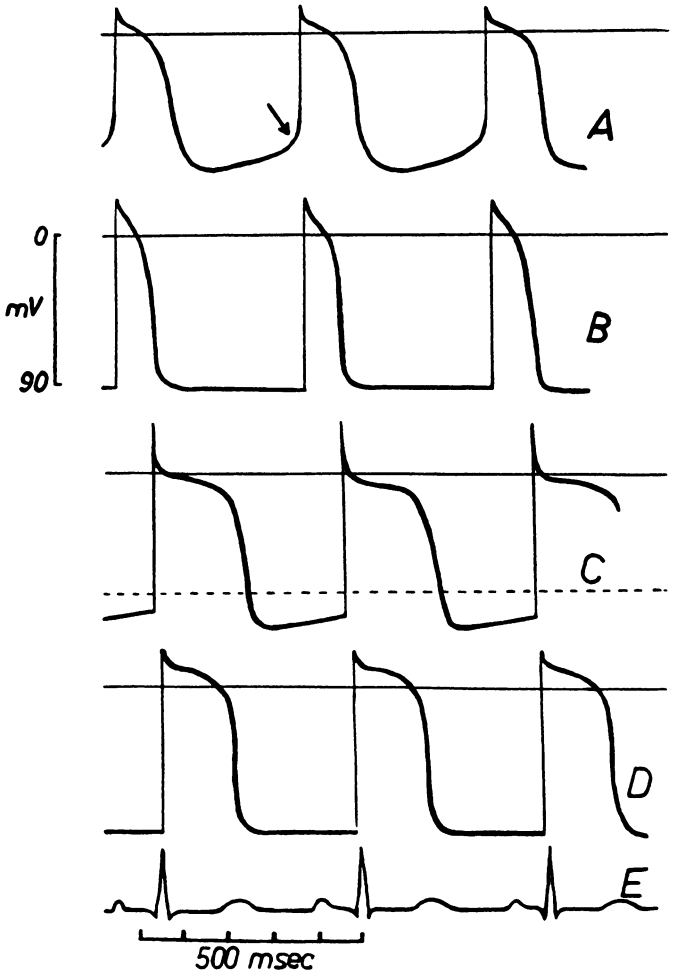

FIG. 1. The membrane potential in the course of two cardiac cycles of a fiber of the sino-atrial node (A), of the atrium (B), of the Purkinje system (C), and of the ventricular myocardium of a dog heart (D) drawn on the same time axis as the electrocardiogram (E). Note diastolic depolarization in (A) and (C), the different shape and duration of the action potentials of different cardiac tissues and the atrioventricular delay indicated by the delay in the upstroke between (B) and (C). Schematic drawing of unpublished records.

sinus node the rate of depolarization may vary from 15 to 60 mV/sec. The potential difference between the maximum diastolic membrane potential at the peak of repolarization and the threshold for the rapid upstroke is about 15 mV and roughly the same in the different spontaneously active structures. Threshold will therefore be reached earliest in the sinus (threshold potential in a Purkinje fiber is indicated by the dashed line in Fig. 1 C). The action potential arising in the sinus is conducted to the latent pacemaker structures and arrives there before their diastolic depolarization reaches threshold. Thus is the coordinated

heart beat achieved, but whenever the activity of the pacemaker in the sinoatrial node ceases, the fastest latent pacemaker will take over.

Even within the sinoatrial node or an excised strand of Purkinje fibers, the slope of the pacemaker varies at different points (42, 145, 326, 334). It is steepest at the locus of the earliest appearance of the propagated action potential. This is a small area of about 3 to 5 mm^2 at which the slow diastolic depolarization passes in an upward concave curve smoothly into the rapid upstroke (see arrow in Fig. 1 A); this is typical for the actual pacemaker. At a few millimeters distance, the slope is less steep and the upstroke of the action potential starts abruptly from the level of the membrane potential; this is typical for latent pacemaker fibers. Such an area is excited by a propagated action potential set up in the actual pacemaker, as can be shown by latency measurements. If the slow diastolic depolarization is enhanced and threshold is reached earlier than in all other parts of the preparation, a latent pacemaker fiber may become the actual pacemaker. The shift of the pacemaker is often accompanied by a change in beat frequency of the preparation for a shorter or longer period. Arrhythmia occurs whenever excitation arising in an ectopic focus interferes with the regular rhythm established in the sinoatrial node. In an excised Purkinje fiber network, it can often be observed that the propagated action potential does not invade a small twig of Purkinje tissue in which independent pacemaker activity is going on. Once in a while an impulse is conducted from this area into the main part of the preparation, disturbing the regular rhythm. Local areas of block and an anatomical situation which favors unidirectional conduction are the conditions for this arrhythmia. The mechanism of ectopic impulse generation is not different from that of spontaneous excitation in the sinoatrial node. Of the many physical factors which may influence the pacemaker potential, temperature and stretching of the pacemaker tissue should be mentioned. Cooling reduces the rate of the slow diastolic depolarization in Purkinje fibers (50, 305), while stretch can have the opposite effect (65). Positive chronotropic effects of probably similar nature have also been observed in the heart-lung preparation of the dog after increasing the right atrial pressure (14).

B. Abnormal impulse generation

Spontaneous excitation is not always preceded by a diastolic period during which the membrane potential depolarizes to threshold. In both ordinary ventricular fibers and Purkinje fibers, action potentials can be observed which start abruptly when repolarization of the preceding action potential has reached a level of -50 mV to -70 mV. These extrasystoles coupled to the preceding beat (as seen in Fig. 2) are frequently observed in deteriorated (20, 306) or drug-treated preparations (67, 214, 276). In Purkinje fibers these extrasystoles are often repetitive and appear as slow oscillations between -50 mV and 0 mV (see Fig. 2, above). Suddenly one of these oscillations may fully repolarize to the resting potential. One of the typical influences eliciting such extrasystoles is a reduction of the extracellular potassium concentration, which is known to decrease the potassium permeability (36, 38). Low potassium conductance or

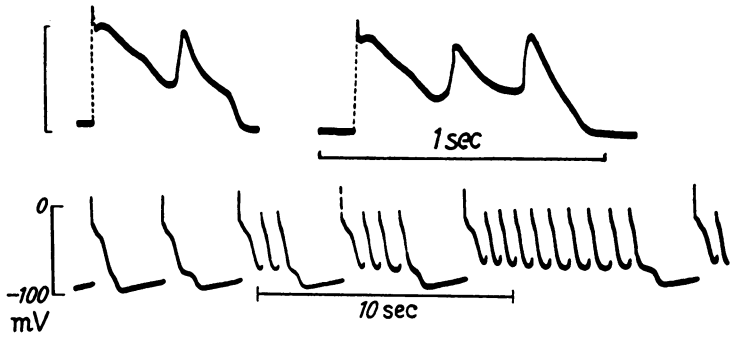

FIG. 2. Purkinje fiber of the dog.

Upper trace (Fig. 7 from 301): in the presence of low oxygen tension, showing action potentials which start during repolarization. Lower trace (Fig. 2a from 276): after administration of aconitine; note the negative afterpotential, on top of which repetitive discharges develop. (Reproduced by permission.)

reduction in the potassium driving force, *i.e.*, a smaller electrochemical potential for potassium due to a loss of intracellular potassium (in a deteriorated preparation), may play a causative role for this disturbance of repolarization.

Action potentials which start before repolarization is complete have slow rising velocity of the upstroke, and the size of the overshoot is reduced. Both these factors cause a reduction of conduction velocity (see p. 287). The latency of the coupled action potentials from the preceding action potentials thus increases with increasing distance from the point of generation of the extrasystole. At the locus where coupled extrasystoles originate, depolarizing afterpotentials are often seen (65, 276). Repolarization shows a hump (see Fig. 2, below); at about −55 mV to −60 mV the rate of repolarization suddenly becomes small and the potential stays relatively constant for 10 to 20 msec. From these humps the coupled extrasystoles take off.

Another possible mechanism for these coupled beats is re-entry of excitation. Under special conditions, excitation could travel in a circular pathway to the fiber under observation and re-excite this fiber, if it arrives after the refractory period. Conditions favoring such re-entry are: 1) an abnormally restricted pathway caused by local areas of block; 2) low conduction velocity; 3) a short refractory period. Such re-entry has clearly been demonstrated in the atria, where ring-like structures around the great vessels occur (200). As a special form of re-entry the electronic spread from an adjacent depolarized area into the already repolarized fiber should be mentioned. Such spread may produce re-excitation during the supernormal phase (20, p. 151; 301, p. 68). There is a continuous transition from an occasional re-entry in a limited region of the heart to repetitive re-entry in many areas of the heart; this is emphasized in the circus movement theory of fibrillation (90, 199, 200, 257, 258, 259). When extrasystoles closely coupled to the preceding beat are seen in a single fiber, it is clearly impossible to decide immediately whether they originated in a heterotopic pace-

maker or as a disturbance of repolarization, or whether they are the result of re-entry.

C. Normal and abnormal impulse generation in experimentally and clinically encountered arrhythmias

The information concerning normal and abnormal impulse generation as presented in the last two sections has mostly been derived from experiments on single fibers, *i.e.*, of small preparations kept in a tissue bath. The significance of such observations on single cells for an explanation of cardiac irregularities *in situ* under experimental or clinical conditions might be questioned. In this review many examples of drug effects will be described which occur in an identical or similar manner in a small preparation of excised cardiac tissue as well as in the whole heart of an animal or in a patient.

Clinical observations of disturbances of rhythm are in general agreement with the experimental findings on the individual cardiac fibers. Experimentally, the rate of the pacemaker in the sinoatrial node can be slowed or accelerated; most important in this connection are the effects of innervation, of the transmitter agents, and of drugs which interfere with the transmitter action (see p. 296). Clinically, sinus bradycardia, sinus tachycardia, and sinus arrhythmias are described, and for most of the cases the reflex nature is emphasized (285, p. 79 ff.). Heterotopic spontaneous impulse generation occurring in the atrioventricular node, in the bundle of His, or in the Purkinje fibers is clinically and experimentally equally well known. In any of these structures a pacemaker can develop which drives the heart in regular rhythm. Clinicians describe an arrhythmia caused by the rhythmic activity of two independent pacemakers, *e.g.*, one in the sinoatrial node and another in the ventricle (parasystole, 274, p. 151 ff.). An electrocardiographic criterion of this arrhythmia is the variable time interval between the dominant systole and the parasystolic activity. Obviously the time course of the membrane potential of the pacemaker which gives rise to the parasystole is similar to that in the sinoatrial node, *i.e.*, activity is preceded by a pacemaker potential. Presumably the parasystolic focus is localized in the Purkinje fiber network and protected by unidirectional block against the propagated action potential from the dominant pacemaker (see section A). In contrast to this form of arrhythmia, which results from the activity of two independent pacemakers, extrasystoles proper are premature beats which occur at a fixed latency after the regular beat. Such premature beats are clinically observed as bigeminy or as multiple extrasystoles. Again this type of impulse generation has also been found in single fibers of excised cardiac tissue and is described in section B as abnormal impulse generation. This type of excitation takes off either from the repolarization phase of the preceding action potential, or close to an area of injured and depolarized fibers; it also occurs in the presence of drugs, especially digitalis (see p. 316).

The large variety of extrasystoles and their electrocardiographic pattern results from the many possibilities of their timing within the dominant cycle, from the localization of their origin, and from special situations regarding the

refractoriness of parts of the heart, as well as the propagation through the heart. As far as the origin of the extrasystole is concerned, however, both the clinical and the experimental aspects point to two types of impulse formation: 1) the automatic and 2) the extrasystolic impulse formation.

III. THEORETICAL CONSIDERATIONS

A. Mechanism of excitation; refractoriness

The ionic theory of excitation (113, 115, 116, 117, 118), as derived from experiments on giant nerve fibers, has been of great value for the understanding of excitation in cardiac muscle. During the last 10 years, much information has become available to show the great similarity in the basic events of excitation in nerve, skeletal muscle and cardiac fiber (*cf.* 280). In this connection, only the theory of excitation and of the pacemaker potential will be considered.

The excitability of the cardiac fiber is based on the ability of the membrane to increase its sodium permeability on depolarization. If depolarization reaches a critical value (threshold), the depolarizing sodium current will be larger than the opposing repolarizing potassium outward current and a regenerative depolarization—excitation—results. Thus the degree of excitability of interest here is determined by the extent of the increase in sodium conductance (g_{Na}) on depolarization. According to Hodgkin and Huxley (116, 117, 118), the increase in g_{Na} can be interpreted as the activation of a sodium carrier system. The availability of this system to activation depends on the level of the membrane potential prior to stimulation (117, 328). If the resting membrane potential is high, the system is fully available: the increase in g_{Na} on depolarization is great and the velocity of the potential change during the rising phase of excitation is at maximum value. If the membrane potential prior to stimulation is low, the availability of the sodium carrier system is reduced. Consequently, the rising phase of the action potential is less steep. Thus, the maximum rate of depolarization of the rising phase is a measure of the availability of the sodium carrier system. A graph of the maximum rate of depolarization against the membrane potential prior to stimulation, as shown in Figure 3, characterizes the sodium carrier system and hence the reactivity of the membrane on stimulation. The graph shows an S-shaped curve. There is little decrease in the rate of rise on decreasing the membrane potential from -100 to -80 mV. Further decrease in membrane potential from -80 to -60 mV sharply reduces the rate of rise from about 500 V/sec to 50 V/sec; and, at membrane potentials less negative than -60 mV, the rate is small and relatively constant again (311, 328, 331). It is obvious that a change in the shape of this curve is a reliable indication of a drug effect on the sodium carrier system. It should be expected from the ionic theory that the size of the overshoot of the action potential is closely related to the maximum rate of rise. In agreement with the theory, a very similar dependence of the size of the overshoot on the resting membrane potential has been found (328). Excitability is determined, therefore, by the membrane potential before stimulation, the availability of the sodium carrier, and the resting potassium outward current opposing depolarization.

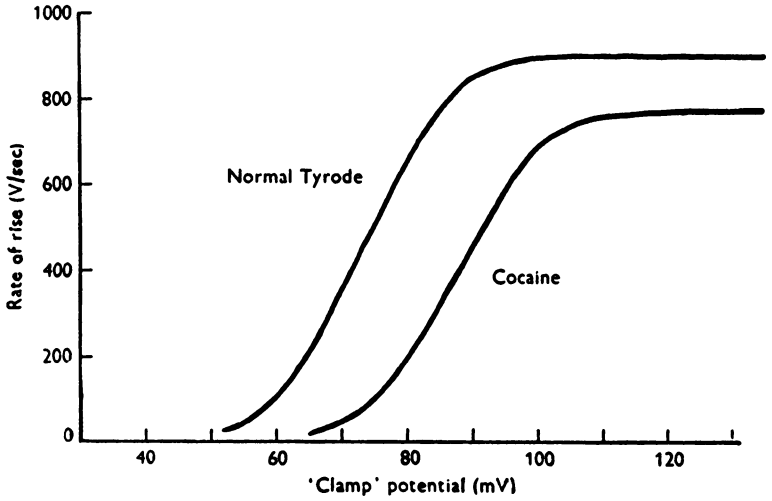

FIG. 3. Relation between the maximum rate of rise of the action potential (ordinates) and the membrane potential prior to stimulation, varied by current flow (clamp potential, abscissae).

Purkinje fiber of the kid in Tyrode solution (left curve), and in the presence of 0.05 mM cocaine hydrochloride (right curve). Redrawn from Weidmann (329).

The maximum rate of rise also is a measure of the intensity of the sodium current (118). Since the electrotonic spread of this current is the stimulus for the quiescent adjacent fiber and therefore the basis of conduction of the action potential, conduction velocity depends strongly on the maximum rate of rise of the action potential. Variation in the maximum rate of rise as affected by drugs will be used extensively in this review for the explanation of changes in conduction velocity. It should be noted that the excitatory sodium current flows only for a very short period. The maximum sodium conductance is maintained only for about 1 msec; afterwards the sodium carrier becomes more and more inactivated, although depolarization is still maintained. Inactivation is complete in heart muscle after about 10 to 15 msec (328). This inactivation means that the sodium carrier is not available on depolarization; therefore, in this state the fiber is inexcitable. This fact is the basis of the absolute refractory period during the plateau of the cardiac action potential. The inactivated carrier is transferred to the state of availability again during repolarization. Thus, during the relative refractory period excitability progressively returns to the resting level. At the end of repolarization a brief supernormal phase of excitability (of a few msec duration) is observed (122, 329). The threshold potential is nearly restored to its diastolic value, but repolarization has not yet reached the diastolic level by about 5 or 10 mV. No theoretical explanation of the supernormal phase is known.

B. The pacemaker potential

The pacemaker potential is a spontaneous slow depolarization. Depolarization occurs if conductance to sodium (g_{Na}) increases relative to conductance to potas-

sium (g_K) either by an increase of g_{Na} or by a reduction of g_K. The observation that the membrane resistance increases during diastole is in favor of the latter alternative (331). It has been shown that this increase can be attributed to a decreasing g_K throughout diastole (68). As to the mechanism of the fall of g_K, two factors seem to play a role. The first of these involves the relation between g_K and the membrane potential; g_K shows a marked decrease on depolarization in the range of the pacemaker potential. This membrane property will enhance depolarization, whatever its cause may be (143, 308, 330). The second factor involves a time-dependent decrease of g_K after repolarization of the preceding beat (308). The membrane resistance immediately after repolarization is smaller than that found later in diastole, when compared at the same membrane potential. The rise in g_K which causes repolarization seems to outlast this phase. The return of g_K to its diastolic value serves to initiate the diastolic depolarization (factor 2), which in turn (factor 1) causes a further decrease in g_K. Considering the time-dependent reduction of g_K during diastole, the pacemaker potential may be called a positive afterpotential.

The depolarizing current during diastole is carried by sodium ions. This rela-

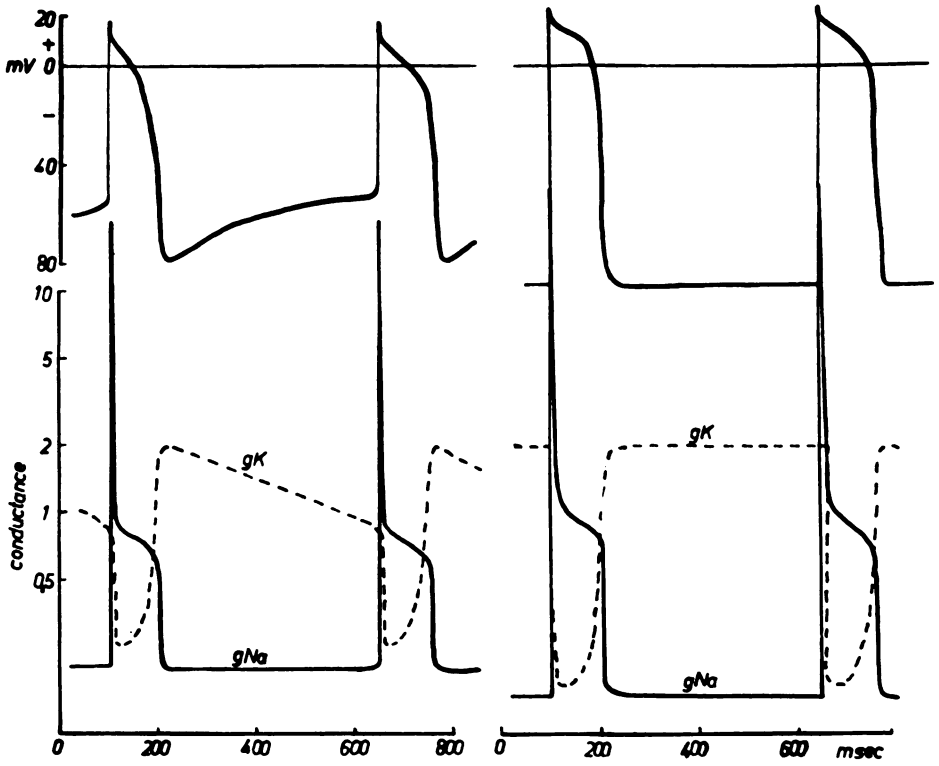

FIG. 4. Schematic diagram of the estimated sodium and potassium conductances (lower tracings) underlying the action potential and pacemaker potential (upper tracing) (using references 68, 142, 156, 308, 331).

Left, in a sinus fiber; right, in a rabbit ventricular fiber.

tively constant current becomes more effective because of the decreasing g_K. The larger the g_{Na} the more effective is a decrease in g_K in depolarizing the membrane. There is indirect evidence of a large "resting" g_{Na} at the pacemaker and "resting" g_{Na} is largest in the fibers of the sinoatrial node (308). Thus, the conditions for pacemaker activity are characterized by a relatively large sodium resting current and a decrease in g_K after repolarization. A schematic diagram of the conductance changes for sodium and potassium and the membrane potential throughout the course of a cycle are shown in Figure 4 for the pacemaker on the left and for a myocardial fiber on the right.

The ionic theory of excitation in the squid giant nerve fiber has been described mathematically in a set of differential equations (118); under special conditions these equations describe spontaneous rhythmic activity (146). Recently, the nerve equations have been modified according to the experimental data derived from the tetraethylammonium-treated squid axon, which also shows a prominent plateau (83, 93), as well as from ventricular cardiac muscle (156, 345) and Purkinje fibers (241, 242). It is of interest that the computed cardiac action potentials show the features of a pacemaker when g_{Na} is made relatively high. Thus, theory and experimental evidence are in good agreement.

C. Conduction of the cardiac impulse

Although the generation of the impulse may be considered as the primary event determining the rhythm of the heart, normal impulse conduction is also of importance for the maintenance of regular activity. Of special interest in this connection is the impulse conduction in naturally narrow and otherwise critical pathways like that of the atrioventricular node. Also, the effectiveness of extrasystolic as well as parasystolic impulse formation in producing arrhythmia depends on whether and how far extrasystolic activity can spread throughout the heart.

1. Syncytium of fibers versus insulated cardiac cells. Cardiac cells have a length of about 200 μ and are separated from each other by the intercalated discs. The latter are considered as anatomical cell borders (81, 232, 246). The functional separation of cardiac cells depends entirely on the effectiveness of the intercalated discs to act as a diffusion barrier for small ions like potassium or sodium, *i.e.*, to present a high resistance to electric current. Obviously, when electrotonic potentials are recorded 2 to 3 mm from the site of application of current (302, p. 141; 327, 347), current flows through the intercalated discs, which, therefore, cannot be a serious hindrance for local circuit current, *i.e.*, for conduction. Arguments to the contrary have been raised (286). These were based on the finding that the ohmic resistance between the interiors of two cardiac cells was always found to be at least twice as large as the resistance between the interior of one cell and the bathing fluid. This result proves that the two cells compared were not syncytially in close connection; however, it does not imply that every cardiac cell is separated from the adjacent cells by an ionic barrier of the order of that of the plasma membrane. More recently it was shown that the intercalated disc is a low barrier for diffusion of K^{42}. The resistance of a disc was estimated

to be of the order of that of the myoplasma between two discs, still being 2000 times smaller than the resistance of the plasma membrane (332). Therefore, under normal conditions conduction should not be affected appreciably by the intercalated discs; but when in a deteriorated fiber the membrane potential and the safety margin for conduction are reduced, the discs may well be the locus where block first occurs.

2. Local circuit theory and conduction velocity in the heart. In a cylindrical fiber with cable-like properties, that is, a membrane with a capacity and high resistance, surrounding a myoplasmic core of low specific resistance (R_i), the current density (I_m) through any patch of membrane is given by

$$I_m = \frac{a}{2R_i \theta^2} \frac{\delta_2 V}{\delta t^2} \qquad (1)$$

where a is the diameter of the fiber, V the potential difference across the membrane and θ is the conduction velocity, which is the same at each point of the fiber (114, 118). No equation is available which gives θ explicitly, for I_m is a complicated function of V and time. By the aid of the Hodgkin-Huxley nerve equations (118) θ could be determined for a giant axon and was found to agree with the θ measured in this structure. According to equation (1), conduction velocity depends on the rate of rise of the action potential and should be slowed when the latter is reduced. Many examples will be given in this review of slowing of conduction due to a reduction in the rate of rise of the action potential. Since the rate of rise is reduced when the membrane potential is less negative, conduction velocity is slowed in a fiber which is slightly depolarized. This is expected to occur not only in a deteriorated or injured fiber, but also in a normal fiber when an extrasystole arises before repolarization of the surrounding fibers is complete, *i.e.*, at a time when the membrane potential is still lower than in diastole. This consideration becomes especially interesting when a premature action potential is conducted along a pathway whose fibers have a progressively longer action potential; examples are orthodromic conduction through the atrioventricular node (129) or retrograde conduction from the ventricle to the Purkinje system (127, 161).

Conduction velocity is proportional to the square root of the fiber diameter (see equation), which varies considerably in the heart. The conduction velocity in both the atrial and ventricular myocardial fibers (average diameter 12 μ) of the canine or human heart is about 0.9 m/sec (302); much slower conduction (0.05 m/sec) has been found in the thin fibers (3 μ or less) of the atrioventricular node (125, p. 159 ff.; 129), whereas in the thicker Purkinje fibers (diameter about 30 μ) conduction velocities of 1.5 to 3 m/sec have been measured (62, 302, 305, 331). Both in the atrioventricular node and in the Purkinje fiber, the diameter of the fibers does not account quantitatively for the conduction velocities measured, a circumstance which suggests that the membrane properties of the fibers compared are different. In Purkinje fibers, for example, the rate of rise for a given membrane potential is considerably steeper than in a myocardial fiber. This suggests a greater increase of g_{Na} on depolarization in the specialized

fiber type than in the ordinary ventricular muscle fiber (302). The higher conduction velocity allows the Purkinje fiber to play its physiological role in distributing excitation, so that the different parts of the heart will be excited nearly simultaneously (normal duration of QRS in the electrocardiogram). After interruption of the distributing network or of one of its branches, the wave of excitation has to make detours through slowly conducting myocardial tissue (prolonged duration of QRS).

In a network of fibers, such as in the heart, "the cable" is anatomically not well defined and the current distribution is difficult to survey. The electrotonic potential spreads farther in the main direction of the fiber bundle than in a lateral direction (347). It should be expected, therefore, that conduction velocity is higher along the bundle, as compared to lateral spread. Complicated situations arise at points where one or several thin fibers merge into one very thick fiber or *vice versa*. At such locations, for instance, at the atrial margin of the atrioventricular node (125, p. 160 ff.), the current density in the adjacent fiber may become suddenly small, and conduction is delayed. Eventually spatial or temporal summation plays a role in conduction under such conditions. Also, when the membrane potential is reduced, conduction might be blocked in one direction, but could still be possible in the opposite direction.

3. Normal and abnormal transmission through the atrioventricular node. Much valuable information about conduction in the atrioventricular node has been derived from surgical interruption (112) and from extracellular recording (172, 247, 270). Recently it became possible to trace the spread of excitation continuously from the right atrium to its arrival in the bundle of His, as well as in retrograde direction (128, 129, 215, 264), by using intracellular electrodes. The major part of the atrioventricular delay could be localized within 1 mm of the atrial margin of the node. In this circumscribed area the fibers have low resting potentials, and both overshoot and rate of rise of the action potential are small. Often the upstroke of the action potential is notched, indicating that the electrode records excitation from different merging fibers in the network (128, 129). The conduction velocity through this area is as slow as 0.05 m/sec or less. Certainly the safety margin for conduction is low. It should be expected, therefore, that drugs which affect the cell membrane will do this very effectively in those fibers in which the passage of an impulse can easily be blocked or enhanced. Also, in all parts of the node, slow diastolic depolarization has been observed (129).

It is well known that the nodal tissue cannot transmit rapid atrial activity. Intracellular recording has shown that failure of transmission is in part due to the fact that the duration of the action potential is increased along the pathway from the atrium to the bundle of His. During rapid atrial rhythm, at the atrial margin of the node every atrial excitation may be followed by a normal nodal action potential, while in the lower parts of the node every second action potential falls into the period of relative refractoriness of the preceding excitation; accordingly it is of smaller amplitude and of shorter duration; ultimately block may occur. Then an action potential arrives in the bundle of His only after every

second atrial excitation (2:1 block of atrioventricular conduction) (129). As stated above, the atrial margin of the node is a very critical pathway through which premature atrial beats are often conducted with delay or are even blocked (126). The action potentials in this region are short, but refractory period seems to last much longer than the repolarization of the action potential.

IV. EFFECT OF INORGANIC CATIONS AND ANIONS ON SPONTANEITY AND CONDUCTION

This chapter will be concerned with the effect on spontaneity of changing the ionic concentrations of the plasma or of the perfusate, as well as those of foreign ions. A change in the concentration of an ion species in the perfusion fluid results in a change of the equilibrium potential for this ion between the extra- and intracellular phases. The membrane potential will be more or less affected, according to the membrane permeability for the ion species. The initial effect of a change in external concentration is a current flow until a new equilibrium for the currents contributed by all ions is established. Thus, both initial and late effects should result from a change in the extracellular ionic concentration. The same holds true when a foreign ionic species is added. If the foreign ion is able to enter the cell there will be an initial inward flow of this ion until a final distribution is reached. Furthermore, the presence of a foreign ion may influence the permeability for the other ions. The results of such experiments, therefore, have to be considered with caution and do not always give a clearcut answer.

Sodium. According to the theory of spontaneity presented above, reduction of the extracellular sodium concentration and hence of the electrochemical potential of sodium between the intra- and extracellular phases should slow the pacemaker activity. Unfortunately this effect has not been systematically studied in the sinoatrial node, but a few observations are available. Generally it can be said that reduction of extracellular sodium reduces the slope of the pacemaker potential and thereby retards spontaneity. When 50 % of the sodium chloride in the perfusion fluid is replaced by an equimolar amount of sucrose or tris(hydroxymethyl)aminomethane chloride, the slope of the pacemaker potential in the sinoatrial node decreases somewhat (115, 125, p. 115; 307). The rate of the guinea pig heart is slowed by only 10 to 20 % when the extracellular sodium is reduced to one half (251). In the sinoatrial node of the rabbit heart, osmotic replacement of 75 to 90 % of the external NaCl by sucrose is followed by an initial transient hyperpolarization and a reduction of the slope of the pacemaker potential (308). After 2 to 3 minutes, however, the preparation deteriorates and depolarization causes block of conduction at a time when spontaneous depolarization and repolarization can still be observed. About 20 minutes after depletion of sodium, spontaneity is abolished. The early effects of sodium depletion are in qualitative agreement with the theory of a sodium inward current being responsible for spontaneity. In the case of the late effects several secondary actions are believed to complicate the picture.

In the excised Purkinje fibers of dog or sheep hearts the rate effect of sodium depletion is more marked than in the sinoatrial node (62). When the extracellular

sodium concentration is halved, the rate of beating is halved within 10 minutes (62). This result is attributable to a reduction in the slope of the pacemaker potential, which is roughly proportional to the sodium driving force, as would be expected if a depolarizing sodium current is a condition for the pacemaker potential (308).

A decrease in the plasma sodium concentration that is compatible with life does not change the excitability of the heart appreciably. When, however, isolated hearts are studied, a severe depletion of extracellular sodium reduces the amplitude of the extracellularly recorded action potential (52) and depresses excitability as well as conduction velocity. These effects result in atrioventricular dissociation and finally render the fiber inexcitable (46). Inexcitability of a frog heart bathed in a sodium-free Ringer solution was observed as early as 1902 (244). On the other hand it was found that in a solution containing 4.8 mM NaCl and no other ions the frog ventricle is still excitable (40). The rate of rise of the action potentials under this condition is very low. When potassium or chloride ions are now added, excitability is lost promptly, probably because the flux of these ions short circuits the small excitatory sodium current (40). It should be noted that all these effects of sodium depletion are reversible when normal Tyrode solution is readmitted.

Lithium. Replacement of the extracellular sodium by lithium ions is of interest, since it has been shown that in skeletal muscle fibers lithium can replace sodium for the spike potential; it cannot, however, be eliminated from the cell "uphill" by active transport (165). A similar situation might occur in cardiac muscle and in the pacemaker. When 90% of NaCl in the bathing solution of a Purkinje fiber or a sinoatrial preparation is replaced by LiCl, the beating rate is slowed progressively after a short period of acceleration. In this phase the slope of the pacemaker potential is reduced. Finally spontaneity ceases, but the preparation remains excitable (163). In this state the action potential is no longer followed by a positive afterpotential. Readmission of Tyrode solution results in diastolic depolarization and spontaneity within 1 to 3 minutes (163, 311). In both ordinary ventricular fibers and Purkinje fibers the duration of the action potential is shortened with a loss of plateau when a larger part of the extracellular sodium is replaced by lithium (163, 208, 288). The mechanism of the lithium effect is not clear.

Potassium. Either increase or decrease of the extracellular potassium concentration is accompanied by a change in the activity of the pacemaker. When the extracellular K is increased from 2.7 mM up to 13.5 mM, an early increase in the frequency of the sinoatrial node is noted (89, 227, 263). In a later phase, impulse generation is slowed. With higher K concentrations in the bathing solution, depression of the pacemaker in the sinoatrial node becomes more and more prominent (65, 263). In Purkinje fibers, elevation of the extracellular potassium also results in a transient rise of the rate, and later the slope of the pacemaker potential is more and more reduced until spontaneous activity ceases (331). If the preparation is still excitable, a positive afterpotential is scarcely detectable. A relative insensitivity towards an increase of the extracellular K in sinus

fibers, as compared to atrial fibers, has been demonstrated (171, 227). Sinus fibers do not depolarize as much as atrial fibers when the extracellular K is elevated, presumably because the ratio g_K/g_{Na} is smaller in the pacemaker than in the myocardium (see p. 286). At 13.5 mM extracellular K, action potentials still originate within the sinus, whereas atrial myocardium is no longer excitable when stimulated electrically (227). Several factors may be responsible for the rate effects of an increase in the extracellular K concentration. 1) At a slight depolarization of the diastolic membrane potential, the pacemaker potential may reach a still unchanged threshold earlier, thereby accelerating the beat frequency. In a later phase or at higher concentrations of the extracellular K, the threshold membrane potential becomes less negative, a factor which slows the rate again. 2) In Purkinje fibers the decrease of g_K on depolarization is smaller when the extracellular K is elevated than when depolarization is due to current flow (36, 38). This effect should slow the rate of the pacemaker potential at high K. 3) Sinus fibers are still excitable at low membrane potentials at which in myocardium or Purkinje fibers the sodium carrier system is inactivated (125, p. 119 ff.). This in an important condition for the sustained spontaneous activity on elevation of extracellular K.

In the sinoatrial node reduction of the extracellular K concentration to zero increases the tendency for spontaneous depolarization, and multifocal activity appears (125). In Purkinje fibers lowering of the extracellular K to zero causes arrest of the preparation at the plateau of the action potential (38). Preceding the arrest on the plateau, extrasystoles coupled to repolarization, as described on p. 281, are often observed. Probably a similar effect in the sinoatrial node is the basis for multifocal activity in this tissue. Reduction of g_K in low extracellular K concentration, which could explain this effect, has been observed both in electrophysiological and radioisotope studies (36).

Increase in serum potassium is known to lead to changes in the human electrocardiogram (285, p. 448). As in the experiments with excised tissue, slowing and speeding of the sinus rhythm, as well as production and suppression of heterotopic pacemakers, have been reported. Furthermore, an increase in the amplitude of the T-wave, atrioventricular block, and an increase in the duration of the QRS complex have been observed. The latter effects are the result of a decrease in conduction velocity, or block of conduction resulting from a low resting potential (see p. 287). The change in the T-wave can be explained by the shortening of the action potential on elevation of the extracellular K concentration (279, 331). Arrhythmia can be produced experimentally in the dog heart *in situ* on elevation of the extracellular K. Factors which contribute to the development of the arrhythmia are: 1) increase in the activity of the heterotopic pacemakers; 2) decrease in conduction velocity with the occurrence of local areas of block and 3) shortening of the refractory period, which favors re-entrance activity. These factors eventually can result in incoordinated independent activity of different regions of the heart and in fibrillation.

Electrocardiographic changes have also been observed when the serum potassium is reduced. The QT interval was found to be prolonged (76), indicating

prolongation of the action potential under this condition. ST depression and the appearance of U waves were also reported (220). It is possible that these U waves are caused by retardation of the terminal part of repolarization, as has been observed in single fiber experiments upon reducing the extracellular K (125). Extrasystoles reported to occur in hypopotassemia might be related to this disturbance of repolarization.

Calcium. Calcium ions do not affect the membrane potential by virtue of their electrical driving force, but they exert an influence on excitability of cardiac fibers. When Purkinje fibers are kept in a Tyrode solution with extracellular Ca reduced to one fourth of normal (2 mM) or increased to 4 times normal, the maximum diastolic membrane potential and the slope of the pacemaker potential for the slow diastolic depolarization do not change appreciably (329). The threshold potential, however, is more negative (−68 mV) when the extracellular Ca is low than it is in a Tyrode (−57 mV) solution with a high extracellular Ca concentration. This change in threshold potential results in a rise of the beat frequency when the extracellular Ca concentration is low and in a slowing when the extracellular Ca is elevated. With a high extracellular Ca concentration spontaneous depolarization might not reach threshold potential. The fiber is arrested and the membrane potential "stabilized" at a level of −70 to −80 mV. The effect of extracellular Ca concentration on threshold potential has also been tested by application of current pulses (329). Again, a marked influence of the extracellular Ca on threshold was found. The fiber has to be depolarized more, *i.e.*, more current is required to reach threshold potential when the Ca concentration is high. In a variant of this experiment, constant depolarizing current is driven through the membrane; the quiescent fiber responds repetitively, each action potential being preceded by a slow depolarization like the pacemaker potential. When the extracellular Ca is reduced, less current is needed to produce a repetitive response (308). The effect of a reduced extracellular sodium concentration on threshold can practically be cancelled by additional reduction of extracellular Ca concentration (308).

It should be noted that in Purkinje fibers the current-voltage relationship of a fiber is not influenced by a change of extracellular Ca (range of 0.45 mM to 7.2 mM) either in normal or in a sodium-free Tyrode solution. This finding suggests that the extracellular Ca does not affect g_K (308, 329), and it is concluded that the stabilizing effect of Ca ions reflects a reduction in the sodium permeability of the membrane. The effect of calcium ions on the sodium permeability can partially explain the influence of calcium ions on the depolarization caused by an increase or decrease of the extracellular potassium concentration. As already mentioned, the diastolic membrane potential of Purkinje fibers bathed in a Tyrode solution of normal potassium content is not affected by a reduction or elevation of Ca concentration. However, when the membrane is depolarized by an increase in the K concentration of the Tyrode solution, an additional lowering of the Ca concentration causes still greater depolarization, but if Ca is raised under this condition, the membrane potential increases (132). The depolarization produced by a *low* potassium concentration in the Tyrode solution

becomes smaller when Ca is lowered and enhanced on elevation of the Ca concentration (132). The mechanism of these effects is not fully understood. They could be explained qualitatively by the effects of Ca on g_{Na}. It is possible, however, that under these conditions g_K is also affected by the Ca concentration in the Tyrode solution.

The Ca concentration also influences the time course of the action potential. When Ca and Mg (which has an effect similar to that of Ca) are reduced, the atrial action potential is prolonged and its shape resembles that of the ventricular action potential (132). The time course of the action potential of the ventricular fiber is reported to be insensitive to a reduction of Ca, from 2 mM normally present in Tyrode solution to 0.02 mM (67, 132); other authors, however, observed prolongation of the plateau when Ca was reduced to 0.5 mM (67). Total depletion of both Ca and Mg prolongs the action potential (125, p. 94; 132) and an increase of Ca by a factor of 4 shortens the duration of the action potential, which now has a time course like that of an atrial fiber (132). Depletion of Ca and Mg by EDTA (ethylenediaminetetraacetate) prolongs the action potential of both ordinary ventricular and Purkinje fibers (44); $CaCl_2$ antagonizes the effect (218). Under these conditions spontaneous action potentials of many seconds duration have been observed. The diastolic period is shortened, since the pacemaker potential is enhanced. Even in ordinary ventricular fibers spontaneous depolarization develops in the presence of EDTA. These findings can also be explained by a strong influence of calcium on sodium permeability.

In contrast to the relative stability of the membrane potential in Purkinje fibers, in nerve and muscle fibers elevation of the extracellular Ca concentration hyperpolarizes the membrane potential, and the opposite can be observed on Ca depletion (19, 280, 287). The latter type of Ca effect seems to occur in the sinoatrial node. This structure is relatively insensitive to a lowering of Ca, but depolarizes when the Ca concentration is markedly decreased (307). Similarly, a marked increase in Ca has the expected effects of hyperpolarization of the maximum diastolic membrane potential and suppression of the pacemaker potential in the rabbit sinus (125, p. 115).

High calcium decreases diastolic excitability in cardiac fibers (329), the same effect it has in nerve and skeletal muscle fibers (280). When, however, excitation occurs starting from a low membrane potential, the maximum sodium inward current is larger on elevation of the Ca concentration (329). This means that the availability of the sodium carrier system is larger at low resting potentials when Ca is raised. Under these conditions the S-shaped curve relating maximum rate of rise of the action potential to the membrane potentials prior to stimulation (see Fig. 3) is shifted to the left, to less negative membrane potentials. This Ca effect may be related to the following observations. When the resting potential is low because of an increase in the extracellular potassium concentration, it is sometimes impossible to elicit a propagated action potential at normal extracellular Ca concentration. When, however, the Ca is raised, a propagated action potential appears at the same membrane potential (125, p. 67). It is known that fibers in high Ca concentration are re-excitable relatively early (at relatively

low membrane potentials) during repolarization (329). This behavior, which sometimes gives rise to coupled extrasystoles (early re-excitation), should be expected when elevation of Ca concentration restores excitability already at low membrane potentials. Arrhythmia can also be observed on elevation of the extracellular Ca concentration in the whole heart *in vitro* and *in situ* (121, 123). In the perfused rabbit heart *in vitro*, fibrillation can be initiated by injection of $CaCl_2$ into the perfusion stream (105); also, in the intact animal injection of $CaCl_2$ causes ventricular extrasystoles and eventually ventricular fibrillation (123, 344).

Strontium and barium. When strontium is added to the normal Tyrode solution its effect is the same as of a similar amount of Ca (89). When, however, the Ca concentration is very low, the addition of strontium affects the action potential in the same way as would a complete removal of Ca. It has been concluded that strontium displaces the remaining Ca from sites within the membrane.

Barium increases the duration of the ventricular action potential and the refractory period in the frog heart (169). Conduction velocity seems to be reduced in the guinea pig ventricle after injection of $BaCl_2$ (2). Occurrence of extrasystoles and ventricular fibrillation in the dog heart has been reported (238, 260, 284). This arrhythmia might be related to the occurrence of spontaneous activity of the abnormal impulse type in excised ventricular fibers in presence of $BaCl_2$ (218). The effect of barium on the sinoatrial node and heterotopic pacemakers *in vitro* is not known. It is not possible at present to relate clearly the arrhythmias in the presence of barium to observations on the single cardiac fiber.

Anions. The significance of the flow of chloride ions for the slow diastolic depolarization has recently been studied (37, 38, 142, 144). In Purkinje fibers the chloride equilibrium potential (E_{Cl}) is at about -50 mV, and at the diastolic membrane potential the conductance to chloride (g_{Cl}) amounts to less than 20% of the total membrane conductance (144). A small Cl current will therefore contribute to the diastolic depolarization in Purkinje fibers. In the sinoatrial node of the rabbit heart, intracellular Cl concentration has been determined as 40 mM and E_{Cl} can be estimated to be between -30 and -40 mV (227). If g_{Cl} were appreciable a depolarizing Cl current could be responsible for part of the diastolic depolarization.

When chloride is replaced by another ion the effect on membrane potential depends on the permeability to the new anion in comparison to g_{Cl}. Sudden depletion of extracellular Cl concentration to zero results in a depolarizing current, the intracellular chloride leaking out. Moreover, the concentration difference for the replacing ion is initially very large; the inflow of these ions results in hyperpolarization. It can be shown experimentally that the anions smaller than Cl, like I^-, Br^-, and NO_3^-, suppress the pacemaker potential, whereas larger anions such as pyroglutamate, methylsulfate, and acetylglycine, produce the reverse effect (144). The membrane resistance in the range of the pacemaker potential is reduced in the presence of the smaller anions and increased when the extracellular chloride is replaced by a larger anion (38, 144). The effects of these ions on the membrane potential are explained by the larger permeability

of the membrane towards I^-, Br^-, and NO_3^-, which results in a hyperpolarizing anion current.

In the case of the large anions to which the membrane is less permeable, there predominates a transient depolarizing chloride current which increases the beat frequency (144). When intracellular chloride then falls, however, late effects appear. The depolarizing current decreases and the frequency is reduced to below its original value. This effect is observed, in the presence of large anions, 5 to 10 minutes after the extracellular chloride has been replaced. The plateau of the action potential is prolonged in the presence of the large anions, often to such an extent that the rate of the Purkinje fiber is reduced, although the slope of the pacemaker potential is still steeper than in normal extracellular chloride (144). The effect of chloride replacement by large anions is less prominent in the sinoatrial node than in the Purkinje fiber. When in the rabbit sinus 90% of the extracellular chloride is replaced by acetylglycine, the rate of the pacemaker increases inconsistently up to 20% within 5 minutes (307); lack of any effect has also been reported (125). These results suggest that the contribution of the chloride current to the diastolic depolarization is not large and is smaller in the natural pacemaker than in the Purkinje fibers.

V. METABOLIC INHIBITORS, OXYGEN LACK

In giant nerve fibers, 2,4-dinitrophenol (DNP), cyanide, and monoiodoacetate are known to inhibit active transport, which can be activated again by intracellular injection of ATP (33, 34, 119). In heart muscle, active transport has not been studied directly by using radioisotopes, but the electrophysiological effects of a few metabolic inhibitors on the pacemaker are known. DNP, in a concentration of 0.2 mM, after a short excitatory period decreases the slope of the slow diastolic depolarization in the sinoatrial node of the rabbit heart; the effect is reversed by appropriate concentrations of ATP (226). The poisoning is accompanied by a depolarization, the preparation being finally arrested at about -50 mV. It is possible that this depolarization is due to an accumulation of potassium outside the cells. Reduction of the intracellular potassium concentration to about one half and increase of the intracellular sodium concentration by up to 30% have been found in sinoatrial fibers in the presence of DNP (188); the effect can be attributed to an inhibition of active sodium and potassium transport, but direct effects of DNP on membrane permeability cannot be excluded. Similar effects have been observed in Purkinje fibers. Bathing a preparation in 1 mM DNP or 1 mM iodoacetate immediately results in increased slope of the pacemaker potential and depolarization; within a few minutes pacemaker activity is abolished. When the preparation is arrested, the membrane potential often increases again to a value of -70 to -75 mV. In this case the fiber is excitable when directly stimulated, but repolarization is no longer followed by a pacemaker potential, *i.e.*, the membrane potential is constant throughout diastole (163). Again, it is not possible to conclude from these observations that a net inward current due to active transport, which is blocked by the inhibitors, is responsible for the pacemaker potential.

An initial excitatory period of increased automaticity has also been observed

on lowering the oxygen tension both in the sinoatrial node and in Purkinje fibers (125, p. 117; 306). Increasing the CO_2 tension in the bathing solution from 0 to 10% decreases pacemaker activity. A further increase up to 20% and higher leads to bigeminy, trigeminy and finally to a regular rhythm at a high rate. Repolarization remains incomplete, *i.e.*, depolarization starts prematurely. Finally the membrane potential oscillates between −50 mV and 0 (47). As judged from the rate changes of the sinoatrial node and a ventricular pacemaker, the latter is more sensitive towards increase of CO_2 or reduction of O_2 tension (296).

In the presence of DNP, iodoacetate, sodium azide, sodium cyanide, or low oxygen tension, conspicuous shortening of the action potential of atrial, ventricular, and Purkinje fibers occurs before the resting potential is appreciably affected (170, 207, 210, 222, 226, 306). All these agents, including low oxygen tension, lower the resting potential, and thereby reduce conduction velocity. In the dog heart *in situ*, reduction of the arterial oxygen saturation to one third or one half results in prolongation of QRS in the electrocardiogram by 20%; the PQ interval is prolonged by 50%, indicating a greater sensitivity of the nodal fibers towards oxygen deficiency (295).

For the occurrence of extrasystoles and fibrillation, the observation of incomplete repolarization and oscillation of the membrane potential in the Purkinje fibers might be of importance. The shortening of the refractory period, together with the slowing of conduction, is expected to favor re-entry activity. The degree of shortening differs considerably in different fibers of the same preparation, a circumstance which results in an inhomogeneity of the refractory state. It is not surprising, therefore, that electrical stimulation leads more often to fibrillation under these conditions (26).

VI. CARDIAC INNERVATION AND TRANSMITTER ACTION

A. Vagal stimulation and acetylcholine

1. The effect on the membrane potential. The effect of vagal stimulation on the membrane potential of the pacemaker has been studied in the sinus venosus of the frog heart (41, 145). If a single shock is applied to the vago-sympathetic trunk in the beginning of diastole, the slope of the pacemaker potential is slightly reduced, resulting in a longer interval between the two beats. If the vagus is stimulated repetitively at a low rate, the slope of the pacemaker potential is reduced, the duration of the action potential is shortened, and on repolarization the membrane potential reaches a value slightly more negative than the control. Repetitive stimulation at a higher frequency (10 to 20/sec) suppresses the pacemaker potential and drives the membrane potential to a level which is more negative by about 10 mV than the maximum diastolic potential prior to stimulation [hyperpolarization, the Gaskell-effect (92)]. When stimulation ceases, the membrane potential depolarizes again to the threshold value and the beat is resumed. Vagal fibers and endings can also be excited by direct stimulation of the sinoatrial node of the rabbit heart by a rapid volley of stimuli, which the cardiac muscle cannot follow. Immediately after the stimulation period the rate

of the pacemaker is reduced or the preparation is arrested and simultaneously the membrane potential is increased. Hyperpolarization and the rate effect are abolished when the preparation has been previously atropinized or when hemicholinium has been administered; the rate effect is enhanced in the presence of physostigmine (3).

Inhibitory effects have also been observed after application of acetylcholine (ACh) to the sinoatrial node of the dog and rabbit heart, as well as to ectopic pacemakers in the right atrium of the human heart (303, 309, 338). In quiescent atrial tissue of the cat, hyperpolarization on application of ACh or carbamylcholine has been reported (23). On application of ACh or stimulation of the vagus nerve in atrial myocardium, very little hyperpolarization may be seen, although the fibers are strongly inhibited and eventually inexcitable (131, 145). It will be shown later that the amount of hyperpolarization depends to a large extent on the level of the membrane potential.

The sensitivity towards ACh varies considerably in different structures of the heart. The inhibitory effects are most pronounced in the sinoatrial node and the atrionodal junction (54, 125, 145, 304). Furthermore, ACh effects are well known to occur in both atria (131, 304) and have also been observed in the upper part of the atrioventricular node (54). With higher concentrations of ACh (10^{-4} g/ml), slight inhibition of the spontaneity in the bundle of His and in Purkinje fibers can occur (275), but this effect is not seen consistently. Some Purkinje fiber strands seem to be slightly sensitive towards ACh, but some are not. This agrees with the observation that in the dog heart with complete atrioventricular block, ACh can affect the ventricular pacemaker (71). A depressant effect of ACh on ventricular pacemakers in rat and rabbit hearts has also been reported (9). Mammalian myocardial fibers are insensitive towards ACh and even concentrations of 10^{-4} g/ml cause no inhibition, *i.e.*, no sign of an increase in the membrane permeability towards potassium ions can be detected (131, 275). This agrees with older reports which indicate that the ventricular pacemaker in complete atrioventricular block could not be affected by vagal stimulation (73, 74, 75, 111, 252). Since it is known that the density of innervation is higher in the sinus than in the atrium (312), we may assume that the differences in sensitivity are related to the density of synapses in the structures compared. The ventricular myocardium does not seem to be innervated by cholinergic nerve fibers. It should be noted that in the ventricular myocardium, negative inotropic effects do occur when high ACh concentrations (10^{-4} to 10^{-5} g/ml) are applied to excised ventricular strips (275) or are administered intravenously to the dog (278); however, this depressant effect is not accompanied by any change in the membrane potentials. This means that the surface membrane of the ventricular fiber has no receptive sites at which ACh can increase the potassium permeability (see below, p. 298), but that ACh can reach sites which control contractility. This direct effect on the contractile process occurs only when higher concentrations of ACh are used. It is doubtful whether this effect has any physiological importance.

2. Mechanism. As a possible mechanism of the effect of ACh, an increase in the

potassium permeability of the cardiac surface membrane has been suggested (23). This could explain the shortening of the duration of the action potential and the hyperpolarization. That a "mobilization" of potassium ions is involved in inhibition was indicated by the observation of a liberation of potassium from the atria as a result of vagus stimulation or in the presence of ACh (135, 137, 195). All these earlier observations, however, did not lead to a coherent hypothesis; this was introduced as soon as Kuffler (184), Fatt and Katz (80), and Eccles (69) put forward a general concept of a change in ionic permeability produced by the transmitter. This hypothesis has since been elaborated with respect to heart muscle both by electrophysiological and radioisotope techniques.

If ACh were to increase g_K, this should cause a fall in membrane resistance independent of the membrane potential. Reduction of membrane resistance in a frog atrial strip has been demonstrated, the length constant being reduced by one third to one half in the presence of ACh (310). Many examples of a drop in membrane resistance by ACh have been reported in the rat, dog, and rabbit atrium (68, 155, 303, 304, 347). An increase in potassium conductance will drive the membrane potential towards the electrochemical equilibrium potential for potassium (E_K) and cause hyperpolarization whenever the membrane potential E is less negative than E_K. This is the case at the site of the pacemaker, where, as stated above, an appreciable resting sodium current—the generator of the heart beat—keeps the diastolic membrane potential 10 to 20 mV more positive than E_K (see p. 285). In a latent pacemaker fiber, however, the difference between E and E_K is smaller and, therefore, the hyperpolarization will be smaller for a given change in g_K. In atrial fibers, which have a high constant diastolic membrane potential, the difference between E and E_K may be small and no hyperpolarization is seen (131, 145). These observations suggested a crucial experiment. It should be possible to show that, at membrane potentials more negative than E_K, depolarization results on application of ACh, *i.e.*, that the ACh effect reverses its direction at E_K. This reversal has been demonstrated (303): depolarization occurs on application of ACh when a strong inward current raised the membrane potential above E_K. Furthermore, the membrane potential at which the hyperpolarizing response reverses to a depolarization became more positive when the extracellular potassium concentration was increased. These results should be expected if the reversal potential is identical with the electrochemical potassium potential between the intra- and extracellular phases. This behavior proves that ACh causes a specific increase in g_K, because a nonspecific increase in membrane permeability could not be expected to have a reversal potential identical with that of a potassium electrode.

The increase in potassium permeability has been directly observed by radioisotope experiments (107, 141, 248). In a sinus venosus, vagal stimulation (10/sec), as well as application of ACh (2×10^{-7} to 2×10^{-6} g/ml), increases the rate of outflow as well as the rate of uptake of K^{42}. Similar experiments in atrial tissue have also shown an increase in g_K, which is much weaker than in the sinus. As to the specificity of the permeability change, it is interesting that no obvious effect of ACh on the movement of Br^- and Cl^- could be detected (141).

Thus, in the heart both electrophysiological and radioisotopic studies show the great specificity of the change in membrane permeability produced by ACh towards potassium ions.

3. "Excitatory" effects of ACh. Several observations suggest that ACh might produce excitatory effects in heart muscle in addition to the well-known inhibition. When ACh is added to isolated atria in Locke's solution containing quinidine, the arrested atria resume the beat. The same concentration of ACh causes inhibition in a control preparation (22, 24). Similar stimulating effects of ACh have been reported in deteriorated arrested atria and in the cooled atrium when the vagus was stimulated (28, 30). It has been concluded from such experiments that ACh might initiate the pacemaker potential at least under special experimental conditions (24). Experimental evidence, however, supports a different explanation. In the cooled or deteriorated atria the membrane potential is so low that conduction is blocked, but local pacemaker activity is still going on. When ACh is administered or the vagus nerve is stimulated hyperpolarization occurs. At the increased resting potential excitability is restored and conduction is possible again. After a short initial inhibition of the pacemaker, the same inhibitory mechanism allows the preparation to contract, for the action potential is conducted again through the preparation (212, 304). A similar explanation may apply to the quinidine "conditioning," another circumstance in which hyperpolarization might overcome the "stabilizing" quinidine effect on the membrane (see p. 311). Clearly, such effects cannot be called excitatory if their mechanism is considered.

Whether the increase in membrane potential following administration of ACh or vagal stimulation fully explains the recovery of excitability and conduction of a deteriorated or cooled preparation is difficult to decide; accordingly, additional mechanisms should be considered. It is possible that ACh can liberate norepinephrine (29), the effect of which might outlast the inhibitory period, consequently produce acceleration of the pacemaker in the sinoatrial node, and improve the spread of excitation over the preparation (see p. 308). Stimulating effects of ACh have been observed in the heart with sinus rhythm (133), as well as on the ventricular pacemaker (after atropinization or when high doses of ACh were used) of hearts with complete block (71). The effects have been considered to be adrenergic; in the case of the ventricular pacemaker, phentolamine, an adrenergic blocking substance, eliminates the stimulatory effect of ACh. It has also been reported that either epinephrine or ACh exerts a positive chronotropic action on isolated rabbit atria treated with quinidine (24). In this case both effects are blocked by dichloroisoproterenol (DCI) or by pretreatment with reserpine (see p. 309). These findings suggest that the stimulating action of ACh is due to a release of epinephrine (6).

True excitatory action of ACh requires evidence for a depolarizing effect (*i.e.*, reduction of g_K or an increase of g_{Na}) or for an effect similar to that of a low Ca concentration on excitability. The latter possibility has been tested by measuring the rate of rise of the action potential in the atrium of the guinea pig heart; this increased in the presence of ACh, concomitantly with a small hyper-

polarization, which is believed not to be responsible for the effect (154). In the dog atrium, however, the increase in rate of rise corresponds to the small increase in membrane potential caused by ACh (307). The available evidence does not support the hypothesis of a direct influence of ACh on the sodium-carrying system.

4. Excitability and refractoriness. The effect of vagal stimulation or ACh on the resting or diastolic excitability has frequently been determined and with variable results. The strength-duration curve for a threshold stimulus in the dog atrium has been found completely unaffected even by strong vagal stimulation (20, p. 203; 130). In the cat heart and in the turtle sinus an increase in rheobase has been reported (61, 96), whereas in the same preparation in the presence of carbamylcholine a decrease of rheobase and a fall of chronaxie have been observed (23). The discrepancies may be attributed in part to differences in the sensitivity to ACh of the structures in which excitability has been determined. There is also some uncertainty as to whether the absolute threshold potential changes when hyperpolarization occurs. No changes of absolute threshold potential were recorded. Defined in terms of the threshold potential, excitability is certainly not increased in the presence of ACh. If, however, chronaxie is used for definition of excitability, the latter is expected to increase, since a fall of the membrane resistance, and hence of the membrane time-constant, increases the stimulating efficiency of short pulses. Finally, the rheobase might increase in fibers whose membrane conductance is increased because the stimulating voltage across the membrane is relevant here. A contrary result might be due to methodological reasons: when the strength-duration curve is determined with extracellular electrodes, the current distribution in the tissues changes as the membrane conductance falls, and a stronger current may flow to some fiber regions.

It is well known that ACh shortens the action potential in the sinus and atria (131, 145, 279), the effects of carbamylcholine and acetyl-β-methylcholine being indistinguishable from those of ACh (23, 211, 325). The degree of shortening could be related quantitatively to the increase in potassium permeability (304). The reduction of the duration of the action potential results in a marked shortening of the absolute refractory period of the atrium, the degree of shortening being proportional to the strength of vagal stimulation (279). The relative refractory period is also shortened to a variable degree, depending on the strength of inhibition. During strong vagus stimulation or in the presence of ACh concentrations of 1.3×10^{-7} g/ml, this period is definitely shorter than the control values [dog atrium *in situ* (20, p. 204); cat atrium *in vitro* (23)]. It should be remembered that during vagal stimulation or application of ACh these changes in the refractory periods are not uniform, and that this results in a considerable inhomogeneity in excitability in atrial fibers. This might explain the decrease in fibrillation threshold and the prolongation of the period during which the atrial preparation responds with extrasystoles or fibrillation when stimulated after the absolute refractory period.

A somewhat surprising observation is the sudden increase in the beating rate of the atria that results from either local application of ACh or vagal stimulation

(90, 131, 257). Thus, ACh or vagus stimulation can cause fibrillation of the non-fibrillating atria or an increase of the rate of fibrillation (257). Also, local application of either acetyl-β-methylcholine (237) or ACh (273) to small areas of the atria produces atrial fibrillation. Likewise, ACh can cause fibrillation when added either to the fluid bathing isolated atria (339) or to the circulation of the heart-lung preparation (25, 31). The cause of these effects is not an increase of the spontaneous sinus rate or the occurrence of a single or multiple pacemakers. It results presumably from early re-excitation (see p. 281) by re-entrance (339). A favorable condition for eliciting atrial fibrillation by ACh injection is hyperpotassemia (196). However, hyperpotassemia can also either prevent or stop fibrillation induced by ACh (25). Often, of course, ACh will stop arrhythmic activity and fibrillation, especially when applied in higher concentrations.

5. Effects of ACh and vagal stimulation on conduction in the atrium and atrioventricular node. The effects of vagal stimulation or ACh on conduction depend on the intensity of the inhibitory effect. In the frog atrium a small area can be inhibited so effectively by stimulation of the branch of the vagus that innervates it, that the action potential cannot invade the inhibited area, *i.e.*, complete block occurs (185). Similar observations have been reported from the electrically driven turtle sinus; it is rendered inexcitable when the vagus is stimulated repetitively at high frequencies (145); the action potentials which arise just before block occurred have a low amplitude. The block of conduction will occur when g_K is increased up to levels comparable to g_{Na} during excitation.

In the right atrium of a dog or a rabbit heart the inhibitory effect of ACh, even in the highest concentrations, is never as strong as the vagal effect in the amphibian atrium, and the conduction velocity of the action potential is not decreased. By contrast, it can be shown by latency measurements that conduction velocity is often increased either in the presence of ACh or when the vagus is stimulated (61, 130, 304). This might be due to a slight increase in the resting potential. It is also possible that conduction velocity rises when the membrane conductance falls to a certain extent, since the current density in the immediate vicinity of excitation increases. No judgment of the quantity of such an effect is possible, since the influence of a change of the membrane resistance on conduction velocity has not yet been worked out theoretically. A situation similar to that in the amphibian sinus is encountered in the transmission through the mammalian atrioventricular node, which is blocked by ACh in low concentrations. Atrioventricular transmission has been extensively studied by a systematic simultaneous recording of the electrical events along the pathway of excitation (54, 125, p. 145 ff). A considerable difference in the ACh sensitivity of fibers in different regions has been demonstrated. ACh strongly affects fibers of the atrionodal junction. In these apparently very thin fibers, ACh produces a great reduction of the amplitude and duration of the action potential, and a block of conduction occurs (54, 125). The lower part of the node does not show signs of inhibition, and the bundle of His is also not inhibited. When the atrioventricular node is blocked, independent rhythmic activity may originate in a pacemaker in the bundle of His.

6. Atropine and cholinesterase inhibitors. Atropine eliminates the inhibitory effect of ACh (211), as well as those of carbamylcholine and acetyl-β-methylcholine (313). ACh in a concentration of 10^{-5} g/ml fails to exert its influence on the pacemaker potential and membrane resistance of an atropinized preparation bathed in a Tyrode solution (atropine 10^{-5} to 10^{-7} g/ml). The ACh effects on potassium fluxes are also abolished in the presence of atropine (107). Immediately after application to an excised dog atrium, atropine (10^{-7} g/ml) causes a slight prolongation of the action potential and a decrease of the resting potential. The effect suggests the existence of a spontaneous "resting" liberation of ACh, as is well known from other synapses (164). In concentrations of 5×10^{-6} and higher, atropine also affects ACh-insensitive membranes of Purkinje fibers and ventricular fibers. These effects are similar to those of local anesthetics (313).

The cholinesterase inhibitors have been used in several studies on the pacemaker. Eserine decreases the rate of the pacemaker activity on the heart-lung preparation and on atria *in vitro* (27, 313, 324). These indirect effects of ACh are abolished by atropine. Neostigmine causes a variable shortening of the action potential of rabbit atria (211). The effect of neostigmine on the changes in membrane resistance of fibers of the frog atrium produced by ACh has been determined (310). When the preparation is immersed in mineral oil and surrounded by a small volume of solution, the membrane resistance falls abruptly on application of ACh through the micropipette. The inhibitory effect disappears or is reduced within a few minutes, however, presumably because the ACh in the small volume is hydrolyzed. In fact, when neostigmine and ACh are applied together, the effect on membrane resistance remains constant for many minutes. Similarly, in the presence of neostigmine the potassium fluxes in rabbit atria are slightly increased (248). Diisopropyl fluorophosphate (DFP) in large concentrations (10^{-3} to 10^{-6} g/ml) added to the fluid in which a dog atrial preparation is beating spontaneously or is driven electrically produces within minutes all signs of inhibition, very much like those caused by ACh. The effects are nullified when atropine or PAM (pyridine aldoxime methiodide) is added (313). The same observation has been reported for pacemaker and nonpacemaker fibers in the isolated rabbit atrium in the presence of tetraethyl pyrophosphate (168). Here again the effect of this drug is the same as that produced by ACh and eliminated by PAM. It is possible that, in the presence of the alkylphosphates, ACh spontaneously released from the nerve endings accumulates, or the liberation is increased, or both, and ACh effects become apparent under this condition. The latter alternative may explain the shortening of the atrial action potential by strychnine, which is also reversible by atropine (211). These effects of DFP could not be observed in ventricular myocardium, in which DFP has a local anesthetic action, which, however, occurs later than the inhibitory effect in the right atrium.

B. The effect of sympathetic stimulation and epinephrine

1. Observations on the pacemaker. The direct electrophysiological observation of the effects of sympathetic stimulation on the natural pacemaker has been

possible only on the sinus venosus of the frog heart (41, 145). No information is available concerning the effects of sympathetic stimulation on the pacemaker of the mammalian heart. The typical effect of the sympathetic stimulation is an increase in the slope of the pacemaker potential, which results in shorter beat intervals, since neither the maximum diastolic membrane potential nor the threshold is appreciably affected. During stimulation at 20/sec for several seconds, the effect develops gradually, being just detectable after 1 to 2 seconds and doubling the heart rate after 10 seconds. The rate effect wanes within 1 to 3 minutes after the end of stimulation. Thus the time course of the sympathetic effect is much slower than that of the vagal effects in the heart. Similarly when either epinephrine or norepinephrine is applied to the sinoatrial node of the rabbit heart, the most marked effect is an increase in the rate of the slow diastolic depolarization (163, 338). Often application through a micropipette to a latent pacemaker area results in a shift of the pacemaker to the site of application. The effect of sympathetic stimulation on the membrane potential and the time course of its development can be judged better when the sinus venosus is quiescent. A single stimulus applied to the sympathetic root causes depolarization within one second. On repetitive stimulation depolarization reaches threshold and spontaneity is re-established (41). A somewhat indirect method of stimulating the sympathetic fibers and nerve endings in the sinoatrial node of the rabbit has been reported recently (3). When the spontaneously beating preparation is stimulated by 20 pulses/second for 5 seconds, immediately after stimulation the beating rate is suppressed, presumably because of the stimulation of vagal fibers in the preparation (see p. 296). This suppression is followed by a positive chronotropic effect, which is most probably the result of the stimulation of sympathetic structures, for it is blocked by pretreatment with reserpine or by dichloroisoproterenol, guanethidine, or bretylium and enhanced in the presence of cocaine and pyrogallol. Cocaine and phenoxybenzamine also increase the chronotropic response of sympathetic stimulation in the sinoatrial node of the rabbit dissected with its innervation (138).

Experiments with excised Purkinje fibers yielded results similar to those gained with the sinoatrial node. When epinephrine is administered to the fluid in which the preparation is bathed, the only consistent observation is the increase in the slope of the pacemaker potential (163, 243). The beat frequency can approach that of the sinus node. This observation agrees well with the observation of extrasystoles and ectopic foci after an intravenous injection of epinephrine. These are seen especially in the intact animal, in which the reflex inhibition of the sinus activity favors the development of heterotopic pacemakers (120). It is important to note that only in Purkinje fibers has epinephrine the depolarizing effect on the diastolic membrane potential, whereas in ventricular myocardium the production of a pacemaker potential has never been observed. Since epinephrine induces spontaneity in cat papillary muscles (63), this preparation is supposed to contain Purkinje fibers. The quiescent Purkinje fiber shows depolarization when epinephrine is applied locally, as in the quiescent sinus after sympathetic stimulation. When the depolarization is large enough,

it leads to oscillations and development of a pacemaker (163, 243). At the pacemaker in a Purkinje fiber strand a few further effects can be observed after local application of epinephrine through a micropipette (surface area of application, 100 μ^2; concentration, 10^{-3} g/ml). In the majority of all applications a slight depolarization of the maximum diastolic membrane potential occurs. Sometimes small changes in the take-off level of the action potential are measured, which might result from small shifts in the location of the pacemaker. Often the action potential "improves" 1 to 2 minutes after the application of the drug. This "improvement" consists of slightly more negative maximum diastolic potentials and threshold potentials, which result in a larger amplitude of the overshoot; often the plateau phase is at a more positive level and prolonged (91).

The relative potencies of *l*-epinephrine and *l*-norepinephrine in increasing the heart rate have not been studied electrophysiologically at the pacemaker in the sinoatrial node, but much is known about the chronotropic effects, as observed by the rate of contraction of isolated hearts. Significant species differences have been reported. Generally *l*-norepinephrine is somewhat more potent than *l*-epinephrine in accelerating the pacemaker (55, 189, 202, 205, 333). When the chronotropic effects of *l*-epinephrine and *l*-norepinephrine were compared with those produced by isoproterenol, the latter agent was found 10 to 15 times more potent in accelerating the pacemaker (155, 189).

2. Arrhythmia in the intact animal after administration of epinephrine. The ability of epinephrine to induce ectopic ventricular beats, when administered intravenously in the dog, cat, or rabbit was recognized early and has often been studied (120, 159, 194, 240, 340). These ectopic beats most probably result from the development of pacemakers in the conductive system, where epinephrine depolarizes the membrane potential to threshold. Obviously, the probability for the occurrence of ectopic beats will be the larger the longer the time available for their development. A favorable condition is the inhibition of the sinus node due to vagal reflex activity, which occurs when the blood pressure rises (240). When the vagi are cut or when atropine is administered, larger doses of epinephrine are necessary to precipitate ectopic beats, which occur less frequently (240). Under these conditions sinus tachycardia may result; thereby the increased sinoatrial rate can still dominate the heart, *i.e.*, the conductive system will be excited by a propagated impulse before a pacemaker potential, originated somewhere in this structure, reaches threshold. The preparation with the sinoatrial node inhibited by stimulation of the vagus nerve has been used in the anesthetized animal to test the effectiveness of sympathomimetic drugs in producing ventricular pacemakers (255). Isoproterenol is ten times more potent than epinephrine or norepinephrine in producing ectopic beats. In the intact animal, however, isoproterenol does not elicit ectopic beats, for it does not increase the blood pressure; accordingly, it produces a marked increase in the rate of the pacemaker in the sinoatrial node, which is not inhibited by vagal reflex action (254). In another study, *l*-epinephrine, *l*-norepinephrine, mephentermine, and methoxamine have been compared in their effectiveness in producing ectopic beats in the exposed heart of a dog whose vagi had been cut. The most

potent drug has been shown to be *l*-epinephrine, while methoxamine neither produces ectopic beats nor increases the normal pacemaker rate of the sinoatrial node (94); this situation holds true even when the dog heart is sensitized with cyclopropane or chloroform (187, 291). In the heart-lung preparation, methoxamine (4 mg) produces a slight depression of the pacemaker rate; doses of 1 mg or more abolish the positive chronotropic action of epinephrine (148). The possible mechanism of the antagonizing effect towards epinephrine is discussed below (see p. 306).

3. Mechanism. The effect of epinephrine on the pacemaker potential is only partially understood. As the basis for the effects of epinephrine one might think of an increase of g_{Na} or a decrease of g_K or of g_{Cl}, or a combination of these. The latter possibilities are not convincing because the epinephrine effect is practically unaltered when the extracellular chloride is replaced by a large anion to which the cell is not permeable (unpublished experiments), and the membrane resistance does not increase as would be expected when g_K decreases (66). Certainly, sodium ions carry the depolarizing current, since depolarization fails when the extracellular sodium concentration is reduced to one-half, or when the membrane is depolarized and thereby the potential is brought closer to E_{Na}. Thus, an increase in the "resting" g_{Na} may be assumed as the basis of the membrane effect of epinephrine. The difficulty in this interpretation is that sometimes no depolarization is observed, although the slope of the pacemaker potential is increased. An increase in resting g_{Na} cannot be the only effect of epinephrine. The hyperpolarization of quiescent atrial fibers (67) and the increase of the maximum diastolic membrane potential in the pacemaker, which is sometimes observed, are contrary to the effect of an increase in g_{Na}.

Epinephrine could have an effect similar to that of depletion of external calcium; but the following observations do not favor this hypothesis: 1) epinephrine does not alter the level of the threshold potential, when this is determined with square pulses in the quiescent Purkinje fiber (163); and 2) the dependence of the rate of rise of the upstroke or the size of the overshoot of the action potential on the level of the membrane potential prior to excitation (see Fig. 3) is not affected by epinephrine in Purkinje fibers (311). In the frog sinus, however, the rate of rise and overshoot seem to increase on sympathetic stimulation (145). These effects could be due to a slight hyperpolarization of the firing level, which, however, was not observed in the experiments. In the frog atrium, partially depolarized by an increase in the extracellular potassium concentration, epinephrine appreciably increases the amplitude of the overshoot without an increase in the resting potential, the rate of rise of the action potential being unchanged (72). For an explanation of these effects of epinephrine on the frog sinus and frog atrium, a decrease in the intracellular sodium concentration by activation of the active transport has been discussed. This hypothesis seems to be supported by a few experiments. Epinephrine fails to have detectable membrane effects in Purkinje fibers poisoned with DNP or iodoacetate (1 to 2 mM) (163, 311); these agents are known to block active transport in giant nerve fibers. Similarly, when sodium is replaced by lithium, which cannot be extruded actively

from the skeletal muscle fiber (165), epinephrine fails to have any effect. However, direct effects of these metabolic inhibitors on the membrane (for example, an increase in g_K) are not excluded. In quantitative terms, an explanation of the epinephrine effects on the basis of an increase of sodium concentration difference is not very convincing, since the reduction of intracellular sodium by 50% (from 50 mM to 25 mM) increases the driving force for sodium ions by only 10%. Larger intracellular changes can scarcely be expected (256). In radioisotope studies a markedly increased outflow of Na^{24} in the presence of epinephrine (at a constant rate of beating) was found in frog sinus venosus and atrium (106).

It is of interest that methoxamine, which is chemically related to epinephrine, antagonizes the chronotropic effect of epinephrine on the pacemaker in the sinoatrial node, as well as on ectopic pacemakers (148); also, it reduces the rate of rise of the upstroke of the ventricular action potential but does not change the resting potential (147). This latter finding suggests a reduction of g_{Na} similar to the "stabilizing" action of quinidine. A depression of the oxygen consumption of heart tissue by methoxamine has also been reported (108). Dichloroisoproterenol, which in small doses (0.01 to 3 mg) in the dog heart-lung preparation has the properties of a sympathomimetic amine, in larger doses produces a depression of the pacemaker in the sinoatrial node; furthermore it inhibits competitively the chronotropic effect of epinephrine (85, 239) whereas the classical adrenergic blocking agents, such as Dibenamine, phenoxybenzamine, azapetine, piperoxan and Hydergine do not block the chronotropic response of the pacemaker towards epinephrine (239). Unfortunately no electrophysiological study on the pacemaker is available with methoxamine or with dichloroisoproterenol.

4. Excitability and refractoriness. In contrast to the strong effects of the sympathomimetic agent on spontaneous impulse generation, the effects of these drugs on resting excitability are often insignificant. It should be stated in this context that automaticity and resting excitability are two different qualities and are by no means directly related. Stimulation of the cardiac sympathetic nerves of the dog heart produces either no change of the ventricular or atrial excitability or a minimal lowering of the diastolic threshold (20, p. 216).

When epinephrine or norepinephrine is administered the most frequent response is a diphasic effect: an initial period (1 to 5 minutes) of increased excitability (10 to 25%) is followed by a longer period (up to 30 minutes), during which the threshold is increased by 25 to 50% of the control value (282). These effects of epinephrine and norepinephrine vary with the rate and volume of the injection. In the dog, small doses of either agent infused at a rate of about 7 μg per kg body weight and min for 3 minutes produce only the initial transitory increase of excitability in both the ventricle and atrium whereas larger amounts (15 to 30 μg per kg and min) produce the long lasting depression of excitability after the initial transitory increase. When equimolar doses are compared, *l*-epinephrine produces a slightly greater change in excitability than does *l*-norepinephrine (282). The diphasic effect on excitability has also been observed with other sympathomimetic agents, such as mephentermine and methoxamine (94, 289). As to the mechanism of this diphasic change in excitability, it has been

shown that a single intravenous injection or a continuous infusion of either *l*-epinephrine or *l*-norepinephrine causes a transitory increase of the serum potassium by a factor of 2 to 3. This increase in serum potassium is in phase with, and may account for, the decrease of the current requirement. The slight depolarization apparently reduces the difference between the threshold membrane potential and the resting potential and, therefore, increases excitability. This explanation is supported by the fact that the same elevation of the serum potassium by injection of KCl also leads to an increase in excitability (20, p. 228). The depression of excitability seems to be a direct effect of these agents on the cardiac fibers, which occurs when larger concentrations are administered. Further effects, such as an increase in vascular volume after the administration of epinephrine, which could change the short-circuiting of an extracellularly applied current pulse, are, however, not fully excluded. Threshold determinations on single ventricular fibers during a period of 30 minutes after epinephrine administration are not available. In the quiescent Purkinje fiber of the sheep, the threshold membrane potential is not altered after local application of *l*-epinephrine through a micropipette, in concentrations that are below those producing a pacemaker. In this structure the resting potential depolarizes by a few mV and, as a result, the current strength of a threshold stimulus led through a second microelectrode into the same fiber is slightly decreased after epinephrine application (163). Unfortunately no such experiments are available on ventricular myocardial fibers.

Rather inconsistent observations have been reported concerning the effects of sympathetic stimulation and sympathomimetic agents on the refractory period or the duration of the cardiac action potential. At a constant beating rate the latter has been found to be slightly shortened in the atrial fibers of the dog and cat, as well as in the right ventricular fibers of the dog (20, p. 221 ff.; 125, p. 87) and in fibers of the embryonic chicken heart (82). Prolongation has been reported, however, in atrial fibers of the rat, guinea pig and rabbit (86, 125, p. 57; 325), as well as in the frog ventricle, when high epinephrine concentrations (0.2 mg/ml) are applied (206). Most often the duration of the normal ventricular action potential is not changed by epinephrine (45). Prolongation after epinephrine application can be observed in the course of the recovery of a hypodynamic or deteriorated preparation, the action potentials of which show a loss of plateau and are of short duration (91, 311). The monophasic action potential led off by a suction electrode from the exposed heart of a dog is shortened by 10 to 20% when *l*-epinephrine, norepinephrine, mephentermine, or methoxamine is administered (94), but this effect may reflect an increase in the serum potassium, which has been reported under this condition (20, p. 224).

Stimulation of the cardiac sympathetic nerves has no significant effect on the refractory period when the heart rate is kept constant (20). When, however, in the dog *l*-epinephrine is administered by infusion at a rate of about 7 μg per kg body weight and min, both the relative and the absolute refractory period in the dog ventricle and atrium are shortened by 10 to 15 msec (282). A stronger effect, shortening of the absolute refractory period by 20 to 50 msec, has been

reported after the infusion of norepinephrine (94). The shortening of the absolute refractory period might be related to a slight decrease in the duration of the action potential, as observed with isolated atrial or ventricular strands after the application of epinephrine. In the intact dog an increase in the serum potassium, after the infusion of either epinephrine or norepinephrine, could produce a similar effect. A decrease in refractory period by 10 to 20% has also been found with mephentermine, but only for a short time after the beginning of the infusion (289). Generally it can be said that the effects of the sympathomimetic agents on the resting excitability and the refractory period are small and play no significant role in the ability of these drugs to produce arrhythmia.

5. Conduction velocity. Epinephrine causes a slight increase in conduction velocity in the dog heart, as measured by close bipolar electrodes in the heart-lung preparation; simultaneously, the QRS complex is shortened (18). A decrease in the conduction time has been observed on the exposed dog heart, *in situ* in both the ventricular and atrial myocardium (94). In equimolar doses (infusion at a rate of about 7 μg per kg body weight and min) the effect is slightly stronger when induced by epinephrine than by norepinephrine (282). The mechanism of this increase of conduction velocity is difficult to understand, since neither the resting potential and action potential nor the resting excitability show consistent changes, except in atrial fibers, in which an increase in resting potential has been observed (66).

In a deteriorated preparation with a low conduction velocity, epinephrine causes a marked increase in conduction velocity. The improvement of the spread of excitation into a partially or completely blocked fiber area has been demonstrated (311). The action potential increases appreciably in amplitude and is accompanied by a slight increase in resting potential. When conduction is blocked in the frog atrium by an increase in the extracellular potassium concentration, the amplitude of the action potential is increased and conduction is restored by epinephrine without a change in the level of the resting potential (72).

The effect of epinephrine on transmission in the atrioventricular node has also been studied in isolated preparations in the tissue bath (216), as well as in the exposed dog heart (94, 294). In this structure the resting potential and the velocity of the rising phase of the action potential are normally low, and the situation is somewhat similar to that in the deteriorated or potassium-treated myocardium. This may explain the marked effect of epinephrine in shortening the atrioventricular delay (182, 216, 294); *l*-epinephrine (3 mg/min) infused for 5 minutes shortens the atrioventricular conduction time by 40%. Ten to twenty times larger concentrations have to be administered to affect intraventricular conduction (294). Pressor amines such as methoxamine and mephentermine prolong the atrioventricular conduction time, as should be expected in the presence of a drug which reduces the rate of the upstroke of the action potential. In the case of methoxamine, this increase amounts to 30 to 100%. Mephentermine initially shortens atrioventricular conduction time and also ventricular conduction time (289), but, when it is infused continuously, a lengthening by as much as 20 msec is consistently observed (94). Here again it should be stated that the

effects of the sympathomimetic drugs on conduction velocity do not explain the arrhythmias produced by these agents. The cause of this arrhythmia lies in the enhanced tendency of the fibers of the conductive system to produce spontaneous excitation.

6. Reserpine. In relation to the action of epinephrine on the pacemaker, the cardiac action of reserpine is of great interest. When small doses (0.03 to 1 mg/liter of blood) are applied to a heart-lung preparation, an increase in the rate of the pacemaker in the sinoatrial node, as well as of a pacemaker in the atrioventricular node, is observed (179). These accelerating effects of reserpine are due to a release of norepinephrine, presumably stored in the sympathetic nerve endings (12, 235, 245, 281a, 315a, 323). A release of catecholamines by reserpine is also found in other tissues (136, 236). The depletion can be so complete as to interfere with transmission between the nerve endings and the pacemaker fibers, and stimulation of the accelerator nerve fails to evoke an increase in rate (316). When larger doses (5 to 15 mg per liter of blood) are applied, a direct depressant effect on the pacemaker is observed (149), which is not affected by atropine. The sensitivity to catecholamines of the reserpine-pretreated pacemaker seems to depend on time; an early reduction of the sensitivity (149, 179) that is followed by periods of unchanged sensitivity (149) and hypersensitivity (7) has been described. A detailed presentation of this subject has appeared (315a).

Only one electrophysiological study of the effect of reserpine on the cardiac pacemaker is available (299). In the presence of reserpine in a concentration of 1 to 10 mg/liter the duration of the action potential is prolonged and the size of the overshoot is reduced in both pacemaker and atrial fibers; in pacemaker fibers the slope of the diastolic depolarization is decreased, the heart rate is slowed, and after one to two hours the pacemaker activity ceases. The effects of reserpine are not reversed by washing, but the addition of norepinephrine restores the normal rate and configuration of the action potential. The sensitivity of the preparation to epinephrine was reduced by a factor 10 to 100; however, after 2 to 3 hours the sensitivity towards catecholamines began to increase. This study shows that reserpine has direct membrane effects. Both electrophysiological and radioisotope work will be necessary before a possible mechanism can be discussed.

In a study of the Rauwolfia alkaloids a group of compounds has been found with a biphasic action on rate similar to that of reserpine. These substances are raunescine, isoraunescine, deserpidine, and rescinnamine. Another group, consisting of ajmaline, serpentine, aricine, and α-yohimbine, causes only the depressant effect on rate, without the initial cardioacceleratory action (56, 57, 151, 158). These agents have an action like that of quinidine (see p. 310). Two other agents with an action related to that of reserpine have become known recently (15, 219). Bretylium and guanethidine produce positive chronotropic effects on the heart-lung preparation similar to those observed with reserpine (87, 352). Bretylium has also been shown to depress considerably the effect of sympathetic stimulation in the right atrium of the rabbit heart dissected with its innervation

intact (139). Both bretylium and guanethidine abolish the positive chronotropic effect seen after the rapid stimulation of the rabbit sinoatrial node (3). It was proposed recently that these effects are due to the release of catecholamines in a manner similar to the action of reserpine (87, 177). The following observations support this hypothesis. Depletion of the catecholamines in the rabbit and dog heart by guanethidine could be shown (39, 177). When the dog used for the heart-lung preparation is pretreated by reserpine in a manner known to deplete the catecholamine storages, the positive chronotropic effects of bretylium and guanethidine are abolished. Furthermore, pretreatment of the dog by guanethidine abolishes the positive chronotropic effect of this agent when subsequently administered in the heart-lung preparation (87). Block of the chronotropic effects of nerve stimulation by guanethidine has, however, been found to occur before depletion of the catecholamine storage (39a, 88a, 220a). These authors attribute the block produced by guanethidine, like that of bretylium, to some action on the nerve other than on the release of catecholamine.

VII. DRUGS WITH ANTIARRHYTHMIC PROPERTIES

The aim of this chapter is not to present a complete review of the numerous papers about drugs with antiarrhythmic effects, but to show a few common features of their action. Several factors contribute to the influence of these agents on rhythmicity of the heart: 1) a direct effect on the rate of the pacemaker potential which is more marked in the heterotopic pacemakers than in the sinus node; 2) a decrease in diastolic excitability; 3) prolongation of the absolute and relative refractory period, and a decrease in vulnerability of the heart to stimuli during these periods; and 4) a reduction in conduction velocity in all cardiac fibers, which becomes prominent when higher concentrations are used. The antiarrhythmic drugs differ in the extent of their influence on these factors, but all their effects can possibly be explained by a common mechanism.

A. Effect of quinidine and procainamide

Quinidine sulfate (6 mg/liter) depresses the rate of the pacemaker potential in the sinoatrial node of the rabbit heart *in vitro* (337). Both quinidine and procainamide also suppress heterotopic pacemakers in Purkinje fibers. In low concentrations quinidine (3 to 6 mg/liter) and procainamide (30 to 60 mg/liter) reduce the slope of the diastolic depolarization (124, 329). The same effect has been observed on Purkinje fibers, the pacemaker activity of which was enhanced by epinephrine (125, p. 195). The depression of the pacemaker potential occurs without any change in the maximum diastolic membrane potential. In the mammalian sinus node both these drugs seem to be less active in suppressing spontaneous activity than in Purkinje fibers (125, p. 118). A comparative study of these drugs in depressing spontaneity in the sinus and heterotopic pacemakers is not yet available.

The duration of the action potential in both ventricular myocardium and Purkinje fibers, as well as in atrial fibers, is slightly prolonged when therapeutic concentrations of quinidine are used, mainly because of a retardation of the

terminal repolarization phase (124, 152, 218, 318, 319, 337). In the presence of quinidine, procaine, or procainamide, the small effect on the duration of the action potential does not fully account for the pronounced increase in the "effective" refractory period (198), *i.e.*, the shortest interval between two maximal stimuli which elicit a response propagated through the whole heart (297, 337). The prolongation of the effective refractory period caused by these agents results mainly from an interference with the depolarizing process (see Mechanism, below). It should be noted that these agents increase the refractory period in concentrations that are equal to those in the blood after clinical use.

Interesting effects of these antiarrhythmic agents on the upstroke of the action potential have been reported. Quinidine sulfate (3 to 4 mg/liter), procainamide (100 mg/liter) and pyrilamine maleate (7.5 mg/liter) reduce the rate of rise of the upstroke and the size of the overshoot of the action potential, but leave the diastolic membrane potential unaffected (152, 318, 337). The effect of quinidine on the rate of the upstroke of the action potential has been reported to depend on the beat frequency, being more pronounced at high rates than at lower rates (153). Whether this is a true rate-effect, as the authors state, or is the result of a slightly less negative membrane potential due to incomplete repolarization at a high beating rate, is difficult to decide on the basis of figure 3 of their paper (153). In Purkinje fibers quinidine sulfate (10 mg/liter), procainamide (550 mg/liter) and diphenhydramine hydrochloride (10 mg/liter) reduced the rate of rise of the upstroke and the overshoot of the action potential (329); clearly, the decrease in the rate of rise could not be attributed to depolarization of the resting potential. When the latter was artificially increased by current flow to the control value, or to even more negative values, the rate of rise of the upstroke was still smaller in the presence of quinidine than in the untreated fiber (329). All these drugs rendered Purkinje fibers inexcitable within 1 to 2 hours; at this time the resting potential was decreased (48), but block occurred before the potential dropped to −72 mV, a level at which untreated fibers were still excitable (329). A decrease in the conduction velocity of the rabbit atrium produced by quinidine, procaine, or procainamide in low therapeutic concentrations has been reported (297).

The observations on the effects of these drugs in the exposed heart *in situ* are in good agreement with the results gained on single fibers. Excitability was determined in these experiments by measuring thresholds at different times of the cycle at a constant heart rate. The atrial and ventricular excitability and fibrillation thresholds, as affected by quinidine sulfate, have been studied with controlled concentrations of the drug in the blood (20, p. 244; 95). Therapeutic concentrations, which did not lower the blood pressure, prolonged the absolute refractory period of the atrium by about 20 msec, and the relative refractory period by 30 msec. During the relative refractory period, the threshold for a single response was consistently elevated and even the strongest of stimuli did not evoke fibrillation. The minimum blood level of quinidine sulfate which prolongs the refractory period, is 3 to 6 mg/liter. This is the same concentration as that used in the Tyrode solution bathing the excised ventricular preparation

(20). The resting excitability is decreased in the atrium (threshold elevated by 40%), and is even more so in the ventricle (threshold elevated by 80 to 110%) (95). The conduction velocity is slightly decreased, but can become very low when higher concentrations are used (294, 300). Very similar results have been obtained with procainamide infused 10 minutes at a rate of 30 to 40 mg per kg and min (350). The atrium seems to be more sensitive towards this drug than the ventricle with respect to the prolongation of the refractory period. Conduction through the atrioventricular node is greatly delayed by all the antiarrhythmic drugs, as might be expected in a structure that has a low safety margin for conduction.

B. Mechanism

The mechanism of action of quinidine, procainamide, procaine, diphenhydramine, and cocaine has been extensively studied on Purkinje fibers (329). When compared at the same resting potential, the rate of rise of the action potential and the size of the overshoot are decreased. The S-shaped curve relating the maximum rate of rise (which is proportional to the sodium inward current on activation) to the membrane potential at the start of the action potential, is shifted to the right (see Fig. 3). This could be interpreted as a block or inactivation of sodium carriers. The maximum transport capacity of the carrier system is also reduced (see the right curve in Fig. 3), for even the action potentials taking off from very large membrane potentials (artificially increased by current flow) show a maximum rate of rise which is smaller than the maximum rates of controls (329). This basic observation holds true for all the other drugs mentioned and possibly explains their antiarrhythmic effects.

As mentioned above, there is a discrepancy between the small increase in the duration of the action potential and the marked prolongation of the "effective refractory period" in the presence of quinidine. It has also been reported that, with high concentrations of quinidine, repolarization can be complete, but a propagated action potential still can not be elicited (154, 297). If this were the case, refractoriness could not be explained on the basis of the S-shaped curve, but an additional effect of the antifibrillatory agents on the time of recovery of the sodium carrier system after repolarization would have to be assumed. In accordance with the sodium-carrier blocking theory of the action of quinidine is the observation that its effect can be inhibited by increasing the extracellular sodium concentration. This change increases the sodium driving force, and this partially compensates for the depression of the carrier system (51). The finding of an inhibition of Na^{24} influx by quinidine agrees with the above-described concept of its mechanism of action (167). Also, the reported depression of K^{42} efflux (134) is not an uncommon observation with "stabilizing" drugs (84, 280). In the heart-lung preparation of the dog, however, a slight net loss of potassium in the presence of quinidine has been reported (21). The somewhat conflicting results reported in the literature concerning this matter have recently been summarized (21). Quinidine exerts its antiarrhythmic properties in a concentration which does not change the intracellular sodium or potassium concen-

tration (101a). The antiarrhythmic effect cannot, therefore, be explained by a change in the difference of the ionic concentration between the extra- and the intracellular phase.

C. Related drugs

Many antiarrhythmic agents have been studied in recent years by testing their ability to prevent experimentally induced fibrillation (59). With some of these drugs excitability has been measured. The compound 1-(2-diethylamino-ethylamine)-3-methylisoquinoline dihydrochloride decreased excitability during diastole by as much as 50% below control values and prolonged the refractory period by 15 to 40%; vulnerability against fibrillation and extrasystoles was markedly decreased (230). The compound 1-(2-diethylaminoethylamino)-3 propylisoquinoline produced smaller changes in excitability and in the refractory period, and had a lower antifibrillatory potency for the ventricle (230). Several local anesthetics prevent ACh- or aconitine-induced auricular fibrillation and ventricular arrhythmias induced by combined hydrocarbon-epinephrine administration (283). In addition to diphenhydramine dihydrochloride, many other antihistamines are known to depress experimental atrial arrhythmias elicited by injury or aconitine (341) as well as ventricular arrhythmias (281). A few antimalarial drugs have been described as very effective in preventing atrial fibrillation (32). Other compounds such as N-(γ-isopropylaminopropyl)-α,α-diphenyl-acetamide hydrochloride; β-diisopropylaminoethyl-4-phenyl-4-tetrahydropyrancarboxylate hydrochloride; and β-diethylaminoethyl-2,6-dimethyl-5,-6-dihydro-4H-pyran-3-carboxylate hydrochloride, prolonged the "effective refractory period" of the cat papillary muscle. These compounds also prevented experimental arrhythmias (342). An antiarrhythmic action is also reported with such ataraxic agents as benactyzine and alsoroxylon; their effects have been compared with that of quinidine (5).

A group of antifibrillatory drugs consisting of ajmalin, serpentine, aricine, reserpinine and α-yohimbine has been found in Rauwolfia. These drugs prolong the relative refractory period in the isolated rabbit atrium and prevent experimental arrhythmias (4, 319). Serpentine and ajmalin also restore the sinus rhythm (209); ajmalin increases the functional refractory period of the atrioventricular transmission (151), as well as the PQ interval (277). Ajmalin also reduces myocardial excitability and conduction velocity. In comparable doses it is about twice as potent as quinidine in prolonging the refractory period (10, 98, 277). Another compound, amotriphen, has recently been reported to decelerate markedly the normal and epinephrine-stimulated isolated rabbit atrium and to decrease the sinus rate of the dog heart; in smaller doses than are required with quinidine this agent stops ventricular flutter and prolongs the relative refractory period (78).

It should be remembered that, in the single fiber experiments, chemically quite unrelated agents with antifibrillatory action such as quinidine, procaine, diphenhydramine and papaverine have basic common properties: they inhibit the sodium permeability on activation, reduce the resting excitability and pro-

long the relative refractory period. One might assume, therefore, that the antiarrhythmic effects of the drugs discussed in this section are based on the same principles. All these drugs probably reduce the resting excitability and thereby exert a protective effect against any kind of stimulus, such as mechanical ones in surgery, electrotonic spread from adjacent depolarized areas, and action potentials of low amplitude which might arise in the relative refractory period. Moreover, these drugs will prevent any kind of early re-excitation by re-entry, re-excitation of the aconitine type (see p. 319) or excitation which might occur during the supernormal phase before repolarization is complete. Finally these agents decelerate the isolated rabbit atrium as well as the sinus of the dog heart both under normal conditions and when stimulated by epinephrine.

VIII. DIGITALIS

A. The effect on the pacemaker in the sinoatrial node

In addition to their inotropic effects, the digitalis glycosides and ouabain exert an important influence on the rate and the rhythm of the heart. A detailed electrophysiological study of the effects on the sinoatrial node is not available. Ouabain (0.01 mg/liter) reduces the rate of the frog sinus (314). Slowing of the pacemaker as an early and consistent effect of digitalis has also been reported for the cannulated heart of both the frog and the turtle (223), but even toxic doses of digitalis do not abolish the pacemaker potential in the sinoatrial node of the rabbit heart (334). Bradycardia, as an early event after administration of digitalis glycosides, is known from experiments in the dog and cat (262, 292, 322), as well as from therapeutic administration in man (285). Experiments on isolated or vagotomized hearts, which still show a bradycardia on application of digitalis, were thought to prove a direct effect of the glycosides on the pacemaker fibers in the sinoatrial node (223). Such experiments are not strictly conclusive, for a liberation of ACh from the nerve endings has been observed in the isolated atrium (313). However, in the atropinized preparation, in which sensitization effects towards ACh seem to be ruled out, digitalis still produces a bradycardia (24, 186, 223). Therefore, a direct effect of the glycosides on the pacemaker seems to exist. An electrophysiological study of this phenomenon should be rewarding.

In addition to the direct effect on the pacemaker fibers a peripheral sensitization toward the action of the vagus, *i.e.*, toward ACh, by digitalis has been shown. The chronotropic effect of ACh in the heart-lung preparation is greatly augmented in the presence of ouabain (88, 192, 193). Also, when the effect of vagal stimulation on the sinus rate before and after administration of ouabain is compared, a marked sensitization of the former by the glycosides can be shown at stimulation rates of 10/sec or higher (88, 221) but not with lower rates (88, 192, 193). As to the significance of the digitalis bradycardia, it has been argued that it cannot be observed using "physiological" impulse rates in the vagal fibers. This rate was assumed to be 2 to 4 action potentials per second (191, 192), judging from the effect of stimulation of all fibers of the vagus nerve in man (35). This conclusion seems doubtful, since single fiber recordings from vagal cardiac

branches show frequencies well within the range of 10 to 40 impulses per second (265).

An interaction between digitalis and the effects of either sympathetic impulses or epinephrine has also been seen. Digitalis reduces the chronotropic effect of an epinephrine infusion on the sinoatrial and the atrioventricular node of a previously decentralized heart; also, the effect of sympathetic stimulation in both these structures is reduced by digitalis (228).

B. Refractory period, excitability and conduction velocity

When a papillary muscle is driven at a constant rate and ouabain (2×10^{-7} g/ml) is added, the force of contraction increases within two minutes; simultaneously, the action potential is slightly prolonged. Thereafter, the force of contraction increases further, but the duration of the action potential becomes progressively shorter. This can be observed either in a hypodynamic preparation or in the presence of a low extracellular concentration of calcium ions. Obviously, the force of contraction cannot be related to the duration of the action potential in the presence of ouabain (67). Shortening of the duration of the action potential has frequently been observed in the presence of toxic doses of ouabain or digitalis (290, 348). In the presence of digitalis in concentrations which produce a positive inotropic effect the extracellularly recorded monophasic action potential can be shortened (109, 110). A digitalis-induced shortening of the QT interval in man also indicates a shortening of the action potential (11).

In digitalis intoxication, the shortening of the ventricular action potential explains the shortening of the absolute refractory period, as observed in the frog ventricle (157, 268), tortoise ventricle (204) and in the dog heart (198). The threshold dose at which the refractory period begins to shorten is about 40% of the lethal dose of digitoxin or ouabain (229). Shortening progresses until 80% of the lethal dose is administered. Digitalis exerts its inotropic effects before the action potential or the refractory period is affected (351). We believe, however, that it is not justified to consider the shortening of the ventricular action potential by 20 to 30% of its duration as a toxic effect of digitalis, for this degree of shortening can be observed in the tissue bath before the drug reaches its maximum inotropic effect and when no sign of contracture is detectable (67). In contrast to the absolute refractory period the relative refractory period is markedly prolonged by the glycosides (198), especially at higher rates (268). We believe that the prolongation of the relative refractory period is due to the fact that repolarization is shortened on the plateau level, resulting in the shortening of the absolute refractory period. The terminal phase of the repolarization, however, is prolonged, and during that time excitability is probably depressed (67).

The plateau of the action potential of the Purkinje fiber is less sensitive towards glycosides than that of ventricular fibers and loss of plateau occurs only on severe intoxication (67). The effect of digitalis on the action potential of the atrial fiber has not been studied to our knowledge. The refractory period of the atria in either the heart-lung preparation or the vagotomized exposed heart of a

dog is increased by digitoxin in an amount that is 30 to 40% of the lethal dose. When, however, in this tissue, the vagal innervation is maintained and an appropriate method of anesthesia is used, the glycosides decrease the functional refractory period (229). Digitalis often reduces diastolic excitability (157, 199, 274, p. 276). This observation was confirmed in more recent experiments in the dog heart with respect to doses higher than 50% of the lethal dose; excitability is slightly increased, however, when smaller doses are used (231). Only small quantitative differences were found when the effects on excitability of digitoxin, lanatoside C, ouabain, or K-strophantoside were compared (231).

Corresponding to the influence on diastolic excitability, the conduction velocity in the ventricular myocardium increased early in the course of digitalis administration and decreased after 50 to 60% of the lethal dose had been given (231–294). In the atrium, 35 to 40% of the lethal dose of K-strophantoside or lanatoside C was sufficient to prolong the conduction time (229a). The relatively late effects of digitalis intoxication on myocardial conduction velocity seem somewhat surprising in the light of the large number of observations concerning deformation and widening of QRS (285). In this connection the greater sensitivity of conduction velocity in Purkinje fibers towards digitalis, as compared to that of ordinary ventricular fibers, should be noted. In Purkinje fibers conduction velocity is slowed markedly at a time when in the myocardium a slight increase of the conduction velocity prevails (294).

Conduction through the atrioventricular node, determined as conduction time from a stimulating electrode placed on the atrium to a recording electrode on the ventricular surface, is affected to a distinctly greater degree by the glycosides than is conduction through either the ventricle or the atrium. Atrioventricular conduction time increases already after 15 to 20% of the lethal dose is given and continues to increase until block occurs at 50 to 60% of the lethal dose (231). In the state of atrial fibrillation the strong depressant effect of digitalis on atrioventricular conduction will reduce the number of atrial excitations conducted to the ventricle, thus slowing the ventricular rate (231).

C. The arrhythmias

In the course of digitalis or ouabain intoxication, arrhythmias occur at an early stage in the dog heart. Arrhythmias result from ectopic beats that originate as a rule in the left ventricle (261). The dose of the glycosides necessary to elicit ectopic pacemakers varies from 20 to 60% of the lethal dose. As pointed out by Scherf and Schott (274), these ectopic beats are not coupled to the preceding beat but occur singly with varying coupling or in groups in irregular sequence. The bigeminal type seen in man on digitalis treatment has been observed experimentally only under special conditions, *e.g.*, increasing the CO_2 tension in the lung (99), and, most obviously, when glycosides are applied topically to the surface of the heart (166, 271).

In single fiber experiments on excised tissue, the effects of digitalis are somewhat different in papillary muscle from those in Purkinje fibers. In the former preparation, which consists of ordinary myocardium and which is supposed to be free of conductive tissue, only extrasystoles coupled to a preceding action

potential occur. Frequently a shortened action potential is observed which is followed by a depolarizing afterpotential, on top of which the coupled extrasystole arises (67). In the Purkinje fibers, however, extrasystoles arise before the action potential is appreciably shortened. They can be independent of the preceding beat and simply be fired into the regular rhythm as a single event, indicating an increase in the spontaneity of the Purkinje fibers (67, 321). Extrasystoles can also arise from the repolarization phase at a membrane potential level of about −50 to −60 mV. They can occur as long trains which appear as oscillations between −50 and 0 mV (49, 67, 321). This terminating disorder is certain to lead to ventricular fibrillation if it occurs, especially when conduction velocity is slowed.

D. Mechanism

No definite statement can be made concerning the mechanism of the electrophysiological effects of digitalis glycosides. In Purkinje fibers the membrane resistance increases in an early stage of the positive inotropic action (67, 162). It is possible that this corresponds to the prolongation of the action potential and the slowing of the pacemaker potential with low doses of ouabain, as well as to the slight increase in excitability and conduction velocity. In a later phase the membrane resistance in a Purkinje fiber decreases progressively (67, 162), the action potential is shortened, and extrasystoles appear.

Cardiac glycosides and related compounds inhibit ionic exchange in red blood cells and other tissues (97, 160, 213, 266, 267, 339a). In concentrations which exert a positive inotropic effect but do not produce arrhythmias, both increases and decreases of the fluxes of potassium and gains and losses of this ion in the coronary blood have been reported (21a, 106a, 319a). Some authors have pointed out that positive inotropic effects occur before changes of the fluxes or of the intracellular potassium concentration can be detected (166a, 190, 249, 317). Higher concentrations clearly cause the cardiac cells to lose potassium and take up sodium (21a, 106a, 166a). In experiments on Purkinje fibers in which both the membrane potential and the potassium flux as well as the intracellular potassium concentration were measured, arrhythmias caused by a toxic dose of ouabain could be attributed to a loss of intracellular potassium. When extracellular potassium was doubled, the intracellular potassium concentration increased and arrhythmias disappeared (234a).

It has been pointed out that many of the electrophysiological effects of digitalis are similar to those of the stabilizers (280). In the presence of ouabain (0.2 mg/liter) the resting potential is not appreciably affected, but the action potential is diminished in size and the rate of rise of the action potential is reduced (290, 348). This observation can be interpreted as reduction of the sodium inward current on activation. The S-shaped curve (see p. 284) relating the maximum rate of rise of the action potential to the membrane potential prior to stimulation is shifted to the right, as in the presence of local anesthetics (67, 162). This fact is certainly the basis of the reduction of conduction velocity, which has often been found in the presence of digitalis.

No theoretical relation can be seen between the positive inotropic effects of

digitalis and the shortening of the action potential, the inhibition of active transport, or increase or decrease of potassium conductance. The effect of ouabain on the sodium carrier system seems to be more interesting in this connection. It has been speculated that ouabain might have a high affinity toward receptors for Ca within the membrane and thereby produce an inotropic effect similar to that of a high Ca or low extracellular sodium concentration (67, 203, 250).

IX. VERATRUM ALKALOIDS

A. The tertiary alkamines

The esters of the tertiary alkamines, veratridine, cevadine, germidine, protoveratrine A and B, and related substances, have cardiac actions similar to those of the cardiac glycosides. These drugs exert a positive inotropic action and produce arrhythmias (8, 176, 180). Injected into the circulation of dogs and cats, they produce a profound bradycardia of reflex nature that is nearly abolished when the vagi are cut. The receptor areas for this reflex (Bezold-Jarisch reflex) in the heart have been extensively studied (58). Here, we are concerned with the direct effect of these drugs on the denervated heart. The effect of these agents on the normal sinoatrial rate is rather insignificant, except for cevadine, which produces a sinus tachycardia (8). However, the frequency of latent pacemakers in the conductive system of the ventricles is markedly increased by the tertiary alkamines; this results in the development of ectopic pacemakers and eventually in ventricular tachycardia. In addition, extrasystoles are observed which evidently start from the repolarization phase of the action potential. The simplest form is the coupled extrasystole of the bigeminal type. Also, in Purkinje fibers, long trains of extrasystoles at a high frequency can be elicited by a regular action potential (100); this effect is very similar to that of aconitine (see p. 319). In larger doses even the ventricular myocardial fibers are able to generate repetitive discharges (214). A well-known effect of these agents is the prolongation of the action potential. This prolongation is probably related to the marked tendency for repetitive discharge. The prolonged action potential of both the atrial and ventricular fibers results in a long-lasting refractory period, which has been observed by many authors (20, p. 262; 293).

Conduction velocity is slowed by veratrine (a mixture of veratridine and cevadine), the effect being stronger in the Purkinje fibers (293). Depression of the diastolic excitability has also been reported (20, p. 263) and should be related to the decreased conduction velocity. Unfortunately no electrophysiological experiments are available concerning the mechanism of these disorders in the heart. Theories concerning the repetitive responses in the veratrinized nerve and skeletal muscle fiber have been discussed recently by Shanes (280).

The great quantitative and qualitative differences in the effects of the individual tertiary alkamines should be noted, but an extensive differentiation is not within the scope of this article. The more prominent action of the germine and protoverin esters is their stimulating effect on ventricular pacemakers (8, 293, 294), whereas the veracevine and zygadinine esters tend more to prolong the action potential (214, 293), to depress excitability leading to various kinds of block (8), and to cause repetitive discharges of the aconitine type. With severe

intoxication all these drugs cause ventricular fibrillation. Three factors contribute to this terminal stage: 1) the increase in the spontaneity of the ventricular pacemakers, 2) the occurrence of repetitive discharges, and 3) the depression of the conduction velocity, especially in the conductive system.

B. Secondary alkamines

Representatives of this group such as veratramine, veratrosine, jervine, and pseudojervine have a marked depressant effect on the rate of the pacemaker in the sinoatrial node (174, 175, 183). When the sinoatrial node has been crushed, a similar depressant effect of veratramine on the spontaneous atrioventricular rhythm can be observed (178). These effects can be shown in the heart-lung preparation and they are apparently not mediated through the sympathetic (149) or vagal system (175). Veratramine and veratrosine greatly reduce the chronotropic effects of an epinephrine infusion (174) or of accelerator nerve stimulation (150, 181), but these drugs do not depress the inotropic effect of epinephrine. The rate-depressant effect is very specific for the pacemakers in the sinoatrial and atrioventricular node; ectopic pacemakers are not affected by veratramine, which does not influence the fibrillating heart (178). In large doses, veratramine can greatly depress or even periodically suppress the rhythm in the sinoatrial node, a circumstance that can result in peculiar periods of arrest and activity of the heart (173).

X. DRUGS INDUCING ARRHYTHMIA AND FIBRILLATION

Most chemical or physical influences can cause arrhythmia or fibrillation when present in a certain concentration or intensity. Since arrhythmia and fibrillation are merely states of disorganized activity, they may be produced in many ways. Here the effects of a few agents which are known to elicit experimental arrhythmia will be discussed.

Aconitine has often been used to elicit experimental arrhythmia (272). When applied locally to the atrium, it produces a focus which fires at a high rate (272, 274). Applied to an excised Purkinje fiber or papillary muscle, aconitine causes a marked depolarizing afterpotential from which short action potentials of low amplitude may arise. Shorter or longer trains of action potentials with incomplete repolarization can be observed, which progressively repolarize to a little more negative membrane potential (276). When repolarization reaches a critical value of -65 mV the action potential suddenly fully repolarizes to the resting level (see Fig. 2 B). Aconitine has the same effect in ordinary ventricular fibers (217, 276). Clearly, the extrasystoles caused by aconitine are coupled. Aconitine does not produce slow diastolic depolarization in the ordinary ventricular fibers; no spontaneous activity can be induced in an arrested fiber (217, 276). After the first stimulus, however, the response is repetitive. If the action potential repolarizes fully, the preparation will not start to beat again unless stimulated. When this drug is applied to a small preparation or to the whole heart, it will cause fibrillation by the appearance of multiple foci. The exact mechanism of this aconitine action is a matter of speculation.

Some hydrocarbons, chloroform (197), ether, and cyclopropane (298) can

cause arrhythmia or, in large doses, ventricular fibrillation (140, 225). In addition, these agents increase the sensitivity of the heart to the fibrillation-initiating effect of epinephrine (60, 64, 254). In the isolated atrium of the cat these drugs depress excitability and shorten the refractory period (1); in the heart *in situ* prolongation of the refractory period and depression of excitability have been described (20, p. 258). Possibly depolarization and a slowing of the conduction velocity leading to local areas of block are important factors for the occasional occurrence of fibrillation; especially is this the case when heterotopic pacemakers develop under the influence of sympathetic innervation or injected epinephrine.

Amarin hydrochloride (2,3,5-triphenyl dihydroiminazole) is also known to cause fibrillation, expecially if its effect is combined with that of epinephrine (79). This drug prolongs the PQ interval, reduces the conduction velocity, and prolongs the absolute and relative refractory periods, in both atria and ventricles (20, p. 260). In the dog heart *in situ* a concentration of 5 to 10 mg/kg of body weight nearly doubles the diastolic threshold of the ventricles (20, p. 260). The effect of this drug offers a good example of how a depression of excitability and conduction, together with prolongation of the refractory periods (which is most probably not homogeneous in different regions), results in a disorganized action of the heart. In this respect the fibrillation-inducing effect of this drug is not different from the effect of low temperature or a toxic dose of quinidine, both of which lower conduction velocity to a critical value, thereby preventing coordinated activation of the heart.

Recently *alpha*-phenoxy-*alpha*-dimethylamino-methyl-propiophenone hydrochloride (U-0882) has been shown either to produce spontaneous ventricular fibrillation or greatly to sensitize the dog ventricle toward the fibrillation-inducing action of epinephrine (233, 234). In the presence of U-0882 the QT interval is lengthened and slight bradycardia occurs. On intravenous administration of epinephrine (4 μg/kg) the sinus rate increases and the QT interval is prolonged further. The QRS-complex gradually encroaches upon the T wave of the antecedent beat. When QRS begins before T is complete, the heart fibrillates. Fibrillation also results both on injection of KCl (10 mg/kg), which speeds the sinus rate, and on ventricular tachycardia produced by direct electrical stimulation. Protection against the fibrillation caused by U-0882 can result from either dichloroisoproterenol or a toxic dose of ouabain; the latter is known to shorten the action potential.

The most probable cause of fibrillation produced by U-0882 is spread of excitation into a ventricle which is still—and to a variable degree—in the state of relative refractoriness because of the great prolongation of the action potential. This should result in action potentials which greatly differ in amplitude and duration, as well as conduction velocity. Under these conditions, coordinated activation of the ventricle is no longer maintained. It is possible that after the long plateau, either repolarization suddenly becomes incomplete, or depolarization occurs spontaneously without the arrival of a propagated action potential. In both of the explanations, fibrillation probably is maintained by conduction at random into adjacent nonrefractory fibers.

XI. CONCLUSIONS

On the basis of the present knowledge of cardiac electrophysiology a rather complete picture of the different aspects of the rhythmic activity of the heart can be given. Especially studies on single cells have made possible a detailed analysis of the excitatory process. A complete theory of excitation and impulse generation was first achieved in nerve fibers. This information could then be applied to cardiac electrophysiology and excitation in cardiac fibers could be described mathematically in terms of changes in ionic conductances, the electromotive forces for the ions being constant. Present electrophysiological concepts use a model of the cell membrane which consists of several "channels" for the flow of ions represented by the parallel membrane conductances for these ions. The electrophysiologist attempts to explain the membrane potential during excitation, as well as all influences altering the excitation process, by a change of one or more of these ionic conductances. The most serious limitation of this concept is the lack of any detailed knowledge of the molecular structures and processes inside the membrane which are the basis for ionic conductances.

Effects of drugs on membrane potential and excitation are explained by the electrophysiologist in terms of changes of ionic conductance or of shifts of the electromotive force for the flow of the ions across the membrane. The most readily explained effects of drugs are those which can be represented by the change of one specific ionic conductance from the normal to a new constant level. The effect of ACh is the best known and most important example: all its membrane effects are based on the increase of the potassium conductance. Such drug effects are characterized by a fast onset and usually a fast reversibility to the normal state.

A more complicated theoretical situation exists if the effect of a drug cannot be explained solely by a static change of conductance. The ionic conductances depend on the membrane potential and the time after a change of the membrane potential; a drug can work by shifting these relationships. Typical representatives of this group of drugs, quinidine and procainamide, reduce the increase of sodium conductance brought about by depolarization and alter the time constant of this effect. In this manner these drugs raise the threshold for excitation and have antiarrhythmic properties. These drugs also change the static membrane conductance for other ions, and have effects on the intracellular ionic concentration as well as those on cell metabolism. The antiarrhythmic effect, however, can be attributed to the interference with the depolarizing mechanism in the membrane.

The last (and, from the theoretical point of view, the most frustrating) group of drug effects is that in which, in addition to changes of membrane conductances, a change in the electromotive force for the flow of specific ions has to be assumed. The latter possibility is equivalent to a change in intracellular ionic concentration; such a change may also affect membrane conductances. The action of epinephrine probably belongs to this group of effects. In addition to an increase in sodium permeability, shifts of intracellular ion concentrations are involved. Typically, these drug effects change in character with time after application and may depend on the metabolic state of the cell. It is quite possible that the diffi-

culties in theoretical interpretation of the effects of drugs such as epinephrine are due to the limitations of the electrophysiological membrane model.

As a concluding remark the author may state his views concerning a useful direction of future work in this field. He has regretted the considerable lack in electrophysiological information about the effects of many drugs, information which could be gained by the application of relatively simple methods. Especially to be deplored is the fact that experiments at the cellular level are very often missing. From the theoretical point of view it does not seem very useful to go on doing elaborate experiments on the heart *in situ* as long as the actions of drugs on single fibers have not been observed. For example, no study is available of the direct effects of either digitalis or veratrum alkaloids on the pacemaker in the sinoatrial node. Thus, it is not known whether veratramine causes bradycardia because of an "inhibitory" effect depressing the pacemaker potential, or whether bradycardia results from a very prolonged action potential in the sinus fibers. Electrophysiological studies of effects at the cellular level should be done with several more local anesthetic agents, the volatile anesthetics, sympathomimetic agents, dichloroisoproterenol, reserpine, and many more fibrillatory drugs, in order to clarify their mechanism of action.

Although the biophysical approach to the problem of drug effects on the cardiac membrane will still lead to much new and valuable information, the trend to a biochemical approach is strong. Many new aspects of drug action would be opened if more information concerning structural and biochemical counterparts of membrane conductances would be provided.

ACKNOWLEDGMENT

The author wishes to thank Dr. G. Acheson and Dr. J. Dudel for reading the manuscript and for helpful suggestions, and Mrs. Lotte Lund for excellent technical assistance.

REFERENCES

1. ACIERNO, L. J. AND DIPALMA, J. R.: The effects of ether cyclopropane and chloroform on the isolated auricle of the cat. Anesthesiology **12:** 567–573, 1951.
2. AGNOLI, R. D. AND BUSSA, D.: Azione cardiaca del bario e delle associazioni bario-cardiocinetici. Arch. ital. Med. sper. **4:** 1–9, 1939.
3. AMORY, D. W. AND WEST, T. C.: Chronotropic response following direct electrical stimulation of the isolated sinoatrial node: a pharmacologic evaluation. J. Pharmacol. **137:** 14–23, 1962.
4. ARORA, R. B. AND MADAN, B. R.: Antiarrhythmics. VI. Ajmaline and serpentine in experimental cardiac arrhythmias. J. Pharmacol. **117:** 62–67, 1956.
5. ARORA, R. B.: Antiarrhythmic, quinidine-like activity of some ataraxic agents. J. Pharmacol. **124:** 53–58, 1958.
6. ASHWNI KUMAR, M. A. AND SHETH, U. K.: Acetylcholine on quinidine treated atria. Arch. int. Pharmacodyn. **126:** 381–385, 1960.
7. BEJRABLAYA, D., BURN, J. H. AND WALKER, J. M.: The action of sympathomimetic amines on heart rate in relation to the effect of reserpine. Brit. J. Pharmacol. **13:** 461–466, 1958.
8. BENFORADO, J. M.: Studies on veratrum alkaloids. XXVI. Comparison of the cardiac action of various tertiary amine ester alkaloids. J. Pharmacol. **120:** 412–425, 1957.
9. BENFORADO, J. M.: Depressant effect of acetylcholine on the idioventricular pacemaker of the isolated perfused rabbit heart. Brit. J. Pharmacol. **13:** 415–418, 1958.
10. BENTHE, H. F.: Beitrag zur Beurteilung der antifibrillären Eigenschaften des Chinidins und ähnlich wirkender Substanzen. Arch. exp. Path. Pharmak. **229:** 83–91, 1956.
11. BERLINER, K.: Observations on the duration of the electrical systole of the heart, with special reference to the effect of digitalis. Amer. Heart J. **7:** 189–202, 1931.
12. BERTLER, A., CARLSSON, A. AND ROSENGREN, E.: Release by reserpine of catechol amines from rabbits' hearts. Naturwissenschaften **43:** 521, 1956.

13. BIRCHER, R., ROTHLIN, E. AND SUTER, E.: Glykosidwirkung auf Elektrokardiogramm und Myokard. II. Mitteilung: Untersuchungen am Elektrokardiogramm und am Myokard bei tödlicher Vergiftung von Katzen nach einmaliger, subkutaner Verabreichung herzwirksamer Glykoside. Helv. physiol. acta **5:** 322–332, 1947.
14. BLINKS, J. R.: Positive chronotropic effect of increasing right atrial pressure in the isolated mammalian heart. Amer. J. Physiol. **186:** 299–303, 1956.
15. BOURA, A. L. A. AND GREEN, A. F.: Actions of bretylium: Adrenergic neurone blocking and other effects. Brit. J. Pharmacol. **14:** 536–548, 1959.
16. BOZLER, E.: The initiation of impulses in cardiac muscle. Amer. J. Physiol. **138:** 273–282, 1942–1943.
17. BRADY, A. J. AND HECHT, H. H.: On the origin of the heart beat. Amer. J. Med. **17:** 110, 1954.
18. BRENDEL, W., GLADEWITZ, H., HILDEBRANDT, F. AND TRAUTWEIN, W.: Elektrophysiologische Untersuchungen am Herz-Lungen-Präparat nach Starling. Cardiologia **18:** 345–359, 1951.
19. BRINK, F.: The role of calcium ions in neural processes. Pharmacol. Rev. **6:** 243–298, 1954.
20. BROOKS, C. MCC., HOFFMAN, B. F., SUCKLING, E. E. AND ORIAS, O.: Excitability of the Heart. Grune & Stratton, Inc., New York, 1955.
21. BROWN, T. E., GRUPP, G. AND ACHESON, G. H.: The effect of quinidine on the potassium balance of the dog heart. J. Pharmacol. **133:** 84–89, 1961.
21a. BROWN, T. E., ACHESON, G. H. AND GRUPP, G.: The saturated-lactone glycoside dihydro-ouabain: effects on potassium balance of the dog heart. J. Pharmacol. **136:** 102–113, 1962.
22. BÜLBRING, E. AND BURN, J. H.: Action of acetylcholine on rabbit auricles in relation to acetylcholine synthesis. J. Physiol. **108:** 508–524, 1949.
23. BURGEN, A. S. V. AND TERROUX, K. G.: On the negative inotropic effect in the cat's auricle. J. Physiol. **120:** 449–464, 1953.
24. BURN, J. H.: Function of Autonomic Transmitters. Williams & Wilkins Company, Baltimore, 1956.
25. BURN, J. H., GUNNING, A. J. AND WALKER, J. M.: The effect of KCl on atrial fibrillation caused by acetylcholine. Circulation Res. **4:** 288–292, 1956.
26. BURN, J. H. AND HUKOVIC, S.: Anoxia and ventricular fibrillation; with a summary of evidence on the cause of fibrillation. Brit. J. Pharmacol. **15:** 67–70, 1960.
27. BURN, J. H. AND KOTTEGODA, S. R.: Action of eserine on the auricles of the rabbit heart. J. Physiol. **121:** 360–373, 1953.
28. BURN, J. H. AND RAND, M. J.: Excitation and inhibition of rabbit atria by the vagus nerves. J. Physiol. **138:** 172–177, 1957.
29. BURN, J. H. AND RAND, M. J.: Sympathetic postganglionic mechanism. Nature, Lond. **184:** 163–165, 1959.
30. BURN, J. H. AND VANE, J. R.: The relation between the motor and the inhibitor action of acetylcholine. J. Physiol. **108:** 104–115, 1949.
31. BURN, J. H., VAUGHAN WILLIAMS, E. M. AND WALKER, J. M.: The effects of acetylcholine in the heart-lung preparation including the production of auricular fibrillation. J. Physiol. **128:** 277–293, 1955.
32. BURNO, F., BURSTEIN, F. AND DIPALMA, J. R.: Comparison of antifibrillatory potency of certain antimalarial drugs with quinidine and procaine amide. Circulation Res. **2:** 414–415, 1954.
33. CALDWELL, P. C., HODGKIN, A. L., KEYNES, R. D. AND SHAW, T. I.: The effects of injecting 'energy-rich' phosphate compounds on the active transport of ions in the giant axons of loligo. J. Physiol. **152:** 561–565, 1960.
34. CALDWELL, P. C., HODGKIN, A. L., KEYNES, R. D. AND SHAW, T. I.: Partial inhibition of the active transport of cations in the giant axons of loligo. J. Physiol. **152:** 591–598, 1960.
35. CARLSTON, A., FOLKOW, B. AND HAMBERGER, C. A.: Cardiovascular effects of direct vagal stimulation in man. Acta physiol. scand. **41:** 68–76, 1957.
36. CARMELIET, E.: L'influence de la concentration extracellulaire du K sur la perméabilité de la membrane des fibres de Purkinje de mouton pour les ions 42 K. Helv. physiol. acta **18:** C15–16, 1960.
37. CARMELIET, E.: Chloride ions and the membrane potential of Purkinje fibers. J. Physiol. **156:** 375–388, 1961.
38. CARMELIET, E.: Chloride and potassium permeability in cardiac Purkinje fibers. Press Acad. Europ. S.C. Bruxelles, 1961.
39. CASS, R., KUNTZMANN, R. AND BRODIE, B. B.: Norepinephrine depletion as a possible mechanism of action of guanethidine (Su 5864), a new hypotensive agent. Proc. Soc. exp. Biol., N.Y. **103:** 871–872, 1960.
39a. CASS, R. AND SPRIGGS, T. L. B.: Tissue amine levels and sympathetic blockade after guanethidine and bretylium. Brit. J. Pharmacol. **17:** 442–450, 1961.
40. CASTEELS, R.: Influence of sodium-poor solutions on the action potential of the frog's ventricle. Arch. int. Physiol. **70:** 297–300, 1962.
41. DEL CASTILLO, J. AND KATZ, B.: Production in membrane potential changes in the frog heart by inhibitory nerve impulses. Nature, Lond. **175:** 1035, 1955.
42. CERVONI, P., WEST, T. C. AND FALK, G.: Multiple intracellular recording from atrial and sino-atrial cells. Correlation with contractile tension. Proc. Soc. exp. Biol., N.Y. **93:** 36–39, 1956.
43. CHAMBERLAIN, F. L., SCUDDER, J. AND ZWENNER, R. L.: Electrocardiographic changes associated with experimental alterations in blood potassium in cats. Amer. Heart J. **18:** 458–470, 1939.
44. CHANG, J. J. AND SCHMIDT, R. P.: Prolonged action potentials and regenerative hyperpolarizing responses in Purkinje fibers of mammalian heart. Pflüg. Arch. ges. Physiol. **272:** 127–141, 1960.
45. CHURNEY, L.: Effect of epinephrine on monophasic action potential of auricular muscle. Amer. J. Physiol. **171:** 516–521, 1952.
46. CLARK, A. J.: The action of ions and of lipoids upon the frog's heart. J. Physiol. **47:** 66–107, 1913.
47. CORABOEUF, E. AND BOISTEL, J.: L'action des taux élevés de gaz carbonique sur le tissu cardiaque, étudiée à l'aide de microélectrodes intracellulaires. C. R. Soc. Biol., Paris **147:** 654–658, 1953.

48. CORABOEUF, E., BOISTEL, J. AND DISTEL, R.: L'action de la quinidine sur l'activité électrique élémentaire du tissu conducteur du coeur de chien. C. R. Acad. Sci., Paris **242**: 1225–1228, 1956.
49. CORABOEUF, E., LOZÉ, C. DE 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. Acad. Sci., Paris **243**: 441–444, 1953.
50. CORABOEUF, E. AND WEIDMANN, S.: Temperature effects on the electrical activity of Purkinje fibers. Helv. physiol. acta **12**: 32–41, 1954.
51. COX, A. R. AND WEST, T. C.: Sodium lactate reversal of quinidine effect studied in rabbit atria by the microelectrode technique. J. Pharmacol. **131**: 212–222, 1961.
52. CRANEFIELD, P. F., EYSTER, J. A. E. AND GILSON, W. E.: Effects of reduction of external sodium chloride on the injury potentials of cardiac muscle. Amer. J. Physiol. **166**: 269–272, 1951.
53. CRANEFIELD, P. F. AND HOFFMAN, B. F.: Electrophysiology of single cardiac cells. Physiol. Rev. **38**: 41–76, 1958.
54. CRANEFIELD, P. F., HOFFMAN, B. F. AND PAES DE CARVALHO, A.: Effects of acetylcholine on single fibers of the atrioventricular node. Circulation Res. **7**: 18–23, 1959.
55. CRISMON, J. M. AND TAINTER, M. L.: Action of sympathomimetic amines on the heart-lung preparation. J. Pharmacol. **64**: 191–208, 1938.
56. CRONHEIM, G., BROWN, W., CAWTHORNE, J., TOEKES, M. I. AND UNGARI, J.: Pharmacological studies with rescinnamine, a new alkaloid isolated from *Rauwolfia serpentina* (21027). Proc. Soc. exp. Biol., N.Y. **86**: 120–124, 1954.
57. CRONHEIM, G., ORCUTT, J. A. AND TOEKES, I. M.: Pharmacological properties of canescine ('recanescine'), a new alkaloid isolated from *Rauwolfia canescens* linn 21701. Proc. Soc. exp. Biol., N.Y. **89**: 21–23, 1955.
58. DAWES, G. S.: Studies on veratrum alkaloids. VII. Receptor areas in the coronary arteries and elsewhere as revealed by the use of veratridine. J. Pharmacol. **89**: 325–341, 1947.
59. DAWES, G. S.: Experimental cardiac arrhythmias and quinidine-like drugs. Pharmacol. Rev. **2**: 43–84, 1952.
60. DETERLING, R. A., NGAI, S. H., LARAGH, J. H. AND PAPPER, E. M.: The cardiovascular effects of continuous intravenous infusion of norepinephrine, epinephrine and neosynephrine during cyclopropane and ether anesthesia in the dog. Anesthesiology **15**: 11–17, 1954.
61. DIPALMA, J. R. AND MASCATELLO, A. V.: Analysis of the actions of acetylcholine, atropine, epinephrine and quinidine on heart muscle of cat. J. Pharmacol. **101**: 243–248, 1951.
62. DRAPER, M. H. AND WEIDMANN, S.: Cardiac resting and action potentials recorded with an intracellular electrode. J. Physiol. **115**: 74–94, 1951.
63. DRESEL, P. E. AND DUNCAN, D. G.: Induction of automaticity in cat papillary muscles by sympathomimetic amines. J. Pharmacol. **133**: 70–75, 1961.
64. DRESEL, P. E., MACCANNELL, K. L. AND NICKERSON, M.: Cardiac arrhythmias induced by minimal doses of epinephrine in cyclopropane-anesthetized dogs. Circulation Res. **8**: 948–955, 1960.
65. DUDEL, J. AND TRAUTWEIN, W.: Das Aktionspotential und Mechanogramm des Herzmuskels unter dem Einfluss der Dehnung. Cardiologia **25**: 344–362, 1954.
66. DUDEL, J. AND TRAUTWEIN, W.: Die Wirkung von Adrenalin auf das Ruhepotential von Myokardfasern des Vorhofs. Experientia **12**: 396–401, 1955.
67. DUDEL, J. AND TRAUTWEIN, W.: Elektrophysiologische Messungen zur Strophanthinwirkung am Herzmuskel. Arch. exp. Path. Pharmak. **232**: 393–407, 1958.
68. DUDEL, J. AND TRAUTWEIN, W.: Der Mechanismus der automatischen rhythmischen Impulsbildung der Herzmuskelfaser. Pflüg. Arch. ges. Physiol. **267**: 553–565, 1958.
69. ECCLES, J. C.: The Neurophysiological Basis of Mind: The Principles of Neurophysiology. Clarendon Press, Oxford, 1953.
70. ECCLES, J. C. AND HOFF, H. E.: The rhythm of the heart beat. I. Location, action potential and electrical excitability of the pacemaker. Proc. Roy. Soc., ser. B **115**: 307–327, 1934.
71. ELIAKIM, M., BELLET, S., TAWIL, E. AND MULLER, O.: Effect of vagal stimulation and acetylcholine on the ventricle. Studies in dogs with complete atrioventricular block. Circulation Res. **9**: 1372–1379, 1961.
72. ENGSTFELD, G., ANTONI, H. AND FLECKENSTEIN, A.: Die Restitution der Erregungsfortleitung und Kontraktionskraft des K^+-gelähmten Froschsinus und Säugetiermyokards durch Adrenalin. Pflüg. Arch. ges. Physiol. **273**: 145–163, 1961.
73. ERLANGER, J.: Über den Grad der Vaguswirkung auf die Kammern des Hundeherzens. Pflüg. Arch. ges. Physiol. **127**: 77–98, 1909.
74. ERLANGER, J. AND BLACKMANN, J. R.: Further studies on the physiology of heart block in mammals. Chronic auriculo-ventricular heart block in the dog. Heart **1**: 177–228, 1909–1910.
75. ERLANGER, J. AND HIRSCHFELDER, A. D.: Further studies on the physiology of heart block in mammals. Amer. J. Physiol. **15**: 153–186, 1905.
76. ERNSTENE, A. C. AND PROUDFIT, W. L.: Differentiation of the changes in the QT interval in hypocalcemia and hypopotassemia. Amer. Heart J. **38**: 260–272, 1949.
77. EYSTER, J. A. E. AND MEEK, W. J.: The origin and conduction of the heart beat. Physiol. Rev. **1**: 1–43, 1921.
78. FARAH, A. AND BIRNBAUM, L.: Decelerator and antiarrhythmic properties of amotriphene. J. Pharmacol. **127**: 128–136, 1959.
79. FASTIER, F. N. AND SMIRK, F. H.: Some properties of amarin, with special reference to its use in conjunction with adrenaline for the production of idio-ventricular rhythms. J. Physiol. **107**: 318–331, 1948.
80. FATT, P. AND KATZ, B.: An analysis of the end-plate potential recorded with an intracellular electrode. J. Physiol. **115**: 320–370, 1951.
81. FAWCETT, D. W. AND SELBY, C. C.: Observations on the fine structure of the turtle atrium. J. biophys. biochem. Cytol. **4**: 63–72, 1958.

82. FINGL, E., WOODBURY, L. A. AND HECHT, H. H.: Effects of innervation and drugs upon direct membrane potentials of embryonic chick myocardium. J. Pharmacol. **104:** 103–114, 1952.
83. FITZHUGH, R.: Threshold and plateaus in the Hodgkin-Huxley nerve equations. J. gen. Physiol. **43:** 867–896, 1960.
84. FLECKENSTEIN, A. AND HARDT, A.: Der Wirkungsmechanismus der Lokalanästhetika und Antihistaminkörper—ein Permeabilitätsproblem. Klin. Wschr. **27:** 360–363, 1949.
85. FLEMING, W. W. AND HAWKINS, D. F.: The actions of dichloroisoproterenol in the dog heart-lung preparation and the isolated guinea-pig atrium. J. Pharmacol. **129:** 1–10, 1960.
86. FURCHGOTT, R. F., SLEATOR, W., JR. AND DE GUBAREFF, T.: Effects of acetylcholine and epinephrine on the contractile strength and action potential of electrically driven guinea pig atria. J. Pharmacol. **129:** 405–416, 1960.
87. GAFFNEY, T. E.: Effect of guanethidine and bretylium on the dog heart-lung preparation. Circulation Res. **9:** 83–88, 1961.
88. GAFFNEY, T. E., KAHN, J. B., VAN MAANEN, E. F. AND ACHESON, G. H.: A mechanism of the vagal effect of cardiac glycosides. J. Pharmacol. **122:** 423–429, 1958.
88a. GAFFNEY, T. E., CHIDSEY, C. A. AND BRAUNWALD, E.: Circulation Res., in press, 1963.
89. GARB, S.: The effects of potassium, ammonium, calcium, strontium and magnesium on the electrogram and myogram of mammalian heart muscle. J. Pharmacol. **101:** 317–326, 1951.
90. GARCIA RAMOS, J. AND ROSENBLUETH, A.: Estudios sobre el flutter y la fibrilación. Arch. Inst. Cardiol. Méx. **17:** 302–336, 1947.
91. GARGOUIL, Y. M., TRICOCHE, R., FROMENTY, D. AND CORABOEUF, E.: Effects de l'adrenaline sur l'activité électrique du coeur de mammifères. C. R. Acad. Sci., Paris **246:** 334–336, 1958.
92. GASKELL, W. H.: The electrical changes in the quiescent cardiac muscle which accompany stimulation of the vagus nerve. J. Physiol. **7:** 451–452, 1886.
93. GEORGE, E. P. AND JOHNSON, E. A.: Solutions of the Hodgkin-Huxley equations for squid axon treated with tetraethylammonium and in potassium-rich media. Aust. J. exp. Biol. med. Sci. **39:** 275–293, 1961.
94. GILBERT, J. L., LANGE, G., POLEVOY, I. AND BROOKS, McC. C.: Effects of vasoconstrictor agents on cardiac irritability. J. Pharmacol. **123:** 9–15, 1958.
95. GILBERT, J. L., SIEBENS, A. A., HOFFMAN, B. F., BELFORD, J., WOSKE, H. M. AND BROOKS, C. McC.: Quinidine, auricular excitability and fibrillation. Fed. Proc. **10:** 298, 1951.
96. GILSON, A. S., JR.: The increased accommodation to electric currents produced by vagal inhibition of the turtle atrium. Amer. J. Physiol. **127:** 333–337, 1939.
97. GLYNN, I. M.: The action of cardiac glycosides on sodium and potassium movements in human red cells. J. Physiol. **136:** 148–173, 1957.
98. GÖING, H. AND KEMPE, H. D.: Beeinflussung experimentell erzeugter Arrhythmien durch herzwirksame pflanzliche Stoffe (Rauwolfia serpentina, Khelline, Campher, R. Valerianae). Arch. int. Pharmacodyn. **107:** 255–269, 1956.
99. GOLDENBERG, M. AND ROTHBERGER, C. J.: Experimentelle Beiträge zur Kenntnis der Strophanthin Extrasystolen. Z. ges. exp. Med. **79:** 705–737, 1931.
100. GOLDENBERG, M. AND ROTHBERGER, C. J.: Über die Wirkung von Veratrin auf den Purkinjefaden. Pflüg. Arch. ges. Physiol. **238:** 136–152, 1936.
101. GOLDENBERG, M. AND ROTHBERGER, C. J.: Über das Elektrogramm der spezifischen Herzmuskulatur. Pflüg. Arch. ges. Physiol. **237:** 295–306, 1936.
101a. GOODFORD, P. J. AND VAUGHAN WILLIAMS, E. M.: Intracellular sodium and potassium concentrations of atrial muscle in relation to the action of quinidine. J. Physiol. **160:** 483–493, 1962.
102. GREMELS, H.: Über die Wirkung des Vagus auf die Herztätigkeit. Arch. exp. Path. Pharmak. **179:** 360–402, 1935.
103. GREMELS, H.: Über den Einfluss von Digitalisglykosiden auf die energetischen Vorgänge am Säugetierherzen. Arch. exp. Path. Pharmak. **186:** 625–660, 1937.
104. GROSSE-SCHULTE, E. AND TRAUTWEIN, W.: Der Einfluss der extrazellulären Na-Konzentration auf das Membranpotential der spontan tätigen Faser aus dem Sinus des Kaninchenherzens. Pflüg. Arch. ges. Physiol. **272:** 39, 1960.
105. GRUMBACH, L., HOWARD, J. W. AND MERRILL, V. I.: Factors related to the initiation of ventricular fibrillation in the isolated heart: effect of calcium and potassium. Circulation Res. **2:** 452–459, 1954.
106. HAAS, H. G. AND TRAUTWEIN, W.: Increase of sodium efflux in the frog heart induced by epinephrine. Nature, Lond. **197:** 80–81, 1963.
106a. HAJDU, S., AND LEONARD, E.: The cellular basis of cardiac glycoside action. Pharmacol. Rev. **11:** 173–209, 1959.
107. HARRIS, E. J. AND HUTTER, O. F.: The action of acetylcholine on the movements of potassium ions in the sinus venosus of the heart. J. Physiol. **133:** 58–59, 1956.
108. HASHIMOTO, K., SHIGEI, T., IMAI, S., SAITO, Y., YAGO, N., UEI, I. AND CLARK, R. E.: Oxygen consumption and coronary vascular tone in isolated fibrillating dog heart. Amer. J. Physiol. **198:** 965–970, 1960.
109. HEGGLIN, R. AND NOBILE, F.: Beeinflussung der Form und Dauer monophasischer Ableitungen. Verh. dtsch. Ges. Kreislaufforsch. **12:** 136–142, 1939.
110. HEINRICH, K. AND WEBER, A.: Klinische und experimentelle Studien über das EKG. XI Mitteilung. Beobachtungen am Herzkammerstreifen. Z. klin. Med. **137:** 272–296, 1940.
111. HERING, H. E.: Über die unmittelbare Wirkung des Accelerans und Vagus auf automatisch schlagende Abschnitte des Säugetierherzens. Pflüg. Arch. ges. Physiol. **108:** 281–299, 1905.
112. HERING, H. E.: Nachweis, dass die Verzögerung der Erregungsüberleitung zwischen Vorhof und Kammer des Säugetierherzens im Tawara'schen Knoten erfolgt. Pflüg. Arch. ges. Physiol. **131:** 572–580, 1910.
113. HODGKIN, A. L.: The ionic basis of electrical activity in nerve and muscle. Biol. Rev. **26:** 339–409, 1951.
114. HODGKIN, A. L.: A note on conduction velocity. J. Physiol. **125:** 221–224, 1954.

115. HODGKIN, A. L. AND HUXLEY, A. F.: Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo. J. Physiol. **116:** 449–472, 1952.
116. HODGKIN, A. L. AND HUXLEY, A. F.: The components of membrane conductance in the giant axon of Loligo. J. Physiol. **116:** 473–496, 1952.
117. HODGKIN, A. L. AND HUXLEY, A. F.: The dual effect of membrane potential of sodium conductance in the giant axon of Loligo. J. Physiol. **116:** 497–506, 1952.
118. HODGKIN, A. L. AND HUXLEY, A. F.: A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. **117:** 500–544, 1952.
119. HODGKIN, A. L. AND KEYNES, R. D.: Active transport of cations in giant axons from Sepia and Loligo. J. Physiol. **128:** 28–60, 1955.
120. HOFF, H. E. AND NAHUM, L. H.: The role of adrenaline in the production of ventricular rhythms and their suppression by acetyl-β-methylcholine chloride. J. Pharmacol. **52:** 235–245, 1934.
121. HOFF, H. E. AND NAHUM, L. H.: An analysis of the cardiac irregularities produced by calcium and their prevention by sodium amytal. J. Pharmacol. **60:** 425–433, 1937.
122. HOFF, H. E. AND NAHUM, L. H.: The supernormal period in the mammalian ventricle. Amer. J. Physiol. **124:** 591–595, 1938.
123. HOFF, H. E., SMITH, P. K. AND WINKLER, A. W.: Electrocardiographic changes and concentration of calcium in serum following intravenous injection of calcium chloride. Amer. J. Physiol. **125:** 162–171, 1939.
124. HOFFMAN, B. F.: The action of quinidine and procaine amide on single fibers of dog ventricle and specialized conducting system. Anais acad. brasil. cienc. **29:** 365–368, 1958 (cited in 125).
125. HOFFMAN, B. F. AND CRANEFIELD, P. F.: Electrophysiology of the Heart. McGraw-Hill Book Company, Inc. New York, 1960.
126. HOFFMAN, B. F., CRANEFIELD, P. F. AND STUCKEY, J. H.: Concealed conduction. Circulation Res. **9:** 194–203, 1961.
127. HOFFMAN, B. K., KAO, C. Y. AND SUCKLING, E. E.: Refractoriness in cardiac muscle. Amer. J. Physiol. **190:** 473–482, 1957.
128. HOFFMAN, B. F., PAES DE CARVALHO, A. AND DE MELLO, W. C.: Transmembrane potentials of single fibers of the atrio-ventricular node. Nature, Lond. **181:** 66–67, 1958.
129. HOFFMAN, B. F., PAES DE CARVALHO, A., DE MELLO, W. C. AND CRANEFIELD, P. F.: Electrical activity of single fibers of the atrioventricular node. Circulation Res. **7:** 11–18, 1959.
130. HOFFMAN, B. F., SIEBENS, A. A. AND BROOKS, C. MCC.: Effect of vagal stimulation on cardiac excitability. Amer. J. Physiol. **169:** 377–383, 1952.
131. HOFFMAN, B. F. AND SUCKLING, E. E.: Cardiac cellular potentials: Effect of vagal stimulation and acetylcholine. Amer. J. Physiol. **173:** 312–320, 1953.
132. HOFFMAN, B. F. AND SUCKLING, E. E.: Effect of several cations on transmembrane potentials of cardiac muscle. Amer. J. Physiol. **186:** 317–324, 1956.
133. HOFFMANN, F., HOFFMANN, E. J., MIDDLETON, S. AND TALESNIK, J.: Stimulating effect of acetylcholine on the mammalian heart and the liberation of an epinephrine-like substance by the isolated heart. Amer. J. Physiol. **144:** 189–198, 1945.
134. HOLLAND, W. C. AND KLEIN, R. L.: Effects of temperature, Na and K-concentration and quinidine on transmembrane flux of 42 K and incidence of atrial fibrillation. Circulation Res. **6:** 516–521, 1958.
135. HOLLAND, W. C., DUNN, E. C. AND GREIG, M. E.: Studies on permeability. VIII. Role of acetylcholine metabolism in the genesis of the electrocardiogram. Amer. J. Physiol. **170:** 339–345, 1952.
136. HOLZBAUER, M. AND VOGT, M.: Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. J. Neurochem. **1:** 8–11, 1956.
137. HOWELL, W. H. AND DUKE, W. W.: The effect of vagus inhibition on the output of potassium from the heart. Amer. J. Physiol. **21:** 51–63, 1908.
138. HUKOVIC, S.: Isolated rabbit atria with sympathetic nerve supply. Brit. J. Pharmacol. **14:** 372–376, 1959.
139. HUKOVIC, S.: The action of sympathetic blocking agents on isolated innervated atria and vessels. Brit. J. Pharmacol. **15:** 117–121, 1960.
140. HUTCHEON, D. E.: Susceptibility to ventricular fibrillation during chloroform and cyclopropane anaesthesia. Brit. J. Pharmacol. **6:** 31–34, 1951.
141. HUTTER, O. F.: Ion movements during vagus inhibition of the heart. In: Nervous Inhibition, ed. by E. Florey. Pergamon Press, Oxford, 1961.
142. HUTTER, O. F. AND NOBLE, D.: The influence of anions on impulse generation and membrane conductance in Purkinje and myocardial fibers. J. Physiol. **147:** 16–17, 1959.
143. HUTTER, O. F. AND NOBLE, D.: Rectifying properties of heart muscle. Nature, Lond. **188:** 495, 1960.
144. HUTTER, O. F. AND NOBLE, D.: Anion conductance of cardiac muscle. J. Physiol. **157:** 335–350, 1961.
145. HUTTER, O. F. AND TRAUTWEIN, W.: Vagal and sympathetic effects on the pacemaker fibers in the sinus venosus of the heart. J. gen. Physiol. **39:** 715–733, 1956.
146. HUXLEY, A. F.: Ion movements during nerve activity. Ann. N.Y. Acad. Sci. **81:** 221–245, 1959.
147. IMAI, S.: Effects of methoxamine upon the membrane resting and action potentials of the guinea pig's ventricular muscle fibers. Jap. J. Physiol. **11:** 54–61, 1961.
148. IMAI, S., SHIGEI, T. AND HASHIMOTO, K.: Cardiac actions of methoxamine. With special reference to its antagonistic action to epinephrine. Circulation Res. **9:** 552–560, 1961.
149. INNES, I. R., KOSTERLITZ, H. W. AND KRAYER, O.: Studies on veratrum alkaloids. XXIV. The inhibition by veratramine and veratrosine of the cardioaccelerator effect of electrical stimulation of the accelerator nerves. J. Pharmacol. **117:** 317–322, 1956.

150. INNES, I. R. AND KRAYER, O.: Studies on veratrum alkaloids. XXVII. The negative chronotropic action of veratramine and reserpine in the heart depleted of catecholamines. J. Pharmacol. **124:** 245–251, 1958.
151. INNES, I. R., KRAYER, O. AND WAUD, D. R.: The action of rauwolfia alkaloids on the heart rate and on the functional refractory period of atrio-ventricular transmission in the heart-lung preparation of the dog. J. Pharmacol. **124:** 324–332, 1958.
152. JOHNSON, E. A.: The effects of quinidine, procaine amide and pyrilamine on the membrane resting and action potential of guinea pig ventricular muscle fibers. J. Pharmacol. **117:** 237-244, 1956.
153. JOHNSON, E. A. AND MCKINNON, M. G.: The differential effect of quinidine and pyrilamine on the myocardial action potential at various rates of stimulation. J. Pharmacol. **120:** 460–468, 1957.
154. JOHNSON, E. A. AND ROBERTSON, P. A.: The stimulatory action of acetylcholine on isolated rabbit atria. Brit. J. Pharmacol. **13:** 304–307, 1958.
155. JOHNSON, E. A. AND ROBERTSON, P. A.: Effect of acetylcholine on the membrane resistance and threshold of atrial muscle fibers. Nature, Lond., **181:** 910, 1958.
156. JOHNSON, E. A., TILLE, J., WILSON, L. AND GEORGE, E. P.: Estimation of ionic conductances during the action potential in rabbit ventricle. In: Biophysics of Physiological and Pharmacological Actions, ed. by A. M. Shanes, pp. 529–540. Pergamon Press, New York, 1961.
157. JUNKMANN, K.: Beiträge zur Physiologie und Pharmakologie der Erregbarkeit des Froschherzens. Arch. exp. Path. Pharmak. **108:** 149–206, 1925.
158. KADATZ, R.: Pharmakologische Eigenschaften des Rauwolfia-Alkaloids Serpentin und einer Kombination von Reserpin mit Serpentin (Vilescon). Arzneim.-Forsch. **5:** 715–719, 1955.
159. KAHN, R. H.: Die Störungen der Herztätigkeit durch Adrenalin im Elektrokardiogramm. Pflüg. Arch. ges. Physiol. **129:** 379–401, 1909.
160. 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.
161. KAO, C. Y. AND HOFFMAN, B. F.: Graded and decremental response in heart muscle fibers. Amer. J. Physiol. **194:** 187–196, 1958.
162. KASSEBAUM, D.: Electrophysiological effect of strophanthin in the heart. J. Pharmacol.**140:** 329–338, 1963.
163. KASSEBAUM, D. AND TRAUTWEIN, W.: The effect of epinephrine on spontaneity in specialized cardiac fibers. (In preparation.)
164. KATZ, B.: Microphysiology of the neuro-muscular junction. A physiological 'quantum of action' at the myoneural junction. Johns Hopk. Hosp. Bull. **102:** 275–295, 1958.
165. KEYNES, R. D. AND SWAN, R. C.: The permeability of frog muscle fibers to lithium ions. J. Physiol. **147:** 626–638, 1959.
166. KISCH, B.: Strophanthin: Clinical and Experimental Experiences of the Past 25 Years. Brooklyn Med. Press, New York, 1944
166a. KLAUS, W., KUSCHINSKY, G. AND LÜLLMANN, H.: Über den Zusammenhang zwischen positiv inotroper Wirkung von Digitoxigenin, Kaliumflux und intracellulärer Ionenkonzentration im Herzmuskel. Arch. exp. Path. Pharmak. **242:** 480–496, 1962.
167. KLEIN, R. L., HOLLAND, W. C. AND TINSLEY, B: Quinidine and unidirectional cation fluxes in atria. Circulation Res. **8:** 246–252, 1960.
168. KLEINFELD, M., MAGIN, J. AND STEIN, E.: The effect of tetraethylpyrophosphate (TEPP) on the transmembrane potentials of pacemaker and non-pacemaker fibers of isolated rabbit atrium. Circulation Res. **8:** 240–245, 1960.
169. KLEINFELD, M., STEIN, E. AND MEYERS, S.: Effects of barium chloride on resting and action potentials of ventricular fibers of the frog. Circulation Res. **2:** 488–493, 1954.
170. KLEINFELD, M., STEIN, E., MAGIN, J. AND KOSSMANN, C. E.: The action of iodoacetate on the electrical and mechanical activities of the isolated perfused frog heart. J. clin. Invest. **34:** 1802–1806, 1955.
171. KOLM, R. AND PICK, E. P.: Über die Bedeutung des Kaliums für die Selbststeuerung des Herzens. Pflüg. Arch. ges. Physiol. **185:** 235–247, 1920.
172. VAN DER KOOI, M. W., DURRER, D., VAN DAM, R. T. AND VAN DER TWEEL, L. H.: Electrical activity in sinus node and atrio-ventricular node. Amer. Heart J. **51:** 684–700, 1956.
173. KOSTERLITZ, H. W., KRAYER, O. AND MATALLANA, A.: Studies on veratrum alkaloids. XXII. Periodic activity of the sino-auricular node of the denervated cat heart caused by veratramine. J. Pharmacol. **113:** 460–469, 1955.
174. KRAYER, O.: Studies on veratrum alkaloids. VIII. Veratramine, an antagonist to the cardioaccelerator action of epinephrine. J. Pharmacol. **96:** 422–437, 1949.
175. KRAYER, O.: IX. The inhibition by veratrosine of the cardioaccelerator action of epinephrine and of norepinephrine. J. Pharmacol. **97:** 256–265, 1949.
176. KRAYER, O. AND ACHESON, G. H.: The pharmacology of the veratrum alkaloids. Physiol. Rev. **26:** 383–446, 1946.
177. KRAYER, O., ALPER, M. H. AND PAASONEN, M. K.: Action of guanethidine and reserpine upon the isolated mammalian heart. J. Pharmacol. **135:** 164–173, 1962.
178. KRAYER, O., ARORA, R. B. AND MEILMAN, E.: Studies on veratrum alkaloids. XXI. The action of veratramine upon impulse generation in the dog heart. J. Pharmacol. **113:** 446–459, 1955.
179. KRAYER, O. AND FUENTES, J.: Changes of heart rate caused by direct cardiac action of reserpine. J. Pharmacol. **123:** 145–152, 1958.
180. KRAYER, O., KUPCHAN, S. M., DELIWALA, C. V. AND ROGERS, B. H.: Untersuchungen über die Veratrumalkaloide. XVIII. Die chemischen und pharmakologischen Beziehungen zwischen den Zygadenusalkaloiden und den Veratrumalkaloiden. Arch. exp. Path. Pharmak. **219:** 371–385, 1953.
181. KRAYER, O. AND VAN MAANEN: Studies on veratrum alkaloids. X. The inhibitions by veratramine of the positive chronotropic effect of accelerans stimulation and of norepinephrine. J. Pharmacol. **97:** 301–307, 1949.

182. KRAYER, O., MANDOKI, J. J. AND MENDEZ, C.: Studies on veratrum alkaloids. XVI. The action of epinephrine and of veratramine on the functional refractory period of the auriculo-ventricular transmission in the heart-lung preparation of the dog. J. Pharmacol. **103:** 412–419, 1951.
183. KRAYER, O. AND REITER, M.: Studies on veratrum alkaloids. XI. Jervine and pseudojervine, antagonists to the cardioaccelerator action of epinephrine and of accelerans stimulations. Arch. int. Pharmacodyn. **4:** 409–425, 1950.
184. KUFFLER, S. W.: Physiology of neuro-muscular junctions: electrical aspects. Fed. Proc. **7:** 437–446, 1948.
185. KUFFLER, S. W.: Excitation and inhibition in single nerve cells. In: Harvey Lectures, 1958–1959, pp. 176–218. Academic Press, Inc., New York, 1960.
186. KRUEGER, E. AND UNNA, K.: Comparative studies on the toxic effects of digitoxin and ouabain in cats. J. Pharmacol. **76:** 282–293, 1942.
187. LAHTI, R. E., BRILL, I. C. AND MCCAWLEY, E. L.: The effect of methoxamine hydrochloride (vasoxyl) on cardiac rhythm. J. Pharmacol. **115:** 268–274, 1955.
188. LAMB, J. F.: The action of 2-4 DNP on the auricular action potential duration and intracellular levels of Na^+ and K^+. J. Physiol. **150:** 4P, 1960.
189. LANDS, A. M. AND HOWARD, J. W.: A comparative study of the effects of *l*-arterenol, epinephrine and isopropylarterenol of the heart. J. Pharmacol. **106:** 65–76, 1952.
190. LEE, K. S., YU, D. H., LEE, D. J. AND BURSTEIN, R.: The influence of potassium and calcium on the effect of ouabain on cat papillary muscles. J. Pharmacol. **132:** 139–148, 1961.
191. LENDLE, L. AND MERCKER, H.: Extrakardiale Digitaliswirkungen. Ergebn. Physiol. **51:** 199–280, 1961.
192. LENDLE, L., MERCKER, H. AND ROHR, H.: Über die Herzvaguswirkung unter dem Einfluss von Digitalisglykosiden. Arch. exp. Path. Pharmak. **219:** 352–361, 1953.
193. LENDLE, L. AND WIENKE, H.: Zur Frage der Sensibilisierung von Vaguswirkungen auf die Herzfrequenz durch Digitalis. Arch. exp. Path. Pharmak. **213:** 373–386, 1951.
194. LENEL, R., VANLOO, A., RODBARD, S. AND KATZ, L. N.: Factors involved in the production of paroxysmal ventricular tachycardia induced by epinephrine. Amer. J. Physiol. **153:** 553–557, 1948.
195. LENNARTZ, E.: Potassium ions and vagus inhibition. J. Physiol. **86:** 37P, 1936.
196. LEVEQUE, P. E.: Auricular fibrillation by acetylcholine injection during hyperpotassemia. J. Pharmacol. **120:** 38–45, 1957.
197. LEVY, A. G.: Further remarks on ventricular extrasystoles and fibrillation under chloroform. Heart **7:** 105–110, 1920.
198. LEWIS, T. AND DRURY, A. N.: Revised views of the refractory period in relation to drugs reputed to prolong it, and in relation to circus movement. Heart **13:** 95–100, 1926.
199. LEWIS, R., DRURY, A. N. AND JLIESCU, C. C.: Further observations upon the state of rapid reexcitation of the auricles. Heart **8:** 311–329, 1921.
200. LEWIS, T., FEIL, H. S. AND STROUD, W. D.: Observations upon flutter and fibrillation. Heart **7:** 127–130, 191, 247, 1918–1920.
201. LING, G. AND GERARD, R. W.: The normal membrane potential of frog sartorius fibers. J. cell. comp. Physiol. **34:** 383–396, 1949.
202. LOCKETT, M. F.: The responses of the heart rate and the systolic blood pressure to a series of sympathomimetic amines in the unanaesthetized, atropinized bitch. J. Physiol. **111:** 18–42, 1950.
203. LOEWI, O.: Über den Zusammenhang zwischen Digitalis- und Calciumwirkung. Arch. exp. Path. Pharmak. **82:** 131–158, 1918.
204. LOVE, W. S.: Effect of quinidine and strophanthin upon refractory period of tortoise ventricle. Heart **13:** 87–92, 1926.
205. LU, F. C. AND MELVILLE, K. I.: Effects of noradrenaline on coronary flow and heart contraction as recorded concurrently in the isolated rabbit heart. J. Physiol. **113:** 365–371, 1951.
206. LUEKEN, B. AND SCHÜTZ, E.: Die relative Refraktärphase des Herzens. III. Mitteilung: Reversibilität und Antagonismus. Z. Biol. **99:** 186–197, 1938.
207. LÜLLMANN, H.: Beeinflussung der cellulären Potentiale des Rattenherzens durch Fermentgifte. Arch. exp. Path. Pharmak. **236:** 157, 1959.
208. LÜTTGAU, H. C. AND NIEDERGERKE, R.: The antagonism between Ca and Na ions on the frog's heart. J. Physiol. **143:** 486–505, 1958.
209. MADAN, B. R. AND SHARMA, V. N.: Serpentine and ajmaline in ventricular ectopic activity. Arch. int. Pharmacodyn. **122:** 323–328, 1959.
210. MARSHALL, J. M.: Action of iodoacetic acid, 2,4-dinitrophenol, and L-tri-iodothyronine on the electrical response of the myocardium. Amer. J. Physiol. **180:** 350–356, 1955.
211. MARSHALL, J. M. AND KATSH, S.: Inhibition by anticholinesterases of the electrical and mechanical activity of isolated rabbit auricles in vitro. Amer. J. Physiol. **190:** 495–499, 1957.
212. MARSHALL, J. M. AND VAUGHAN WILLIAMS, E. M.: Pacemaker potentials. The excitation of isolated rabbit auricles by acetylcholine at low temperatures. J. Physiol. **131:** 186–199, 1959.
213. MATCHETT, P. A. AND JOHNSON, J. A.: Inhibition of sodium and potassium transport in frog sartorii in the presence of ouabain. Fed. Proc. **13:** 384, 1954.
214. MATSUDA, K., HOFFMAN, B. F., ELLNER, C. N., KATZ, M. AND BROOKS, C. MCC.: Veratrine induced prolongation of repolarisation in the mammalian heart. Abstr. 19th int. physiol. Congr. 596–597, 1953.
215. MATSUDA, K., HOSHI, T. AND KAMEYAMA, S.: Action potential of the atrio-ventricular node (Tawara). Tohoku J. exp. Med. **68:** 8, 1958.
216. MATSUDA, K., HOSHI, T. AND KAMEYAMA, S.: Action of acetylcholine and adrenaline upon the membrane potential of the atrio-ventricular node (Tawara). Tohoku J. exp. Med. **68:** 16, 1958.

217. MATSUDA, K., HOSHI, T. AND KAMEYAMA, S.: Effects of aconitine on the cardiac membrane potential of the dog. Jap. J. Physiol. **9:** 419–429, 1959.
218. MATSUMURA, M. AND TAKAORI, S.: The effect of drugs on the cardiac membrane potentials in the rabbit. Jap. J. Pharmacol. **8:** 134–142, 1959.
219. MAXWELL, R. A., PLUMMER, A. J., SCHNEIDER, F., POVALSKI, H. AND DANIEL, A. L.: Pharmacology of (2-(octahydro-1-azocinylethyl)guanidine sulfate (Su 5864). J. Pharmacol. **128:** 22–29, 1960.
220. MCALLEN, P. M.: The electrocardiogram associated with low levels of serum potassium. Brit. Heart J. **13:** 159–166, 1951.
220a. MCCUBBIN, J. W., KANEKO, Y., AND PAGE, I. H.: The peripheral cardiovascular actions of guanethidine in dogs. J. Pharmacol. **131:** 346–354, 1961.
221. MCEWEN, L. M.: The effect on the isolated rabbit's heart of vagal stimulation and its modification by cocain, hexamethonium and ouabain. J. Physiol. **131:** 678–689, 1959.
222. MCFARLANE, W. V.: The plateau of the action potential of the frog ventricle. Circulation Res. **8:** 47–55, 1960.
223. MCLAIN, P. L., KRUSE, T. K. AND REDICK, T.: The effect of atropine on digitoxin bradycardia in cats. J. Pharmacol. **126:** 76–81, 1959.
224. MEEK, W. J. AND EYSTER, A. E.: The origin of the cardiac impulse in the turtle's heart. Amer. J. Physiol. **39:** 291–296, 1915–1916.
225. MEEK, W. J., HATHAWAY, H. R. AND ORTH, O. S.: The effects of ether, chloroform and cyclopropane on cardiac automaticity. J. Pharmacol. **61:** 240–252, 1937.
226. DE MELLO, W. C.: Metabolism and electrical activity of the heart: action of 2,4-dinitrophenol and ATP. Amer. J. Physiol. **196:** 377–380, 1959.
227. DE MELLO, W. C. AND HOFFMAN, B. F.: Potassium ions and electrical activity of specialized cardiac fibers. Amer. J. Physiol. **199:** 1125–1130, 1960.
228. MÉNDEZ, C., ACEVES, J. AND MÉNDEZ, R.: Inhibition of adrenergic cardiac acceleration by cardiac glycosides. J. Pharmacol. **131:** 191–199, 1961.
229. MÉNDEZ, R. AND MÉNDEZ, C.: The action of cardiac glycosides on the refractory period of heart tissue. J. Pharmacol. **107:** 24–36, 1953.
229a. MÉNDEZ, C. AND MÉNDEZ, R.: The action of cardiac glycosides on the excitability and conduction velocity of the mammalian atrium. J. Pharmacol. **121:** 402–413, 1957.
230. MIRKIN, B. L., BELFORD, J., HOFFMAN, B. F., SILVERSTEIN, E. AND BROOKS, C. MCC.: The action of 1-(2-diethylaminoethylamino)-3-methylisoquinoline dihydrochloride (SKF # 531) and related compounds on excitability, refractoriness, conduction and fibrillation thresholds to electrical stimulation of mammalian heart. Fed. Proc. **13:** 389, 1954.
231. MOE, G. K. AND MÉNDEZ, R.: The action of several cardiac glycosides on conduction velocity and ventricular excitability in the dog heart. Circulation **4:** 729–734, 1951.
232. MOORE, D. AND RUSKA, H.: Electro-microscope study of the mammalian cardiac muscle cell. J. biophys. biochem. Cytol. **3:** 261–268, 1957.
233. MOORE, J. I. AND SWAIN, H. H.: Sensitization to ventricular fibrillation. I. Sensitization by a substituted propiophenone, U-0882. J. Pharmacol. **128:** 243–252, 1960.
234. MOORE, J. I. AND SWAIN, H. H.: Sensitization to ventricular fibrillation. II. Sensitization by amarine and congeners of U-0882. J. Pharmacol. **128:** 253–258, 1960.
234a. MÜLLER, P.: Kalium und Digitalistoxizität. Cardiologia **42:** 1–13, 1963.
235. MUSCHOLL, E.: Die Wirkung von Reserpin auf die Konzentration von Adrenalin und Noradrenalin im Katzenherz. Arch. exp. Path. Pharmak. **237:** 371–374, 1959.
236. MUSCHOLL, E. AND VOGT, M.: The action of reserpine on the peripheral sympathetic system. J. Physiol. **141:** 132–145, 1958.
237. NAHUM, L. H. AND HOFF, H. E.: Production of auricular fibrillation by application of acetyl-β-methylcholine chloride to localized regions on the auricular surface. Amer. J. Physiol. **129:** 428, 1940.
238. NALEFSKI, L. A., GILBERT, N. C. AND FENN, G. K.: Cardiovascular changes observed following the experimental administration of barium chloride. J. Lab. clin. Med. **34:** 1733, 1949.
239. NICKERSON, M. AND CHAN, G. C. M.: Blockade of responses of isolated myocardium to epinephrine. J. Pharmacol. **133:** 186–192, 1961.
240. NOBEL, E. AND ROTHBERGER, C. J.: Über die Wirkung von Adrenalin und Atropin bei leichter Chloroformnarkose. Z. ges. exp. Med. **3:** 151–197, 1914.
241. NOBLE, D.: Cardiac action and pacemaker potentials based on the Hodgkin-Huxley equations. Nature, Lond. **188:** 495, 1960.
242. NOBLE, D.: A modification of the Hodgkin-Huxley equations applicable to Purkinje fiber action and pacemaker potential. J. Physiol. **160:** 317–352, 1962.
243. OTSUKA, M.: Die Wirkung von Adrenalin auf Purkinje-Fasern von Säugetierherzen. Pflüg. Arch. ges. Physiol. **266:** 512–517, 1958.
244. OVERTON, E.: Beiträge zur allgemeinen Muskel- und Nervenphysiologie. Pflüg. Arch. ges. Physiol. **92:** 346–386, 1902.
245. PAASONEN, M. K. AND KRAYER, O.: The release of norepinephrine from the mammalian heart by reserpine. J. Pharmacol. **123:** 153–160, 1958.
246. POCHE, R. AND LINDNER, E.: Untersuchungen zur Frage der Glanzstreifen des Herzmuskelgewebes beim Warmblüter und beim Kaltblüter. Z. Zellforsch. **43:** 104–120, 1955.
247. PRUITT, R. D. AND ESSEX, H. E.: Potential changes attending the excitation process in the atrioventricular conduction system of bovine and canine hearts. Circulation Res. **8:** 149–174, 1960.

248. RAYNER, B. AND WEATHERALL, M.: Acetylcholine and potassium movements in rabbit auricles. J. Physiol. **146:** 392–409, 1959.
249. REITER, M.: Wirkung von Frequenz, Natriumentzug und Strophanthin auf Kontraktionskraft und Alkaligehalt des Herzmuskels. Arch. exp. Path. Pharmak. **227:** 300–315, 1956.
250. REITER, M.: Die Entstehung von 'Nachkontraktionen' im Herzmuskel unter Einwirkung von Calcium und von Digitalisglykosiden in Abhängigkeit von der Reizfrequenz. Arch. exp. Path. Pharmak. **242:** 497–507, 1962.
251. REITER, M. AND NOE, J.: Die Bedeutung von Calcium, Magnesium, Kalium und Natrium für die rhythmische Erregungsbildung im Sinusknoten des Warmblüterherzens. Pflüg. Arch. ges. Physiol. **269:** 366–374, 1959.
252. RIHL, J.: Über Vaguswirkung auf die automatisch schlagenden Kammern des Säugetierherzens. Pflüg. Arch. ges. Physiol. **114:** 545–552, 1906.
253. RIJLANT, P.: The pacemaker of the mammalian heart. J. Physiol. **75:** 28P, 1932.
254. RIKER, W. F., DEPIERRE, F., ROBERTS, J., ROY, B. B. AND REILLY, J.: The epinephrine and hydrocarbon-epinephrine disturbance in the cat. J. Pharmacol. **114:** 1–9, 1955.
255. ROBERTS, J., STANDAERT, F., YUNG IN KIM AND RIKER, W. F.: The initiation and pharmacologic reactivity of a ventricular pacemaker in the intact animal. J. Pharmacol. **117:** 374–384, 1956.
256. ROBERTSON, W. VAN B. AND PEYSER, P.: Changes in water and electrolytes of cardiac muscle following epinephrine. Amer. J. Physiol. **166:** 277–283, 1951.
257. ROSENBLUETH, A.: The mechanism of auricular flutter and auricular fibrillation. Circulation **7:** 612–613, 1953.
258. ROSENBLUETH, A. AND GARCIA RAMOS, J.: Studies on flutter and fibrillation. II. The influence of artificial obstacles on experimental auricular flutter. Amer. Heart J. **33:** 677–684, 1947.
259. ROSENBLUETH, A. AND GARCIA RAMOS, J.: Estudios sobre el flutter y la fibrilacion. Arch. Inst. Cardiol. Méx. **17:** 441–457, 1947.
260. ROTHBERGER, C. J. AND WINTERBERG, H.: Über die experimentelle Erzeugung extrasystolischer ventrikulärer Tachykardie durch Akzeleransreizung. Pflüg. Arch. ges. Physiol. **142:** 461–522, 1911.
261. ROTHBERGER, C. J. AND WINTERBERG, H.: Über den Einfluss von Strophanthin auf die Reizbildungsfähigkeit der automatischen Zentren des Herzens. Pflüg. Arch. ges. Physiol. **150:** 217–261, 1913.
262. ROTHLIN, E. AND SUTER, E.: Glykosidwirkung auf Elektrokardiogramm und Myokard. I. Mitteilung: Vergleichende elektrokardiographische Untersuchungen verschiedener herzwirksamer Glykoside an der Katze bei intravenöser Infusion. Helv. physiol. acta **5:** 298–321, 1947.
263. ROTHSCHUH, K. E.: Elektrophysiologie des Herzens. Steinkopf, Darmstadt, 1952.
264. SANO, T., TASAKI, M., ONO, M., TSUCHIHASHI, H., TAKAYAMA, N. AND SHIMAMOTO, T.: Resting and action potentials in the region of the atrio-ventricular node. Proc. Jap. Acad. **34:** 558–563, 1958.
265. SCHAEFER, H.: Elektrophysiologie der Herznerven. Ergebn. Physiol. **46:** 71–125, 1950.
266. SCHATZMANN, H. J. AND WITT, P. N.: Action of K-strophanthin on potassium leakage from frog sartorius muscle. J. Pharmacol. **112:** 501–508, 1954.
267. SCHATZMANN, H. J.: Herzglykoside als Hemmstoffe für den aktiven Kalium- und Natriumtransport durch die Erythrocytenmembran. Helv. physiol. acta **11:** 346–354, 1953.
268. SCHELLONG, F.: Der Einfluss der Digitalis auf die Refraktärphase der Erregbarkeit und der Erregungsgrösse des Herzmuskelelements. Z. exp. Med. **75:** 789–826, 1931.
269. SCHELLONG, F.: Die Refraktärphase der Erregungsfortpflanzung im normalen und Digalen vergifteten Herzmuskel. Z. exp. Med. **78:** 1–14, 1931.
270. SCHER, A. M.: Excitation of the heart. In: Handbook of Physiology, section 2, vol. 1, Circulation, ed. by W. F. Hamilton and P. Dow, pp. 287–322. American Physiological Society, Washington, D.C., 1962.
271. SCHERF, D.: Experimental digitalis and strophanthin extrasystoles. Exp. Med. Surg. **2:** 170–181, 1944.
272. SCHERF, D.: Studies on auricular tachycardia caused by aconitine administration. Proc. Soc. exp. Biol., N.Y. **64:** 233–239, 1947.
273. SCHERF, D. AND CHICK, F. B.: Abnormal cardiac rhythms caused by acetylcholine. Circulation **3:** 764–769, 1951.
274. SCHERF, D. AND SCHOTT, A.: Extrasystoles and Allied Arrhythmias. William Heinemann, London, 1953.
275. SCHMIDT, R. F.: Über die Acetylcholin-Empfindlichkeit verschiedener Herzabschnitte. Arch. exp. Path. Pharmak. **233:** 531–541, 1958.
276. SCHMIDT, R. F.: Versuche mit Aconitin zum Problem der spontanen Erregungsbildung im Herzen. Pflüg. Arch. ges. Physiol. **271:** 526–536, 1960.
277. SCHMITT, H. AND SCHMITT, H.: Sur la pharmacologie de l'ajmaline. Arch. int. Pharmacodyn. **127:** 163–179, 1960.
278. SCHREINER, G. L., BERGLUND, E., BORST, H. AND MONROE, R. G.: Effect of vagus stimulation and of acetylcholine on myocardial contractility, O_2 consumption and coronary flow in dogs. Circulation Res. **5:** 562–567, 1957.
279. SCHÜTZ, E.: Elektrophysiologie des Herzens bei einphasischer Ableitung. Ergebn. Physiol. **38:** 493–620, 1936.
280. SHANES, A. M.: Electrochemical aspects of physiological and pharmacological action in excitable cells. Pharmacol. Rev. **10:** 59–164, 165–273, 1958.
281. SHARMA, V. N. AND SINGH, K. P.: Effect of some antihistaminic compounds in experimental cardiac arrhythmias. Arch. int. Pharmacodyn. **131:** 24–30, 1961.
281a. SHORE, P. A.: Release of serotonin and catecholamines by drugs. Pharmacol. Rev. **14:** 531–550, 1962.
282. SIEBENS, A. A., HOFFMAN, B. F., ENSEN, J. E., FARRELL, J. E. AND BROOKS, C. MCC.: Effects of *l*-epinephrine and *l*-nor-epinephrine on cardiac excitability. Amer. J. Physiol. **175:** 1–7, 1953.
283. SINGH, K. P. AND SHARMA, V. N.: Arrhythmia combating properties of some local anaesthetics. Arch. int. Pharmacodyn. **131:** 1–9, 1961.
284. SMITH, P. K., WINKLER, A. W. AND HOFF, H. E.: Cardiovascular changes following intravenous administration of barium chloride. J. Pharmacol. **68:** 113–122, 1940.

285. SPANG, K.: Rhythmusstörungen des Herzens. Georg Thieme, Stuttgart, 1957.
286. SPERELAKIS, N. AND HOSHIKO, T.: Electrical impedance of cardiac muscle. Circulation Res. **9:** 1280–1283, 1961.
287. STÄMPFLI, R. AND NISHIE, K.: Effects of calcium-free solutions on membrane-potential of myelinated nerve fibers of the Brazilian frog *Leptodactylus ocellatus*. Helv. physiol. acta **14:** 93–104, 1956.
288. STEIN, E., KLEINFELD, M., GREENE, H. AND MEYERS, S.: Action of lithium chloride on the isolated perfused frog heart. Amer. J. Physiol. **183:** 121–124, 1955.
289. STEWART, G. H., LYNCH, P. R., BARRERA, F. AND OPPENHEIMER, M. J.: Changes in properties of heart muscle due to mephentermine. Amer. J. Physiol. **186:** 513–517, 1956.
290. 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.
291. STUTZMAN, J. W., PETTINGA, F. L. AND FRUGGIERO, E. J.: Cardiac effects of methoxamine (β-[2,5-dimethoxyphenyl]-β-hydroxyisopropyl amine HCl) and desoxyephedrine during cyclopropane anesthesia. J. Pharmacol. **97:** 385–387, 1949.
292. SUTER, E., ROTHLIN, E. AND BIRCHER, R.: Glykosidwirkungauf Elektrokardiogramm und Myokard. III. Mitteilung: Wirkung von mehrmalig verabreichten toxischen und therapeutischen Dosen. Helv. physiol. acta **7:** 1–36, 1949.
293. SWAIN, H. H. AND MCCARTHY, D. A.: Veratrine, protoveratrine and andromedotoxin arrhythmias in the isolated dog heart. J. Pharmacol. **121:** 379–388, 1957.
294. SWAIN, H. H. AND WEIDNER, C L : A study of substances which alter intraventricular conduction in the isolated dog heart. J. Pharmacol. **120:** 137–148, 1957.
295. SZEKERES, L.: Wirkung von Pharmaka auf die Erregungsleitung am hypoxischen Herzen. Arch. exp. Path. Pharmak. **233:** 338–342, 1958.
296. SZEKERES, L., FALLER, J. AND LICHNER, G.: Empfindlichkeit der einzelnen Reizbildungszentren des Herzens gegenüber Kohlensäure und Hypoxie. Arch. exp. Path. Pharmak. **233:** 343–347, 1958.
297. SZEKERES, L. AND VAUGHAN WILLIAMS, E. M.: Antifibrillatory action. J. Physiol. **160:** 470–482, 1962.
298. THIENES, C. H., GREENLEY, P. O. AND GUEDEL, A. E.: Cardiac arrhythmias under cyclopropane anesthesia. Anaesthesiology **6:** 611–621, 1941.
299. TODA, N.: Effects of adrenaline, noradrenaline and reserpine or the transmembrane potentials in both pacemakers and non-pacemaker fibers of the rabbit atrium. Jap. J. Pharmacol. **10:** 78–91, 1960.
300. TRAUTWEIN, W.: Über die Veränderungen der elementaren Daten der elektrischen Erregungswelle des Herzens bei der Insuffizienz des Myocards. Pflüg. Arch. ges. Physiol. **252:** 573–589, 1950.
301. TRAUTWEIN, W.: Physiologie der Herzirregularitäten. In: Rhythmusstörungen des Herzens, ed. by K. Spang. Georg Thieme, Stuttgart, 1957.
302. TRAUTWEIN, W.: Elektrophysiologie der Herzmuskelfaser. Ergebn. Physiol. **51:** 131–198, 1961.
303. TRAUTWEIN, W. AND DUDEL, J.: Zum Mechanismus der Membranwirkung des Acetylcholin an der Herzmuskelfaser. Pflüg. Arch. ges. Physiol. **266:** 324–334, 1958.
304. TRAUTWEIN, W. AND DUDEL, J.: Hemmende und erregende Wirkungen des Acetylcholin am Warmblüterherzen. Zur Frage der spontanen Erregungsbildung. Pflüg. Arch. ges. Physiol. **266:** 653–664, 1958.
305. TRAUTWEIN, W., GOTTSTEIN, U. AND FEDERSCHMIDT, K.: Der Einfluss der Temperatur auf den Aktionstrom des excidierten Purkinje-Fadens, gemessen mit einer intrazellulären Elektrode. Pflüg. Arch. ges. Physiol. **258:** 243–260, 1953.
306. TRAUTWEIN, W., GOTTSTEIN, U. AND DUDEL, J.: Der Aktionsstrom der Myokardfaser im Sauerstoffmangel. Pflüg. Arch. ges. Physiol. **260:** 40–60, 1954.
307. TRAUTWEIN, W. AND GROSSE SCHULTE, E.: Unpublished observations.
308. TRAUTWEIN, W. AND KASSEBAUM, D. G.: On the mechanism of spontaneous impulse generation in the pacemaker of the heart. J. gen. Physiol. **45:** 317–330, 1961.
309. TRAUTWEIN, W., KASSEBAUM, D. G., NELSON, R. M. AND HECHT, H. H.: Electrophysiological study of human heart muscle. Circulation Res. **10:** 306–312, 1962.
310. TRAUTWEIN, W., KUFFLER, W. S. AND EDWARDS, C.: Changes in membrane characteristics of heart muscle during inhibition. J. gen. Physiol. **40:** 135–145, 1956.
311. TRAUTWEIN, W. AND SCHMIDT, R. F.: Zur Membranwirkung des Adrenalin an der Herzmuskelfaser. Pflüg. Arch. ges. Physiol. **271:** 715–725, 1960.
312. TRAUTWEIN, W. AND UCHIZONO, K.: Abstr. XXII Int. Congr. Physiol. Sci., Leiden, 1962.
313. TRAUTWEIN, W., WAHLEN, W. J. AND GROSSE SCHULTE, E.: Elektrophysiologischer Nachweis spontaner Freisetzung von Acetylcholin im Vorhof des Herzens. Pflüg. Arch. ges. Physiol. **270:** 560–570, 1960.
314. TRAUTWEIN, W. AND WITT, P. N.: Der Einfluss des Strophanthins auf das Ruhe- und Aktionspotentials der geschädigten Herzmuskelfaser. Arch. exp. Path. Pharmak. **216:** 197–199, 1952.
315. TRAUTWEIN, W. AND ZINK, K.: Über Membran- und Aktionspotentiale einzelner Myokardfasern des Kalt- und Warmblüterherzens. Pflüg. Arch. ges. Physiol. **256:** 68–84, 1952.
315a. TRENDELENBURG, U.: Supersensitivity and subsensitivity to sympathomimetic amines. Pharmacol. Rev. **15:** 225–276, 1963.
316. TRENDELENBURG, U. G. AND GRAVENSTEIN, J. S.: Effect of reserpine pretreatment on stimulation of the accelerans nerve of the dog. Science **128:** 901–903, 1958.
317. TUTTLE, R. S., WITT, P. N. AND FARAH, A.: The influence of ouabain on intracellular sodium and potassium concentrations in the rabbit myocardium. J. Pharmacol. **133:** 281–287, 1961.
318. VAUGHAN WILLIAMS, E. M.: The mode of action of quinidine on isolated rabbit atria interpreted from intracellular records. Brit. J. Pharmacol. **13:** 276–287, 1958.

319. VAUGHAN WILLIAMS, E. M. AND SZEKERES, L.: A comparison of tests for antifibrillatory action. Brit. J. Pharmacol. **17**: 424–432, 1961.

319a. VICK, R. L.: Effects of some steroid and non-steroid lactones on potassium exchange and physiological properties of the isolated perfused guinea pig ventricle. J. Pharmacol. **125**: 40–48, 1959.

320. VICK, R. L. AND KAHN, J. B.: The effects of ouabain and veratridine on potassium movement in the isolated guinea pig heart. J. Pharmacol. **121**: 389–401, 1957.

321. WACHSTEIN, M.: Untersuchungen am Purkinjefaden. Z. ges. exp. Med. **79**: 653–672; **83**: 491–536, 1931–1932.

322. WALTHER, R.: Beiträge zur Differenzierung der pharmakologischen Wirkungen von Stoffen der Digitalisgruppe. I. Mitteilung: Vergleich der Wirkung von Digitoxigenin und Digitoxin auf das Elektrokardiogramm der Katze. Arch. exp. Path. Pharmak. **195**: 709–720, 1940.

323. WAUD, D. R., KOTTEGODA, S. R. AND KRAYER, O.: Threshold dose and time course of norepinephrine depletion of the mammalian heart by reserpine. J. Pharmacol. **124**: 340–346, 1958.

324. WEBB, J. L.: The action of acetylcholine on the rabbit auricle. Brit. J. Pharmacol. **5**: 335–375, 1950.

325. WEBB, J. L. AND HOLLANDER, P. B.: The action of acetylcholine and epinephrine on the cellular membrane potentials and contractility of rat atrium. Circulation Res. **4**: 332–336, 1956.

326. WEIDMANN, S.: Effect of current flow on the membrane potential of cardiac muscle. J. Physiol. **115**: 227–236, 1951.

327. WEIDMANN, S.: The electrical constants of Purkinje fibers. J. Physiol. **118**: 348–360, 1952.

328. WEIDMANN, S.: The effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system. J. Physiol. **127**: 213–224, 1955.

329. WEIDMANN, S.: The effects of calcium ions and local anaesthetics on electrical properties of Purkinje fibers. J. Physiol. **129**: 568–582, 1955.

330. WEIDMANN, S.: Rectifier properties of Purkinje fibers. Amer. J. Physiol. **183**: 671, 1955.

331. WEIDMANN, S.: Elektrophysiologie der Herzmuskelfaser. Huber, Bern, 1956.

332. WEIDMANN, S.: Die funktionelle Bedeutung der Glanzstreifen im Myocard. Helv. physiol. acta **19**: C35–36, 1961.

333. WEST, G. B.: Quantitative studies of adrenaline and noradrenaline. J. Physiol. **106**: 418–425, 1947.

334. WEST, T. C.: Personal communication cited in reference 125, p. 118, *ibid.*

335. WEST, T. C.: Ultramicroelectrode recording from the cardiac pacemaker. J. Pharmacol. **115**: 283–290, 1955.

336. WEST, T. C.: Auricular cellular potentials: Ultramicroelectrode recording of drug effects on nodal and extranodal regions. Fed. Proc. **14**: 393, 1955.

337. WEST, T. C. AND AMORY, D. W.: Single fiber recording of the effects of quinidine at atrial and pacemaker sites in the isolated right atrium of the rabbit. J. Pharmacol. **130**: 183–193, 1960.

338. WEST, T. C., FALK, F. AND CERVONI, P.: Drug alteration of transmembrane potentials in atrial pacemaker cells. J. Pharmacol. **117**: 245–252, 1956.

339. WEST, T. C., TURNER, L. D. AND LOOMIS, T. A.: Effects of acetylcholine on mechanical and electrical properties of isolated rabbit auricle; their relationship to genesis of arrhythmias. J. Pharmacol. **111**: 475–482, 1954.

339a. WILBRANDT, W., AND ROSENBERG, T.: The concept of carrier transport and its corollaries in pharmacology. Pharmacol. Rev. **13**: 110–183, 1961.

340. WILBURNE, M., SURTSHIN, A., RODBARD, S. AND KATZ, L. N.: Inhibition of paroxysmal ventricular tachycardia by atropine. Amer. Heart J. **34**: 860–870, 1947.

341. WINBURY, M. M. AND ALWORTH, B. L.: Suppression of experimental atrial arrhythmias by several antihistamines. Arch. int. Pharmacodyn. **122**: 318–322, 1959.

342. WINBURY, M. M. AND HEMMER, M. L.: Action of quinidine, procaine amide and other compounds on experimental atrial and ventricular arrhythmias in the dog. J. Pharmacol. **113**: 402–413, 1955.

343. WINKLER, A. W., HOFF, H. E. AND SMITH, P. K.: Electrocardiographic changes and concentration of potassium in serum following intravenous injection of potassium chloride. Amer. J. Physiol. **124**: 478–483, 1938.

344. WINKLER, A. W., HOFF, H. E. AND SMITH, P. K.: Electrocardiographic changes and concentration of calcium in serum following intravenous injection of calcium chloride. J. Physiol. **125**: 162–171, 1939.

345. WOODBURY, J. W.: Time dependent conductance changes in cardiac muscle. In: Biophysics of Physiological and Pharmacological Actions, ed. by A. M. Shanes, pp. 501–527. Pergamon Press, New York, 1961.

346. WOODBURY, J. W.: Cellular electrophysiology of the heart. In: Handbook of Physiology, section 2, vol. 1, Circulation, ed. by W. F. Hamilton and P. Dow, pp. 237–286. American Physiological Society, Washington, D. C., 1962.

347. WOODBURY, J. W. AND CRILL, W. E.: On the problem of impulse conduction in the atrium. In: Nervous Inhibition, Proc. 2nd Friday Harbour Symposium, University of Washington, Seattle, ed. by E. Florey. Pergamon Press, Oxford, 1961.

348. WOODBURY, J. W. 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.

349. WOODBURY, L. A., WOODBURY, J. W. AND HECHT, H. H.: Membrane resting and action potentials from single cardiac muscle fibers. Circulation **1**: 264–266, 1950.

350. WOSKE, H., BELFORD, J., FASTIER, F. N. AND BROOKS, C.McC.: The effect of procaine amide on excitability, refractoriness and conduction in the mammalian heart. J. Pharmacol. **107**: 134–140, 1953.

351. WOSKE, H., FASTIER, F. N., BELFORD, J. AND BROOKS, C.McC.: The effect of induced failure and digitalization on the excitability and rhythmicity of the dog heart. J. Pharmacol. **110**: 215–220, 1954.

352. YELNOSKY, J. AND MORTIMER, L. C.: A brief study of the sympathomimetic cardiovascular effects of bretylium. Arch. int. Pharmacodyn. **130**: 200, 1961.