The effect of changes in heart rate on the refractory period of the heart

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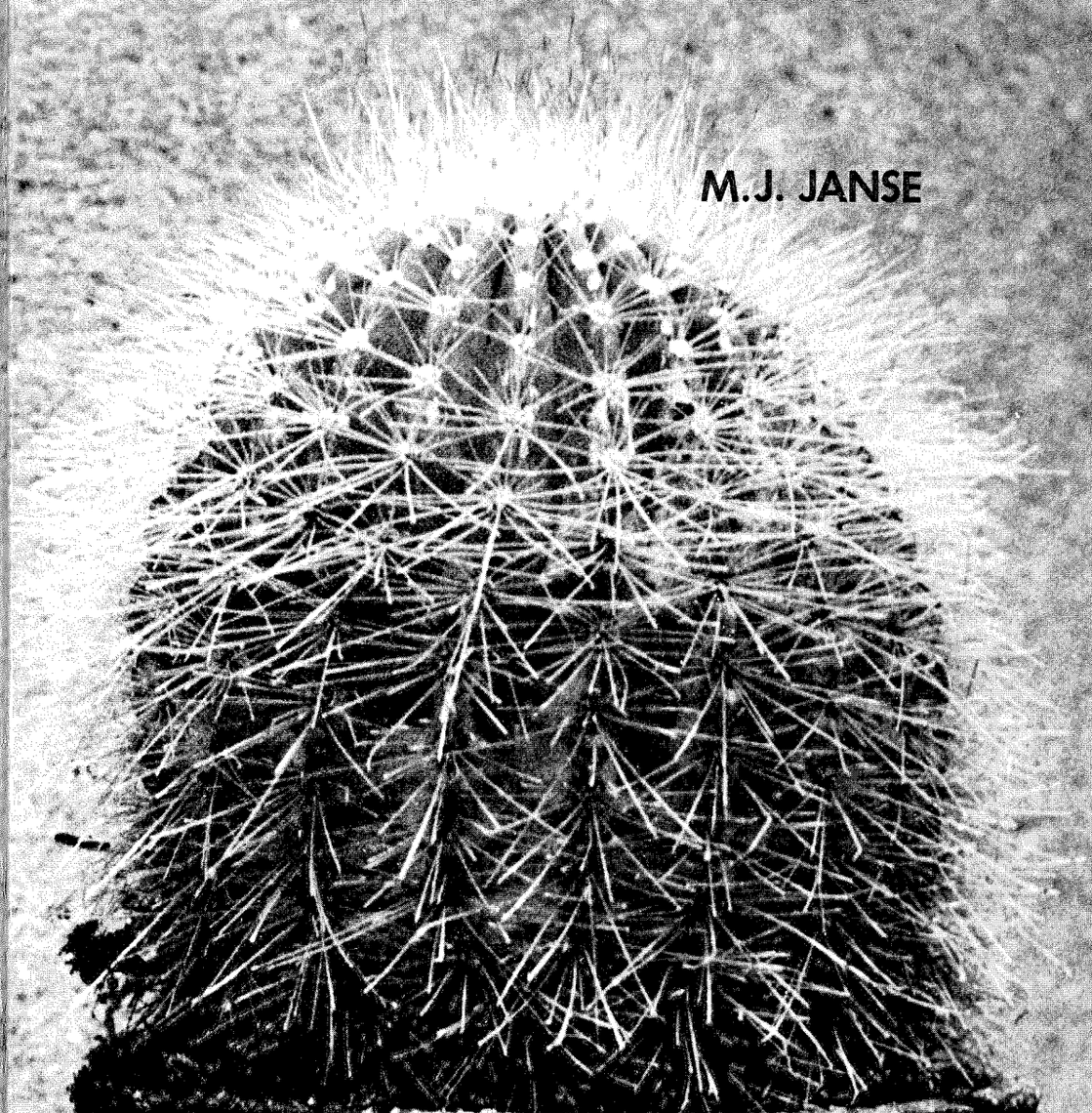

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ACADEMISCH PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE UNIVERSITEIT VAN AMSTERDAM, OP GEZAG VAN DE RECTOR MAGNIFICUS, Mr. A. D. BELINFANTE, HOOGLERAAR IN DE FACULTEIT DER RECHTSGELEERDHEID, IN HET OPENBAAR TE VERDEDIGEN IN DE AULA DER UNIVERSITEIT (TIJDELIJK IN DE LUTHERSE KERK, INGANG SINGEL 411, HOEK SPUI) OP DONDERDAG 18 MAART 1971 DES NAMIDDAGS TE 5 UUR

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voor Josje, Joris en Tom

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CHAPTER I

INTRODUCTION

One of the characteristic properties of cardiac tissue is that during a large part of the cardiac cycle it is not responsive to stimuli. This long refractory period can be regarded as one of the mechanisms by which the heart is protected to some degree from rhythm disturbances like excessively rapid rates or ventricular fibrillation. In fact, if the refractory period of the ventricles was of constantly long duration, these arrhythmias could not occur. However, it has been known for a long time that the duration of the refractory period of heart muscle is not constant, but is influenced to a large extent by heart rate, the refractory period being short at a rapid rate and long when the heart beats slowly. At the fastest possible heart rate, the interval between successive activations is actually much shorter than the refractory period at slow rates.
This suggests that following the change in rate there is a transient period during which the refractory period gradually changes, thus permitting the heart to be driven at progressively faster rates. The first purpose of this study was to investigate the time course of the change in duration of the refractory period following sudden changes in heart rate, and to measure the beat to beat changes in refractoriness that occur during these alterations in rate. This seemed all the more desirable since clinical experience shows that sudden acceleration of the heart rate, such as the occurrence of multiple premature beats, or the initiation of paired stimulation, can sometimes induce serious arrhythmias e.g. ventricular fibrillation. This suggests that the electrical stability of the heart is upset by sudden changes in rhythm. Knowledge of the transient changes in refractoriness could lead to a better understanding of the mechanisms leading to ventricular fibrillation. In recent years there has emerged the concept that asynchronism in recovery of excitability in closely adjacent areas of the heart may favour the occurrence of ventricular fibrillation by creating local re-entry pathways. Thus, the second purpose of this investigation was to study the refractory period of both the myocardium at multiple sites and of the specialized conduction system during the initial stages of sudden changes in heart rate.

REVIEW OF THE LITERATURE

Introduction

The general concept that the heart is not responsive to stimuli during a certain period of the cardiac cycle is quite old.
According to Hoff [52], Fontana in 1785 was the first to suggest that at the

end of a contraction the heart cannot contract immediately again because it needs time to recover its normal irritability. Objective studies about this irresponsive period and its relation to the cardiac cycle became possible when graphic methods to record the activity of the heart were introduced. In 1867 Marey [74] recorded the contractions of a spontaneously beating frog heart on a kymograph. He stimulated the heart with single break shocks from an induction coil, applying the stimuli at random throughout the cardiac cycle. Marey found that stimuli applied to the heart early in systole failed to elicit a contraction. Increasing the interval between the onset of systole and moment of stimulation resulted in a contraction which appeared after a long latency. Stimulation in diastole always caused a contraction with minimal latency. Marey concluded that the heart was inexcitable during the initial part of systole and he called this part of the cycle the refractory period. The main characteristics of cardiac excitability were well known around the beginning of this century. The absolute refractory period, during which the heart is not excitable, occupies most of systole; then follows a period during which the heart gradually recovers its excitability and will respond to strong shocks only, the relative refractory period. In diastole the heart regains its normal excitability and keeps it until the next contraction [13, 78, 105]. The development of techniques to record the electrical activity of the heart [11, 39] resulted in studies which emphasized the relationships between the refractory period and the electrical phenomena rather than the contractile process. As early as 1879, Burdon-Sanderson and Page [10] showed the relationship of the refractory period to the excitatory process. In 1912 Trendelenburg [116] noted the relationship between the duration of the refractory period and the duration of the monophasic current of injury, recorded between an electrode placed on an injured part of the heart and an electrode located on the intact surface of the heart. He found that strong stimuli could elicit propagated responses in the frog heart before the monophasic current of injury was completed. In 1913 Mines [85, 86] related the duration of the refractory phase to the length of the QT interval of the electrocardiogram. Further investigation on cardiac excitability depended on the development of instrumentation techniques, which made it possible on the one hand to apply stimuli of variable strength and duration at exactly defined moments in the cardiac cycle, and on the other hand to record the electrical activity of the heart with accuracy.
By applying current pulses of fixed shape and duration at various points in the cardiac cycle, and determining for each point in the cycle the minimal intensity (threshold value) necessary to evoke a propagated response, so-called strength-interval curves are obtained. At the end of the absolute refractory period, monopolar cathodal stimuli of 10 to 20 times diastolic threshold strength will evoke a propagated response. During the relative refractory period threshold values decrease rapidly to a low value which remains constant during diastole. These strength-interval curves accurately depict the time course of the recovery of excitability as a function of the cardiac cycle [9, 23]. The introduction of the microelectrode [72] made possible the recording of the potential difference which exists across the

membranes of single cells. The so-called resting potential during the diastole is of the order of 60-90 mV, the inside of the membrane being negative with respect to the outer surface. Upon excitation this potential difference is quickly lost, the membrane is depolarized and even becomes opposite in sign, the interior of the cell becoming 20-40 mV positive. The initial part of the action potential is called phase 0, or the upstroke. Repolarization starts immediately after phase 0, the potential difference tending to its original value. At first repolarization is rapid (phase 1) and then slows down, resulting in a plateau during which the transmembrane potential remains around the 0 mV level and declines but slowly (phase 2). In phase 3, repolarization proceeds rapidly until the resting potential is reached. The diastolic period is called phase 4.

Modern concepts of cardiac excitability

As was first shown for skeletal muscle [66], a threshold stimulus initiates an action potential by depolarizing the cell membrane to a critical level, the threshold potential. When the stimulus has lowered the membrane potential to this threshold level, the depolarization becomes selfsustaining and the upstroke of the action potential is inscribed. Subliminal stimuli which do not carry the membrane potential to the threshold level cause only so-called local responses: a small, "active" depolarization, superimposed on the "passive" displacement of the membrane potential by the stimulating current. These local responses are not propagated; they are sometimes called graded responses because their amplitude can vary with the strength of the stimulus [67]. A normal action potential propagates because it depolarizes adjacent cells to their threshold potential. The ability of the action potential to excite adjacent cells is to a large extent determined by its amplitude and the maximal rate of rise of the upstroke (dV/dt) [112]. The greater the amplitude and maximal rate of rise of the action potential, the quicker neighbouring cells will be depolarized and the greater the area which will be depolarized to threshold level.
The amplitude and maximal rate of rise of the action potential can be said to be parameters of the stimulating efficacy of the action potential, to be compared with magnitude and rate of rise of a stimulating current, and they determine largely the conduction velocity. As was first shown by Weidmann [121], and later confirmed by others [107], the amplitude and rate of rise of the action potential are dependent on the level of the membrane potential at the moment of excitation. Weidmann changed the resting potential of Purkinje fibers by electrical currents and elicited action potentials at different levels of membrane potentials.
At levels lower than approximately - 50 mV application of a depolarizing stimulus usually does not result in an active response (one speaks of a "low" membrane potential when the potential difference across the membrane is small, i.e. when the cell is partially depolarized; the minus sign refers to the interior of the cell). As the level of the membrane potential increases, the maximal rate of rise and the amplitude of the response

also increase, until a near maximal response is generated at levels about - 90 mV (it should be added here that this relationship does not hold for fibers of the sino-atrial and atrio-ventricular node). Hoffman and co-workers use the term "membrane responsiveness" to indicate the relationship between the level of membrane potential at the time of excitation and response [5, 60, 110]. They also emphasize that a description of cardiac excitability only in terms of stimulus requirement gives an incomplete picture. During the latter phase of repolarization the level of the membrane potential changes parallel with changes in stimulus requirement, but also with changes in the resulting response. Bearing this in mind we will now describe the different periods of cardiac refractoriness following the terminology proposed by Hoffman and Cranefield [56], which was later slightly modified [61].

Terminology

Beginning at the moment of the upstroke of the action potential the following periods can be distinguished:

1) The absolute refractory period during which no active response can be obtained by electrical stimulation.
2) A short period during which local responses may be obtained by strong stimuli. Since these responses are not propagated the heart is still effectively refractory. As soon as propagated responses can be obtained the effective refractory period ends (the effective refractory period is identical to the "old" absolute refractory period). According to Weidmann [121], the effective refractory period of Purkinje fibers ends when the membrane has repolarized to the level of - 59 mV. Hoffman, Kao and Suckling [59] found for Purkinje fibers that propagated responses became obtainable at potential levels of - 58 to - 65 mV, and for papillary muscle fibers at levels of - 62 to - 67 mV.
3) The relative refractory period begins at the end of the effective refractory period and ends when the threshold of the stimulus reaches the same value as found during phase 4. At the end of the relative refractory period repolarization is not yet complete. The level of membrane potential is lower than in phase 4 and therefore the response obtained by a stimulus of diastolic threshold strength is not normal with respect to its amplitude and rate of rise. Throughout the relative refractory period there is an increased stimulus-response latency. Three different mechanisms are responsible for this longer latency:
   a) Upon stimulation a graded response develops which needs time to rise to an effective level from which the action potential arises [67]. This especially occurs early in the relative refractory period.
   b) A threshold stimulus during the relative refractory period may elicit a local response in its immediate vicinity, while the propagated action potential arises from a graded response at a more distal site [28].
   c) Because the level of membrane potential is low at the moment of excitation, the resulting action potential has an amplitude and maximal rate of rise which are lower than normal. Therefore its efficacy in stimulating adjacent cells is reduced and propagation will be slow. This factor plays a role when the response is recorded some distance from the stimulus site.

4) The supernormal period begins at the end of the relative refractory period. Stimulus requirement is less than during phase 4, probably because the level of the membrane potential is closer to the level of the threshold potential at the time than it is during phase 4 [122]. Because repolarization is still not complete, the action potentials obtained in this period are not quite normal [56]. Experimental workers do not quite agree about the duration of the supernormal period. Weidmann [122] showed that for Purkinje fibers the supernormal period is short and ends when repolarization is completed. Other studies however indicate a much longer duration for the supernormal period: Brooks et al [9] found a period lasting from 50 to 200 msec, in which thresholds were 5 to 15% lower than later in the cycle; Hoff and Nahum [53] found for the cat's ventricle a supernormal period lasting from 40 to 100 msec; Van Dam, Hoffman and Stuckey [27] found in the specialized conduction system of the dog's heart "a long period of supernormality with thresholds of 10 to 15% below the diastolic value"; van Dam [23] found in some experiments a supernormal period lasting about 60 msec, following the relative refractory period during which thresholds were at the average 77% of the diastolic threshold. So far, no explanation for the long-lasting period of supernormality has been given.
5) When repolarization is complete both stimulus requirement and the resulting response are equal to the values found throughout phase 4. This is called the end of the full recovery time.

Some experimental results do not quite fit in this scheme. Thus, Van Dam [23] showed that at the time thresholds are 1.2 to 1.5 times diastolic threshold, the stimulus-response latency is equal to the diastolic value. It would seem that the response, elicited prior to completion of repolarization, was normal with respect to its ability to excite adjacent cells. In agreement with these findings are the results of Childers et al [18], who demonstrated that there exists a supernormal phase of excitability in the specialized atrial fibers of Bachmann's bundle, but did not find any significant changes in maximum upstroke velocity of the action potential elicited in the supernormal period, although repolarization was not complete.

Steady state relationship between heart rate and the duration of the refractory period

It has been recognized that the duration of the electrical activity of the heart is a function of heart rate. Different parameters of the duration of electrical activity of the heart - the QT interval of the electrocardiogram, the duration of the transmembrane potential, the duration of the refractory period as determined by strength-interval curves - are all short at fast

heart rates and long when the heart beats slowly. Mines [86] in 1913 was the first to show that the QT interval in the electrocardiogram was short at fast rates and lengthened upon slowing of the heart frequency. In 1920 Fridericia [44] presented a formula in which the QT interval in the human ECG is proportional to the third root of the length of the cardiac cycle; whereas Bazett [4] in the same year said that it was proportional to the square root.
This relationship holds only for a restricted range of frequencies; especially for long cardiac cycles the QT interval according to these formulae tends to become much longer than is the case in reality.
Records of transmembrane potentials of single cardiac cells in many different species demonstrate that the duration of the action potential varies inversely with the heart rate (frog's ventricle: 128, 115, 15, 16; dog's papillary muscle: 115, 62, 92; cat's papillary muscle: 113, 115; rabbit's ventricular muscle: 46, 97; dog's false tendon: 92; trabeculae carnae human right ventricle: 114; intact human heart: 43).
Determinations of classical strength-interval curves [106, 23] and determinations of the refractory period by means of stimuli of a fixed intensity [80, 111] at various heart rates show that at higher rates the refractory period of the heart is shorter than at slower rates. Siebens et al [106] showed that although at faster rates the heart begins to recover its excitability earlier and earlier in the cycle, once recovery starts, it proceeds to completion at the same velocity. In agreement with these findings are the results of Hoffman and Suckling [62] and of Brady and Woodbury [8] who demonstrated that at high rates the duration of the transmembrane potential shortens but that the time course of repolarization is unchanged: the phases 3 of the action potentials at different heart rates were practically superimposable, the shortening was due only to a shortening of the plateau (phase 2). Records of transmembrane potentials of human ventricular fibers show the same super-imposability of the final phase of the action potential at different rates [43, 114]. Thus, both the shape of the strength-interval curve and the shape of phase 3 of the action potential are unaffected by changes in heart rate. Several attempts have been made to describe the relation between action potential duration and heart rate more accurately. Woodbury et al [128] found a linear relationship between the log of the reciprocal of the action potential duration and the log of the heart rate. Trautwein and Zink [115] stated that there is a linear relationship between the log of the heart rate and the log of the action potential duration, while Hoffman and Suckling [62] detected a linear relationship between heart rate and action potential duration. The formula which described the duration of the action potential as a function of heart rate accurately for the frog's ventricle is the one presented by Carmeliet [15]. This formula is: $A = A\infty (1 - e^{-\alpha T})$ in which A is the duration at the given rate, $A\infty$ the duration at an infinitely slow rate, T the interval between beats, α a constant and e the base of the natural logarithm. One consequence of this formula is that at slow rates the action potential duration reaches a plateau and does not increase appreciably upon further slowing of the heart rate. In those studies in which the action potential duration, or the

duration of the refractory period, have been determined at slow heart rates, the results of most studies are in good agreement with Carmeliets formula [15, 16, 97, 92, 80]. Only the findings of Gibbs and Johnson are different [46]. They found that the action potential of the rabbit ventricle has a maximal duration at rates ranging from 1 to 3 impulses per second. At both faster and slower rates the action potential duration decreases. Carmeliet and Lacquet [16] studied the influence of changes in ionic concentrations on the relationship between rate and action potential duration. They found that raising the external K concentration, lowering the external Na concentration, or raising the internal Na concentration all caused the action potential to shorten and that this shortening was more marked at higher rates. In other words, these changes in ionic concentrations not only changed the action potential duration at a given rate, but also changed the sensitivity of action potential duration to changes in rate. As has been pointed out by Hoffman and Cranefield [56] this implies that studies of factors which influence action potential duration or duration of the refractory period should be carried out at several different heart rates.

The adaptation of the refractory period to sudden changes in rate

The question whether the duration of the refractory period reaches a steady state immediately after a new frequency is initiated, or develops slowly over several beats, has not been solved. Mines [86] in 1913 observed that following a change in frequency the electrocardiogram attained a constant form after about 10 beats of the new rhythm. This suggests that some time is needed before an equilibrium is reached after a sudden change in rate. Observations by several authors support this view. Thus, Carmeliet [15] noted that a stable value for the duration of the action potential of the frog's ventricle was established only after 40 to 50 systoles of a new rate. Carmeliet and Boulpaep [17] showed that upon rhythmic stimulation after a period of quiescence, 3 to 5 minutes elapsed before the action potential duration reached a constant value; during the first few contractions the action potential shortened considerably, later the duration changed much more slowly. Brooks, Hoffman, Suckling and Orias [9] reported that upon increasing the driving rate the action potential shortens "slightly on each successive cycle and reaches a condition of equilibrium only after a considerable lapse of time". Nolasco and Dahlen [97] saw that the first action potentials of a new rate differed from the steady state action potentials, the difference in duration being greater as the change in rate was larger. Moore, Preston and Moe [92] found in some papillary muscle fibers of the dog's heart evidence of a gradual change in action potential duration after an increase in rate, while in other cells the duration reached a stable value at the very first beat. In the latter instance, action potential duration was determined only by the length of the immediately preceding cycle, and no cumulative effect of earlier cycles was apparent. The same conclusion was arrived at by Mendez, Gruhzit and Moe [80] who measured the refractory period of dog hearts after 1 premature beat and compared

the results with those obtained during regular driving of the heart with the same cycle length as the interval preceding the premature beat. In most instances they found no difference and therefore concluded that the refractory period is determined only by the length of the immediately preceding cycle. Thus, two opposing concepts emerge from the literature:

1. The majority of observations suggests that the action potential duration, and thus the duration of the refractory period, changes gradually following a change in heart rate. This means that the effect of a previous rate persists for some time, or, to quote Mines [86], that "the previous history of the tissue may influence its immediate behaviour".

2. According to the other concept, the "memory" of the heart extends no further than the immediately preceding cycle. This latter concept played an important role in theories and computer models devised to explain the mechanism of atrial fibrillation in which the formula $D = K\sqrt{C}$ was used to define at any given instant the refractory period (D) as a function of the length of the immediately preceding cycle (C), K being a constant [87, 90].

Alternation in action potential duration following a change in heart rate

Several studies report on an alternation in the duration of electrical activity of the heart following changes in heart frequency. In 1930 Pohl [100] published a paper containing a record of a series of monophasic currents of injury, recorded when a sequence of rapid stimuli was applied to the frog's ventricle (it is not clear whether the preparation was beating spontaneously or was quiescent prior to stimulation). The second action current has a short duration, the third is longer, and the fourth is shorter than the third and also shorter than the fifth. Thus, a sudden increase in rate resulted in an alternation in duration of the electrical activity of subsequent beats. Only after the sixth beat a stable duration was established. Pohl related the duration of these action currents to the length of the preceding "electrical diastole" and obtained a characteristic curve, showing that the shorter the diastole, the shorter the subsequent action current.
In 1954 Hoffman and Suckling [62], using microelectrodes to record transmembrane potentials from an isolated dog's papillary muscle, found the same phenomenon. The preparation was stimulated after a period of rest and the resulting action potentials exhibited a similar alternation in duration: the second action potential was shorter than the first, the third slightly longer than the second, etc. Nolasco and Dahlen [97] also found an alternation in action potential duration after an increase in rate.

Changes in the shape of the action potential following changes in rhythm

As was mentioned earlier, in steady states the shape of the strength-interval curves and time course of the final phase of repolarization are identical at different rates [62, 114, 106, 8]. However, in the experiments of Hoffman and Suckling [62] slight changes in action potential configuration occurred when interpolated extrasystoles were introduced: The plateau of the extra action potential was slightly prolonged, the slope of the final repolarization phase was increased.
The subsequent regularly driven beat (which can be considered as the second beat of a new faster rate) was even more altered, repolarization starting earlier and progressing more gradually to completion. Edmands, Greenspan and Fish [38] stated that the direct relationship between the preceding diastolic interval and duration of phase 2 of the subsequent action potential exists only when the heart is driven at a constant rate. They observed that an action potential following a suddenly shortened cycle showed a prolonged phase 2 and a more precipitous phase 3. Conversely, a suddenly prolonged cycle was followed by an action potential with a shortened phase 2 and a prolonged phase 3. According to these authors total action potential duration remained essentially unchanged in either instance. The same authors described the same changes in action potential configuration after sudden changes in cycle length for fibers of human papillary muscles [41]. Contrary to their statement however, it is evident from the figures in both papers, that changes in cycle length do influence total action potential duration, the action potential being longer after a long diastolic interval and shorter after a short preceding cycle. Moreover, in the case of an interpolated action potential there are two action potentials with abbreviated preceding cycles (the interpolated premature action potential and the subsequent regularly driven action potential). The action potential following the first shortened cycle does indeed show a prolonged phase 2 and a shortened phase 3, the next action potential however is not only shorter but its phase 2 is also shorter. Nevertheless changes in action potential configuration do occur in the first action potentials of a suddenly initiated faster rate, and conceivably the shape of the strength-interval curve might not be the same for premature beats (or the first beats of a new faster rate) as for regularly driven beats.
Lewartowski et al [71] found that changes in rate and rhythm induced striking changes in the configuration of the action potential of rabbit atria. They mention, however, that these changes could not be found in cardiac tissues of cat and dog.

CHAPTER II

METHODS

Operation techniques

Mongrel dogs of both sexes, weighing 15 to 20 kg, were anaesthetized with sodiumpentobarbital (30 mg/kg intravenously). Under artificial respiration a right-sided thoracotomy was performed. The pericardium was opened and a pericardial cradle was constructed. Because of the need to explore longer cycle lengths than provided by the sinus rhythm, a total atrio-ventricular block was produced. This was accomplished in the following way. The anterior wall of the right atrium was opened and a pencil-like electrode, insulated except at the tip, was introduced into the atrial cavity. A purse string suture, previously laid, prevented the outflow of blood. The electrode was manoeuvred in such a way until its tip was in contact with the atrio-ventricular node or with the bundle of His. This became apparent by the temporary occurrence of atrio-ventricular block when pressure was exerted on the electrode tip. The tissue under the electrode tip was then destroyed by electrocoagulation, resulting in permanent total A-V block. The idio-ventricular rhythm which was then established was slow, the interval between ventricular beats exceeding as a rule 1200 msec. For the in situ experiments, intramural electrodes for stimulation and recording were introduced into the left ventricular wall [32, 33, 34]. The thorax was then closed. Adequate control of heart and body temperature was provided by a D-C operated heating blanket wrapped around the animal and by covering the thorax with cotton pads soaked in warm saline. Temperatures in the pericardial cavity were frequently checked and varied from 37 to 38° Celsius.
In the experiments in which the heart was to be isolated and perfused, a ligature was laid around the ascending aorta well above the level of the aortic valve, and prior to isolation 2 cc of heparin was administered intravenously. The heart, together with parts of the ascending aorta, was removed and a teflon canula was inserted in the aorta.

The perfusion of the isolated heart

The technique to perfuse isolated mammalian hearts according to the Langendorff principle [68] was introduced in our department by Meijler [84]. The essence of this technique is to introduce a perfusion fluid under a pressure, about equal to the blood pressure, into the aorta. This causes the aortic valves to close so that the perfusion fluid is forced into the coronary arteries. The fluid leaves the heart via the coronary sinus and the Thebesian veins, most of which drain into the right ventricle.

The perfusion fluid

The perfusion fluid consisted of a Tyrode solution modified by Meijler [84] to which a suspension of bovine erythrocytes was added. The erythrocytes were prepared according to a method devised by Drs. A.F. Willebrands and F.L. Meijler. Fresh, citrated (20 vol% of a sodium citrate solution containing 3.8 g/100 ml), bovine blood is used as soon as possible after the bleeding of the animal. After filtration through nylon 37 μ gauze (400 - 37 - ASTM, Schleitz Seidengaze Fabrik A.G. Zürich) 15 l. of citrated blood is centrifuged in a De Laval blood separator, type BP/KR.
The machine is run at low speed (2500 r.p.m.) in order to reduce the number of leucocytes and thrombocytes in the preparation and to prevent damage of the erythrocytes. The separated erythrocytes are collected in 20 l. of a 10% NaCl solution while agitating the fluid (by careful mechanical stirring or with a stream of air) to suspend and wash the cells. After centrifuging again at 2500 r.p.m., the cells are washed twice more in the same way with 20 l. of fluid, the first time with 1% NaCl, the second time with perfusion fluid according to Meijler. After the last washing the separated erythrocytes are collected in bottles containing a solution of 10 g. of glucose in 3 l. perfusion fluid; 2 x 10^6 units of penicillin and 2 g. streptomycinsulfate are added and the volume made up to 10 l. with more perfusion fluid. The approximate composition of the suspension thus obtained is: erythrocyte count 8 to 12 x 10^6 per mm^3; leucocyte count $<1000/mm^3$; thrombocytes 1 to 2 x 10^4 per mm^3; haemoglobin 15-25 g/100 ml; haematocrit 50-65. The ion composition of supernatant is Na 150 meq/l, K 5 meq/l, Ca 2.5 meq/l, Cl 140 meq/l, HCO_3 16-18 meq/l, and glucose 350 mg/100 ml.
All proceedings are carried out in a cold room. The suspension keeps stable for 7 to 10 days, if stored at 0 to -4° C. Before use in the perfusion apparatus, 2 liters of well mixed suspension is diluted to 10 liters with perfusion fluid. The final composition of the perfusion fluid is:
Na 149.1 meq/l, Ca 2.6 meq/l, Mg 2.1 meq/l, K 4.7 meq/l, Cl 137.6 meq/l, HCO_3 20.2 meq/l, H_2PO_4 0.7 meq/l, glucose 2 g/l.
The final haemoglobin concentration is 3 to 5 g/100 ml. (These figures are not to be taken too literally. The solution is made by putting convenient numbers of grams of several salts into a large volume of distilled water. Obviously, slight variations in the concentrations of the ions may occur.)
Despite the obvious fact that this perfusate is a poor substitute for blood, the heart perfused with it remains fairly constant, as far as electrophysiological parameters are concerned, for a period of up to 6 hours. At the end of that period the heart deteriorates as becomes apparent by the occurrence of sinus tachycardia, partial or total A-V block, ectopic rhythms, sometimes even ventricular fibrillation, and a weakening of the contractions. Several factors present themselves as possible causes for the deterioration: marked oedema due to the lack of colloid osmotic pressure in the perfusion fluid; microthrombi in the small vessels, probably caused by small amounts of denatured protein (haemoglobin?); depletion of metabolic "stores".

The perfusion apparatus

To start the perfusion a roller pump pumps the perfusion fluid through the system; first to a heat exchanger (mark: Sarns, 60 ml priming volume) which brings the perfusate to a temperature of 38° C. Next the perfusate flows over a finger like, hollow glass rod, which is located in the centre of a double walled perspex cylinder. The perfusate flows as a thin film over the glass rod and is oxygenated on its passage, a mixture of O_2 and carbogen (95% O_2 and 5% CO_2) being introduced under controllable pressure to the cylinder. Water at 38° C, derived from a Lauda Thermostat warm water bath, circulates between the two walls of the cylinder and also in the interior of the glass rod. The cylinder serves as an oxygenator and as a reservoir. In order to keep the level of the fluid in the reservoir constant, a floater regulates the speed of the roller pump by a pressure transducer (Sanborn). The gas pressure, combined with the hydrostatic pressure of the fluid, constitute the perfusion pressure, equalling about 70 mm Hg. The perfusion fluid enters the aorta via a stop cock provided with 30 μ filters. The perfusion fluid which leaves the heart is collected and led again to the roller pump, and in this way a small volume (2-3 liter) can be recirculated for about 2 hours. The pH of the perfusate was frequently checked and was 7.35 ± 0.05.
The perfusion apparatus was designed by G.E. Freud, M.D., Mr. P. Brekelmans and Mr. G.F. Douma.

Perfusion with heparinized blood

In later experiments the isolated heart was perfused with blood obtained from a second dog. After induction of anaesthesia with sodiumpentobarbital and intubation of the trachea, cannulae were inserted into the femoral artery and vein of the second dog (referred to now as the "pump" dog), and an external arteriovenous shunt made by connecting the cannulae with tygon tubing. Heparin (2 ml) was administered intravenously to keep the shunt free of clot. The isolated heart was first washed with perfusion fluid before the cannula in the aorta was connected to the artery line of the "pump" dog. The aortic perfusion pressure was kept constant in the range of 80 to 100 mm Hg by controlled clamping of the femoral artery line if necessary. Blood from the heart was collected in a reservoir beneath it and returned to the femoral vein cannula by gravity. If necessary, extra blood or fluids to correct volume loss, or drugs such as heparin or sodiumpentobarbital, could be introduced by infusion into this venous line. The temperature of the "pump" dog was kept constant at 37 ± 0.5° C by a DC heating blanket. The advantages of using homologous fresh blood are that it eliminates the need to introduce oxygen into the perfusion circuit and greatly prolongs the time before oedema occurs. With this method the heart beats strongly for a period of up to 40 hours, and can be studied for as many hours as desired.

Recording and stimulation

Multi-electrodes of the type developed by Durrer [32, 33, 34] were used for stimulation and recording. These electrodes have a diameter of 0.9 mm, are 25 mm long and have a sharp point. They contain 10 platinum electrode terminals, each of 0.1 mm diameter, the inter-terminal distance being 2 mm. If the electrode needle is inserted perpendicularly into the left ventricular wall, terminals 1 and 10 are situated near the endocardium and epicardium respectively. The polarity of bipolar complexes was chosen to give an upward deflection for a positive signal. Thus, if excitation spreads from endocardium to epicardium, a bipolar complex, taken between, for example, terminals 5 and 6, is upward. The method of measuring com-

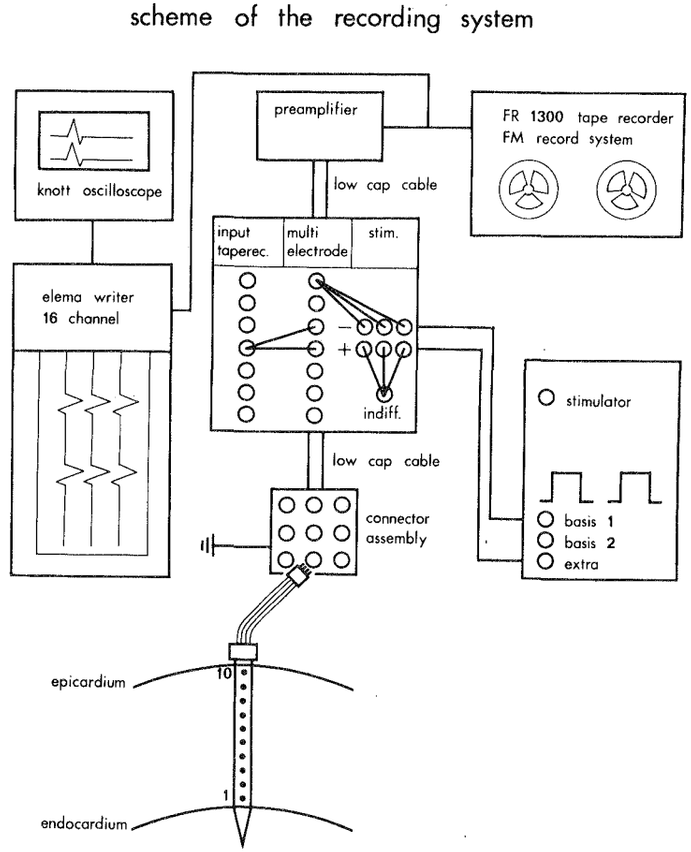

Fig. 1. Block diagram of the recording system. For explanation see text.

plexes and criteria for acceptance of tracings was adopted as outlined by Durrer and van der Tweel [35, 36, 37]. These electrodes do not damage the muscle in such a way that appreciable changes in excitation or excitability occur [23, 34]. Van Dam [23] has discussed in detail the use of these multi-electrodes for stimulation purposes.
Fig. 1 shows a schematic representation of the experimental set-up. Each terminal was connected through a connector assembly, in which up to 40 different multi-electrodes could be plugged, to a low capacitance cable and to a master switch. This master switch was designed and made by Mr. L. Schoo of the Laboratory of Medical Physics of the University of Amsterdam (Director: Prof. Dr. L.H. van der Tweel). It enabled us to connect any desired terminal with either the cathode of the stimulator, or with the positive or the negative input site of a recording channel. An indifferent electrode inserted in the shoulder of the animal, or attached to the root of the aorta of the isolated heart, was also connected to the master switch. From the master switch a low capacitance cable leads to preamplifiers, consisting of a modified DAS 100 system. Unipolar leads (taken between a single electrode terminal and the indifferent electrode), or bipolar leads (taken between two consecutive electrode terminals) were tape-recorded on a 14 channel FR 1300 Ampex physiological tape-recorder at a tape speed of 15 inches per second, and were also monitored on a 8 channel Knott oscilloscope and printed on line by a 16 channel Elema ink writer. For the purpose of time measurements of the recorded complexes the tape was played back at a speed of 1 $^{7}/_{8}$ inch/sec. The complexes were printed out on the Elema writer, running at maximal speed, allowing a time resolution of 1 msec. Proper grounding of the object and of the recording and stimulating apparatus was made. The stimulator was designed and developed at the Laboratory of Medical Physics. It delivered rectangular current pulses of variable strength and duration which were separated from ground. Various stimulation patterns could be delivered; these will be discussed in the section Results.
For a description of the stimulator, see the addendum. In later experiments a new stimulator, which was even more versatile, was used. This stimulator was also designed in the Laboratory of Medical Physics [104].

Recording and stimulation of the specialized conduction system in the isolated heart

Apart from the fact that the Langendorff technique makes it possible to study the heart without nervous, humoral or haemodynamic influences, another advantage of this method lies in the accessibility of the interior of the heart. By cutting a small hole in the wall of the outflow tract of the right ventricle, the junction of the right bundle branch with the septal papillary muscle becomes visible. By tying off the cut vessels, shunts were prevented so that the rest of the heart would be perfused adequately. Electrodes of the type described by Hoffman et al [57] were placed on the right bundle in the following way: Parallel to the bundle two stitches were

made with atraumatic nylon. The threads were passed through holes in the electrode and then through a small rubber tube. By gently pushing the rubber tube, the electrode was brought into contact with the bundle; care was taken to exert minimal pressure. The electrode was held in place by clamping the rubber tube with haemostats. Generally two or more terminals were in contact with the bundle branch, as could be judged by the appearance of the typical 'Purkinje spike" in the unipolar electrogram (the indifferent electrode being connected to the root of the aorta), which preceded the larger deflection due to the activation of the septal myocardium. In the same way an electrode was placed on the posterior division of the left bundle branch, after cutting away most of the left atrium and the mitral valve. An electrode terminal was selected for unipolar cathodal stimulation (the anode being a large clip attached to the root of the aorta) and bipolar leads were recorded from the other terminals in contact with the bundle branch to judge the success of the stimuli. Multi-electrode needles were inserted in the right anterior papillary muscle, in the left posterior papillary muscle and in the free wall of the left ventricle.

Determination of the refractory period

The stimuli used in our experiments to determine the duration of the refractory period were monopolar, cathodal, square wave current pulses of 1 or 2 msec duration. These pulses were applied to the heart by an electrode terminal of 0.1 mm diameter, the anode being a large needle inserted in the shoulder of the animal or a large clip attached to the root of the aorta.

Monopolar stimulation

It is known that the heart can be stimulated by both cathodal and anodal stimuli [23, 21]. Usually, threshold values for anodal stimuli are higher than for cathodal stimuli. However, during a phase in the relative refractory period threshold values for anodal stimuli are lower than for cathodal stimuli. If strong cathodal stimuli are applied to the heart in the relative refractory period, the anode being located in the shoulder of the animal, it is conceivable that the heart will be stimulated at some unknown site, at a "virtual anode". Cranefield, Hoffman and Siebens [21] stated that "the use of a so-called indifferent electrode placed on the body of the animal has been found to be completely inadequate since the current density is often sufficient to stimulate at some unknown point of junction between body and heart. This is especially likely when strong shocks are employed during the relative refractory period". These authors therefore recommended the use of a special bipolar stimulating electrode, one terminal being much larger than the other. The current density at the smaller electrode would then be about 20 times larger than at the large electrode, so that one could be certain to stimulate the heart at all times at the site of the small electrode terminal. We have not followed this advice since we are certain that in our method of stimulation the heart was excited at the site of the

stimulating electrode for the following reasons:
1) Since the diameter of the stimulating electrode terminal was small (0.1 mm) one can expect current density to be maximal at the site of the electrode.
2) We rarely used strong stimuli. As will be explained later, test pulses of 1.5 times diastolic threshold strength were usually used.
3) The succes of a test pulse was determined by the appearance of a propagated response at an electrode terminal close to the stimulating electrode. From bipolar leads, taken between the two electrode-terminals located 2 and 4 mm from the stimulating electrode, one could easily distinguish between the stimulus artefact and the intrinsic deflection caused by the activation front moving under the electrodes. Thus, it could be established that responses elicited by a test pulse of 1.5 times diastolic threshold strength in the relative refractory period arrived at the electrode terminal 2 mm from the stimulating electrode maximally 5 msec later than a basic response. Moreover, the shape of the bipolar complex was always the same, indicating that the direction of the activation was constant, i.e. all responses elicited in the relative refractory period originated at, or very close to, the stimulating electrode.

Cathodal stimulation
Cathodal stimuli excite cardiac cells by depolarizing them to a critical level, the threshold potential. In essentially the same way, the cardiac action potential serves as a stimulus to quiescent adjacent cells, namely by providing depolarizing current by which the adjacent cells are passively depolarized to their threshold potential. Once the threshold level is reached, regenerative depolarization occurs and the action potential emerges. Cathodal stimulation therefore seems the most "physiological" way of stimulation.

The intensity of the stimulus
In general, it can be stated that three factors, which are interrelated, decide whether at a given instant excitation and propagation will take place: 1) the strength of the stimulus, i.e. the amplitude and rate of rise of the action potential; 2) the level of excitability of the cell about to be excited, determined largely by the level of the membrane potential of the cell; and 3) the quality of the response, i.e. the amplitude and rate of rise of the action potential of the newly excited cell. In our study, we can only give an estimate of the second factor, namely the level of excitability at a given instant, or rather, we wanted to know at what point in the cardiac cycle a certain level of excitability existed. This level should be "physiologically" meaningful, i.e. it should be a critical level of excitability at which a normal action potential would just be able to elicit a propagated response. This level of excitability would mark the end of the "physiologically effective refractory period". Hoffman, Kao and Suckling [59] ingeniously estimated the "strength" of a normal action potential. The duration of the action potential of a Purkinje fiber is much longer than the duration of an action potential of a papillary muscle fiber. A preparation, containing both elements, was driven by regular stimuli applied to the Purkinje fibers. By a well-timed premature stimulus, applied to the papillary muscle, a propagated action potential of a papillary muscle fiber could be used to stimulate a Purkinje fiber long before it had completed its repolarization. It appeared that the earliest moment at which a propagated muscle action potential could elicit a propagated response in a Purkinje fiber, coincided with the moment the membrane potential of the Purkinje fiber was at -58 to -65 mV. Cathodal stimuli applied directly to the Purkinje fiber at the same moment and at the same level of membrane potential had to be 4.5 to 6.5 times diastolic threshold strength to evoke a propagated response in the Purkinje fibers. This suggested that the propagated action potential has a safety factor of at least a similar magnitude, i.e. a normal action potential would have an "intensity" of 4.5 to 6.5 times diastolic threshold. Recent experiments by Mendez et al [81] cast some doubts about the validity of these estimates. Premature excitation of the papillary muscle can be used to assess the physiologically effective refractory period of Purkinje cells only when there exists an abrupt change in duration of action potentials at the junction of muscle fibers and Purkinje fibers.
The results of Mendez et al show however, that there is a gradual change in action potential duration at the Purkinje-muscle junction and that premature muscle action potentials, depending on their timing, can be blocked at many different levels. Nevertheless it seems obvious that the use of excessive stimulus strength to determine the physiologically effective refractory period is meaningless. We chose a stimulus intensity of 1.5 times diastolic threshold strength which seems well below the safety factor of a normal action potential. Unless otherwise indicated, we will refer to the refractory period as that part of the cardiac cycle, during which monopolar cathodal stimuli of 1 to 2 msec duration and 1.5 times diastolic threshold are unable to elicit a propagated response. This "refractory period" will probably be somewhat longer than the physiologically refractory period. Another reason for chosing a stimulus intensity of 1.5 times diastolic threshold was that, according to Van Dam and Durrer [24], the refractory period determined with this intensity also marks the time at which conduction velocity is restored to its normal value, the stimulus-response latency being the same as that of a basic beat.

The duration of the stimulus
Diastolic threshold determinations of stimuli with different durations, resulting in so called strength-duration curves, show that thresholds for stimuli of short duration are higher than for stimuli of long duration [9]. However the shorter pulses are more effective in terms of microcoulomb requirement. This is probably due to accomodation occurring during long stimuli [9]. In our experiments a stimulus duration of 1 or 2 msec has been chosen. In commercial pacemakers a pulse duration of 2 msec is frequently used, so that this choice gives results possibly relevant to the clinical situation in which the heart is artificially paced. Furthermore, 1 or 2 msec is close to the deflection point of the strength-duration curve for the dog's ventricle [9], so that by using these pulses extremes are avoided.

They are short enough to prevent much accomodation, yet they are long enough to be able to stimulate with relatively small currents. In our experiments, with electrode diameters of 0.1 mm, diastolic thresholds were in the order of 10 to 50 micro-ampères.

CHAPTER III

RESULTS - VENTRICULAR MYOCARDIUM

Steady state refractory period

In initial experiments* the relationship between heart rate and strength-interval curves was established. The refractory period was considered to be in a steady state when the heart had been driven at the given frequency for at least 500 beats. Test stimuli were then given after every 10th beat at a fixed interval and the thresholds were determined by gradually increasing stimulus strength, until the minimal intensity was found at which the heart responded. As shown in fig. 2, the duration of the effective refrac-

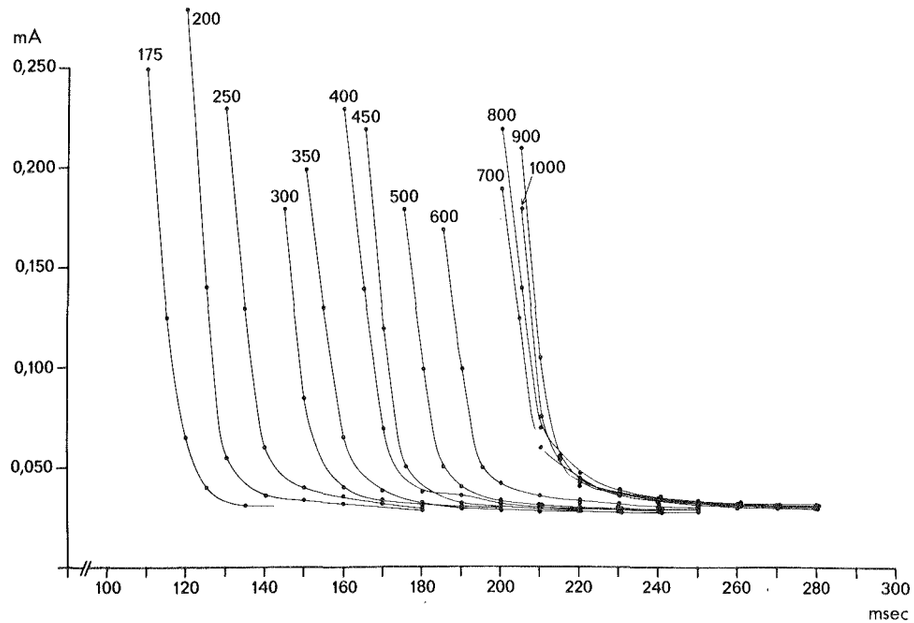

Fig. 2. Steady state strength-interval curves for cathodal stimuli at different driving frequencies. Abscissa: interval between basic driving stimulus and test stimulus in msec. Ordinate: strength of test stimulus in mA. The basic cycle length of each driving frequency is indicated at each strength-interval curve. Note the similarity in shape of the different curves.

* Many of the experiments, of which the results are to be described, were performed together with A.B.M. van der Steen, D.V.M., and, or, with R.Th. Van Dam, M.D., Ph.D.

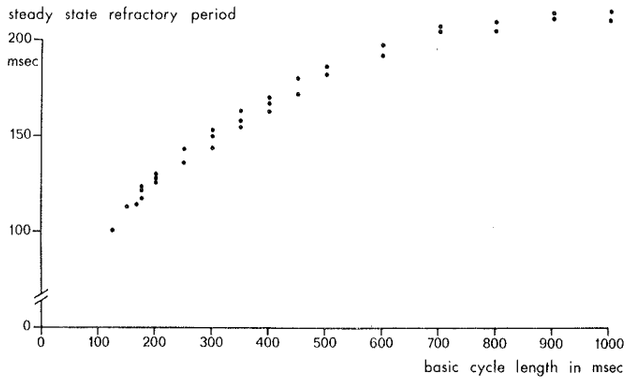

Fig. 3. Steady state relationship between basic cycle length of driving frequency and duration of the refractory period in three dogs.

tory period differed for the various frequencies; the shape of the strength interval curves for cathodal stimuli, however, was similar for every frequency tested. Therefore, in further experiments we determined the duration of the refractory period by measuring the shortest interval preceding a test pulse at 1.5 times diastolic threshold intensity, that did evoke a propagated response. Fig. 3 shows the data acquired in three dogs. At frequencies with a basic cycle length of 800 msec and greater, the duration of the refractory period reaches a plateau. At the fastest possible driving rate (basic cycle 150 msec) the refractory period is about 100 msec shorter than at the slowest rate. At still faster rates ventricular fibrillation invariably occurs.

Shortening of the refractory period

A sudden increase in driving rate from 100/minute (600 msec stimulus interval) to 200/minute (300 msec stimulus interval) shortens the refractory period by approximately 50 msec. In preliminary experiments we observed that a maximal shortening does not occur in the first beats of a new rate, but develops gradually. To determine the time course of this shortening ("on effect") the following procedure was applied. Immediately after changing the driving frequency from the slower to the faster rate test pulses were applied continuously after every second driving stimulus. Because of the properties of the stimulator it was easier to give the test pulse after every second driving stimulus instead of after every basic stimulus. The intensity of the test pulse was 1.5 times the diastolic threshold strength. The interval between driving stimulus and test pulse was shorter than the steady state refractory period at the slow rate and longer than the steady state refractory period at the fast rate. The number of beats at the

fast rate was counted, necessary to shorten the refractory period to such an extent that the ventricle would follow the test pulse with this particular interval. At that point the refractory period of the ventricle was equal to, or slightly shorter than, the interval preceding the test pulse. After allowing the heart to return to a steady state at the slow rate for at least 500 beats, this procedure was repeated for another test interval. The shortest interval tested was equal to the steady state refractory period at the fast rate. A schematic representation of this method is seen in fig. 4. In this way a curve was obtained that relates the progressive shortening of the refractory period after a sudden increase in driving rate to the number of beats at the fast rate. Fig. 5 shows the results of experiments in three dogs in which the time course of shortening of the refractory period was investigated for the frequency jump of 600 to 300 msec basic cycle length. The differences between the two steady state refractory periods were 48, 50 and 51 msec. The interval preceding the test stimulus is expressed as a percentage of this difference. In the first 20 beats about 40 to 60% of the total shortening occurs; the final value is reached after 400 to 500 beats.

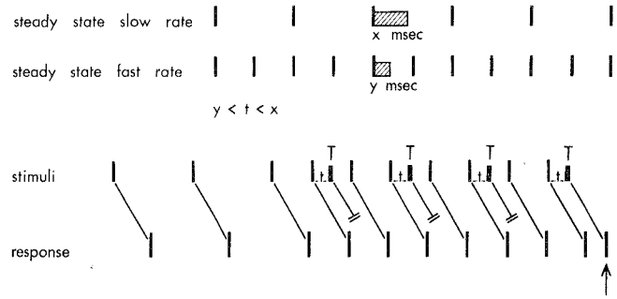

Fig. 4. Schematic representation of the stimulus pattern used to determine the time course of adaptation of the refractory period when the driving frequency is suddenly increased. Every second driving stimulus of the faster rate is followed by a test stimulus (T) after an interval of t msec. t is chosen to be shorter than the steady state refractory period of the slower rate (x), and longer than the steady state refractory period of the faster rate (y). Only after a certain number of beats of the faster rate, the heart responds to the test stimulus T. At that moment, the refractory period is equal to, or slightly shorter than, the interval t.

The "on" effect was investigated in other dogs after doubling the driving frequency for several basic driving rates of 150, 100, 75, 60 and 50/minute (equivalent to basic cycle lengths of 400, 600, 800, 1000 and 1200 msec). Essentially the same curves were obtained (fig. 6). This indicates that the rate of adaptation of the refractory period to a new frequency is dependent rather on the number of beats of the new rate than on the time during which the new rate exists.

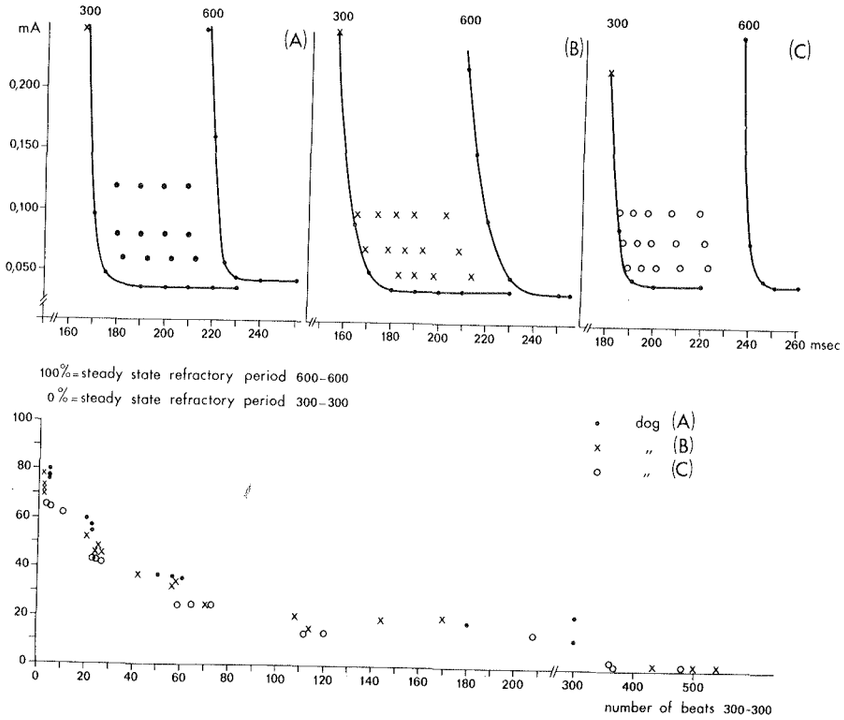

Fig. 5. Top: Steady-state strength-interval curves for two driving rates (600-msec and 300-msec basic cycle length) in three dogs (A, B and C). Abscissa: interval following the basic driving stimulus. Ordinate: intensity of a test stimulus of 1 msec. In the area between the ascending parts of both curves, test stimuli of three intensities and with different intervals, used for determining the progressive shortening of the refractory period following a sudden increase in driving rate from 600 to 300 msec basic cycle length ("on" effect), are indicated. Bottom: "On" effect: the number of beats of the faster rate after which the heart responds to a test pulse with chosen delay and strength. The interval of the test pulse is expressed as a percent of the difference between the steady-state refractory periods at the slow and fast rates. Note that 40% to 60% of total shortening of the refractory period is achieved after about 20 beats of the new rate, and that the steady-state value is reached after a few hundred beats.

Similar curves were found when the "on" effects for different frequency jumps were determined. Fig. 7 shows the time course of shortening of the refractory period when, departing from steady state frequencies of 700, 600, 500, 400, and 300 msec basic cycle length, the rate was augmented to 200 msec basic cycle length.

Control experiments were performed in three dogs, to explore the possible influence of the repeated application of subliminal test stimuli during the refractory period, on the rate of change in refractory period. After deter-

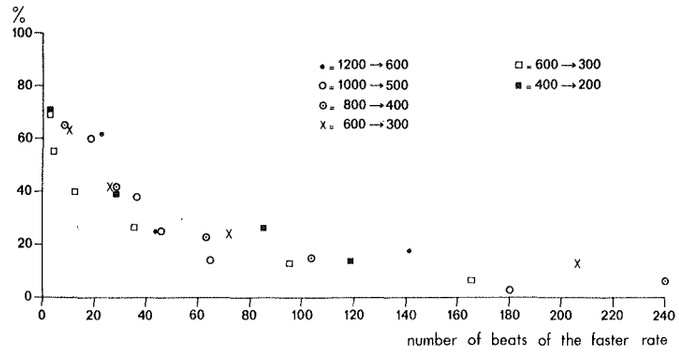

Fig. 6. "On" effect for doubling the driving frequency, starting from different frequencies. 100% = steady state refractory period at the slower rate (basic cycle length of 1200, 1000, 800, 600, or 400 msec). 0% = steady state refractory period at the faster rate (basic cycle length of 600, 500, 400, 300, or 200 msec).

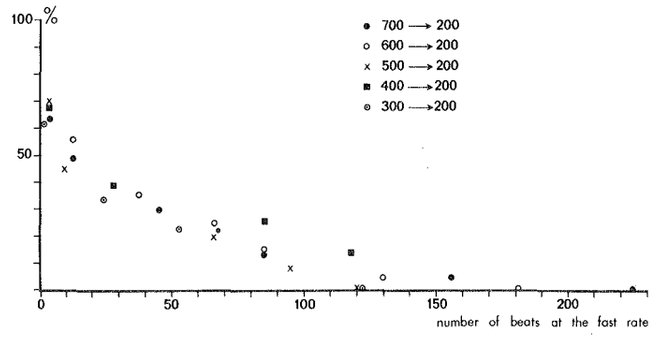

Fig. 7. "On" effect for different frequency jumps. 100% = steady state refractory period of the slower rate (basic cycle length of 700,600, 500, 400 or 300 msec). 0% = steady state refractory period of the fast rate (200 msec basic cycle length).

mining the "on" effect by giving the test stimulus repeatedly after every second basic driving stimulus of the fast rate until the heart responded to it, the "on" effect was determined in a different way. This time the test stimulus was applied only once after a selected number, n (ranging from 5 to 120), of beats of the fast rate. This number was chosen to be slightly lower than the number of beats determined for a given test interval with the first method. If the heart did not respond to the test pulse, it was brought back to the steady state at the slow rate and then driven for n + 2 beats at the fast rate, after which the test stimulus would be given once more. This was repeated with increments of two beats until the heart

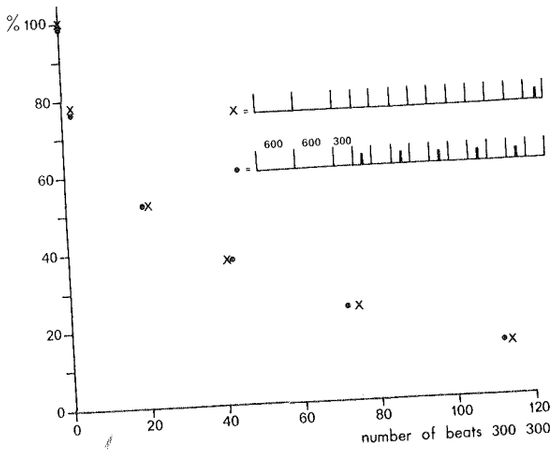

Fig. 8. Control experiment on the effect of repeated subliminal stimulation. Inset: stimulus pattern used for determining the "on" effect after a change in frequency from 600 msec to 300 msec basic cycle length. X: one test pulse follows a series of beats of the faster rate. Solid circles: test pulse applied after every second beat of the faster rate.

responded to the test pulse. In fig. 8 the results of one control experiment are shown; both curves are in good agreement. No important effects of the repeated application of subliminal stimuli was thus observed on the rate of change of the refractory period.

Lengthening of the refractory period

A similar stimulus pattern was used to determine the over-all time course of lengthening of the refractory period which occurs upon sudden decrease of the heart rate. Again, several test pulses were selected with a preceding interval longer than the steady state refractory period of the fast rate. Obviously, these test pulses could not be applied continuously during the slow drive until the heart ceased to respond, because the occurrence of premature beats elicited by those test pulses, would counteract the effect of the slower driving rate on the refractory period. Therefore a more time consuming procedure had to be followed in which the heart, departing from a steady state (300 msec basic interval), was driven at a slower rate (600 msec basic interval) for a number of beats after which the test stimulus was applied. If the heart still responded to the test stimulus, it was brought back to the steady state of the faster rate and then driven for a slightly larger number of beats at the slow rate before the same test stimulus was applied again. This sequence had to be repeated many times to find

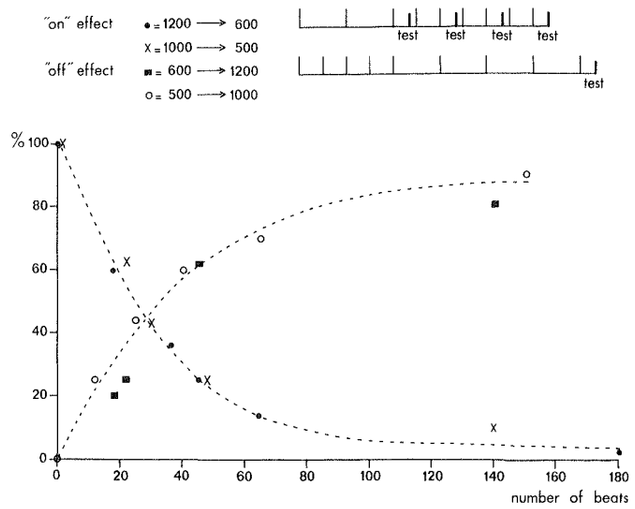

Fig. 9. "On"- and "Off"effects for 2 ranges. Interval of test pulse, expressed as percent of the difference between the steady state refractory periods of the slower rate (100%) and faster rate (0%), is plotted against number of beats of the new (fast or slow) rate. For the "off" effect the number of beats of the slower rate is indicated after which a test pulse is no longer followed by the heart. The curve indicates the progressive lengthening of the refractory period following a sudden change from a fast to a slow driving rate.

the number of beats at the slower rate, after which the refractory period would be lengthened to a point, where the heart would not respond to the test pulse. Fig. 9 shows the "on" and "off" curves for several frequency jumps (1200 ⟷ 600 and 1000 ⟷ 500 msec cycle length); it appears that the "off" effect is to a certain degree the mirror image of the "on" effect.

Influence of premature beats on the refractory period of subsequent beats

Having so far outlined the overall time course of the adaptation of the refractory period following sudden acceleration or deceleration of the heart, we set out to study the beat to beat variations in the refractory period during the first ten, or more, beats of a new rate.
Since in the foregoing paragraph it was shown that the refractory period is to a large extent determined by the "history of the heart", it is essential that, when measuring the refractory period at a given instant (for example after two successive short cycles), the heart has "forgotten" changes in rhythm that occurred previously. It would therefore be useful to know how long the effect of any number of successive short cycles persists after the original slower rate has been restored. Fig. 10 illustrates the experimental

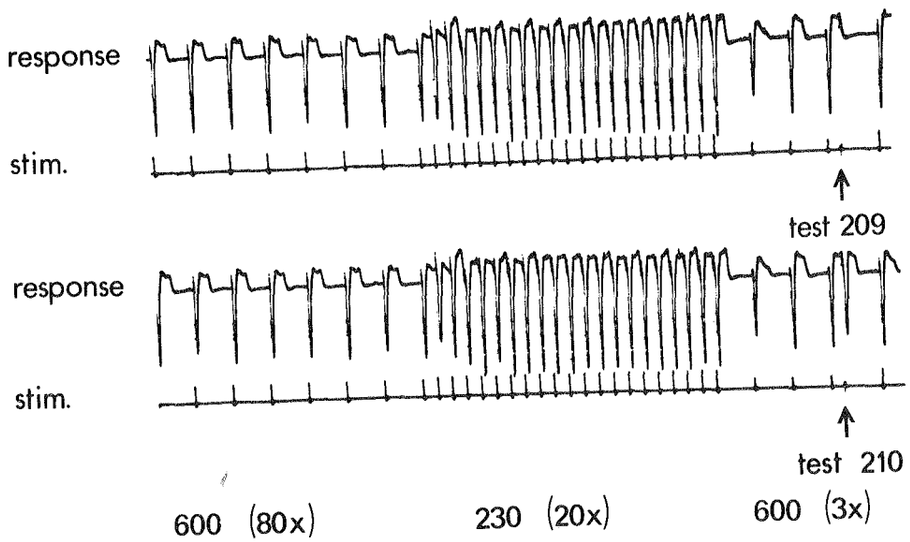

Fig. 10. Method to determine the return of the refractory period to its steady state level after a series of short cycles. The heart is driven for 80 beats at a slow rate (cycle length 600 msec), then for 20 beats at a fast rate (cycle length 230 msec), after which the slow rate is resumed. The third beat of the slow rate is followed by a test pulse, given after 209 msec. This test pulse has no effect (Top). When the sequence is repeated, a test pulse with an interval of 210 msec elicits a propagated response (Bottom).

procedure followed to determine the return to steady state conditions after a series of repetitive premature beats. The heart was driven at the basic rate (600 msec interval) for a large number of beats (80); then 20 stimuli with an interval of 230 msec were applied, after which the basic rate was resumed. The test pulse of 1.5 times diastolic threshold strength was in this case applied after the third basic beat following a series of beats at a rapid rate. No response was obtained when the interval of the test pulse was 209 msec. After repeating this stimulation pattern, and resetting the interval of the test pulse to 210 msec, the heart responded to the test pulse: the refractory period at that instance was therefore 210 msec.
Fig. 11 shows the rate of change in refractory period after several episodes of successive short cycles. Generally speaking, the refractory period of the first basic beat following the rapid impulses is already close to the steady state level, but in the following beats the refractory period shortens again, to return during the next beats gradually to the steady state value. The influence of one premature beat on the refractory period of subsequent basic beats can be detected for at most five beats. After for example 4 short cycles, about 12 basic beats are needed to restore the refractory period to the steady state level; after 10 short cycles it takes about 18 basic beats to do so; after 20 short cycles a stable value is reached after 30 to 40 basic beats.

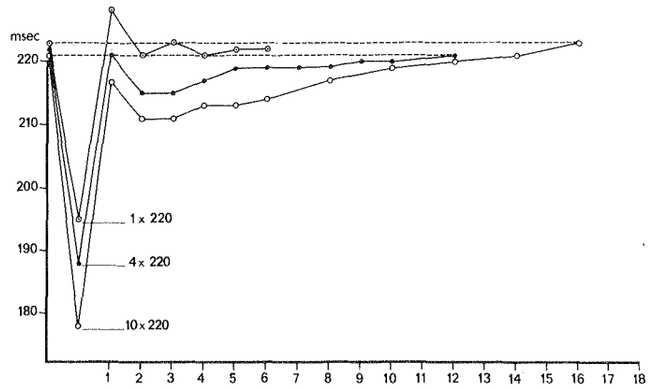

Fig. 11. Time course of the return of the refractory period to its steady state level following different episodes of short cycles, introduced during a regular driving frequency of 600 msec basic cycle length. Ordinate: refractory period in msec. Abscissa: number of basic beats following the episode of rapid beats. The dotted line indicates the level of the steady state refractory period.
1 x 220 = refractory period after one premature beat (preceding cycle length 220 msec.)
4 x 220 = refractory period after four repetitive beats with an interval of 220 msec.
10 x 220 = refractory period after 10 beats with an interval of 220 msec.

Interestingly, after one short cycle, the refractory period of the first basic beat is longer than the steady state value. Similar "overshoots" were also observed in other experiments. These results demonstrate clearly the cumulative effect of repetetive short cycles on the refractory period. In practical terms, it is important to realize that determinations of the refractory period after any number of short cycles is meaningful only, when the heart, in the time interval between subjection to a rapid drive, has had ample time to restore its equilibrium. As a rule, when the number of rapid impulses was 5 or less, we drove the heart for 10 to 20 basic beats before applying the rapid pulses again; 20 to 25 basic beats were interpolated between series of 6 to 10 successive short cycles; 30 to 40 basic beats for series of 10 to 20 short intervals.

Beat to beat changes in refractory period following a sudden increase in heart rate

Strength-interval curves

The stimulation pattern used to determine the refractory period after any number of beats of a new rate is shown in fig. 12. During a steady state rhythm, a certain number (ranging from 1 to 80) of beats of a new rate was introduced. The last of these beats was followed by a test pulse at a preset interval. When this test pulse evoked a response, the refractory period was evidently shorter than, or equal to, the interval of the test

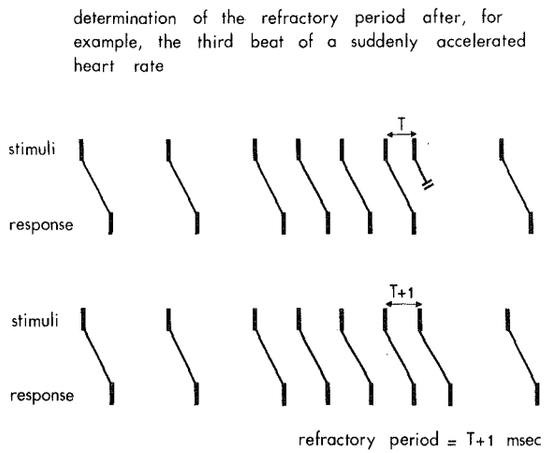

Fig. 12. Schematic representation of the method to determine the refractory period after any desired beat of a new rate. In this case, a test pulse is applied T msec after the third beat of a new, faster driving rate, and no response is obtained. After a sufficient number of beats of the slow rate, again three stimuli of the fast rate are given, and the test pulse is now applied T + 1 msec after the third beat.

pulse. The heart was then brought back to the steady state of the original frequency and the same procedure was repeated with shorter delays of the test pulse, until the shortest interval was found at which the test pulse was successful. This could be done of course with different intensities of the test pulse, so that strength-interval curves could be constructed after for example the first, second and third beat of a new rate. Fig. 13 shows strength-interval curves for the steady state frequency of 100/min. (600 msec basic interval) and for the first, second, third, fourth, fifth, and tenth beat of a new, faster rate with a basic cycle length of 250 msec. It seems as though the final phases of recovery of excitability occur somewhat more slowly during the first beats of a new, faster rate than during steady state conditions. These curves were obtained from an isolated, perfused heart; curves made during in situ experiments did not differ essentially from those shown. At the intensity level of 1.5 times diastolic threshold strength the slopes of the different strength-interval curves are about equal. We feel therefore that the use of a test pulse of 1.5 times diastolic threshold intensity to determine the duration of the refractory period is justified. In the experiments to be described this intensity has been used (see also page 24 and 25).

The initial part of the "on" effect

In fig. 14 the initial part of the "on" effect is depicted. Data were taken

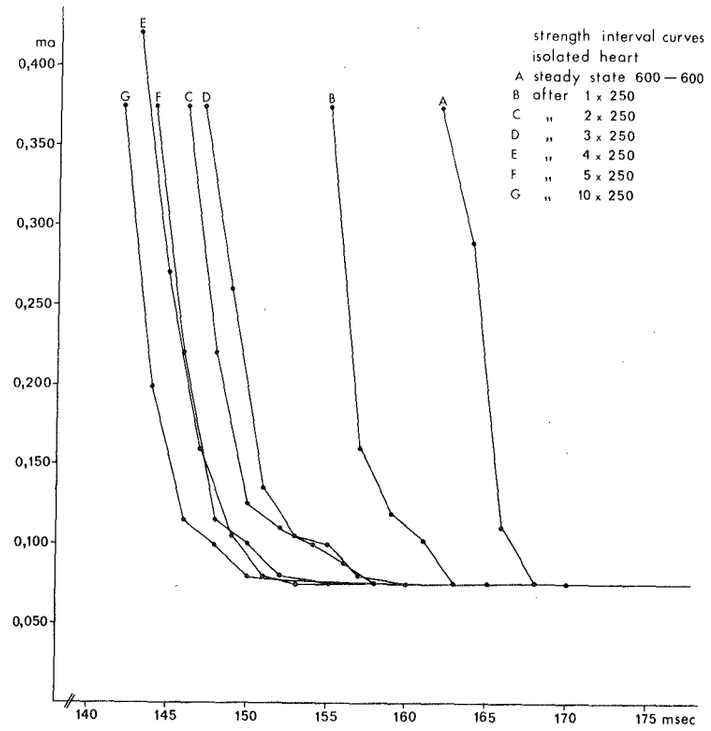

Fig. 13. Strength-interval curves for cathodal stimuli of 2 msec, during a steady state frequency (basic cycle length 600 msec) and after the first, second, third, fourth, fifth and tenth beat of a new, faster rate with a basic cycle length of 250 msec.

from three dogs in which the heart rate was doubled from 100/min. to 200/min. The refractory period is shortened considerably by the first beat of the faster rate (about 27% of total shortening), while up to 45% of total shortening is obtained by the second beat. After the first two beats, the rate of change suddenly becomes slower, and also longer and shorter refractory periods alternate in subsequent beats. Thus, the refractory period of the third beat is longer than of the second beat, the difference being in the order of 3 to 10 msec. In 18 other dogs, the same type of curve was obtained: shortening by the first two beats of the faster rate, alternation between longer and shorter refractory periods after the third beat, the odd beats having a longer refractory period than the even beats. In fig. 15 the beat to beat changes in refractory period for several frequency jumps are shown. Departing from a steady state interval of 600 msec, the stimulus interval was decreased to 400, 300, 250, 230 and 225 msec. For each frequency jump essentially the same curve emerged. It

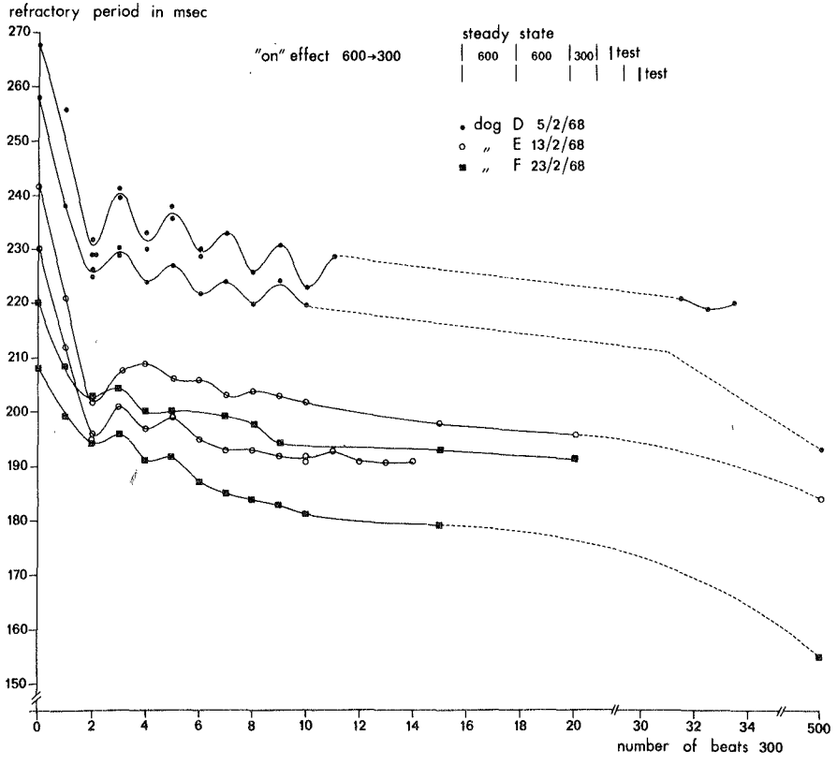

Fig. 14. Immediate changes in refractory period upon sudden doubling of the heart rate in three dogs. Inset: stimulation pattern used for determining the refractory period after the first beat (top) and second beat (bottom) of the new rate. In each dog the "on"effect was determined twice, with a time interval of 2 to 4 hours; the same pattern was found at a slightly different length of refractory period. An alternation in refractory periods of subsequent beats occurs after the second beat, in varying degree in individual experiments.

is evident that the faster the new rate, the greater the shortening by the first two beats, and the more pronounced the oscillation between longer and shorter refractory periods after the second beat. In some dogs however the pattern of change in refractory period following large frequency jumps was different, as can be seen in fig. 16.

For the transition from 600 msec basic cycle length to 400 and 300 msec basic interval, the same curves as in the other dogs were found. Upon further augmentation of the heart rate however, different curves emerged: the refractory period of the second beat being the same as after the first beat (600 to 240 msec), and even longer than after the first beat (600 to 230 and 600 to 220 msec). In the last cases alternation between longer and

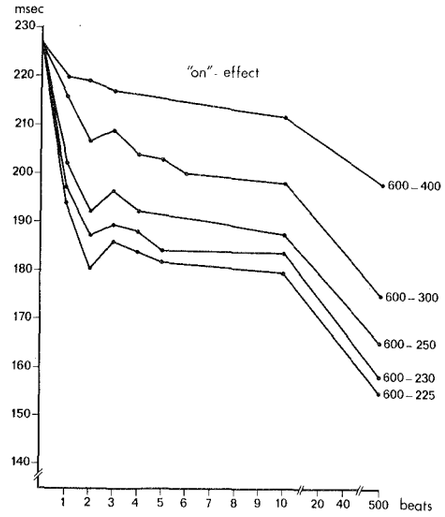

Fig. 15. "On"effect for the sudden transition to different fast rates (cycle lengths 400, 300, 250, 230, 225 msec), departing from the same basal rate (cycle length 600 msec). The alternation in duration of subsequent refractory periods after the second beat increases as the new rate becomes faster.

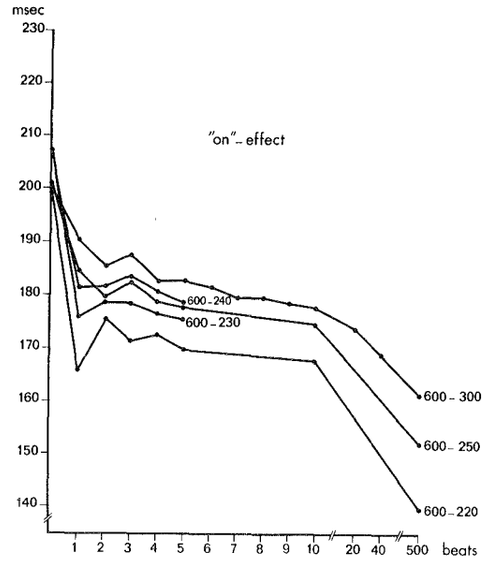

Fig. 16. "On"effect for the sudden transition to different fast rates (cycle lengths indicated in the fig.), departing from a basal rate of 600 msec cycle length. As the new rate becomes faster, the phase of the alternation in refractory periods changes in this dog.

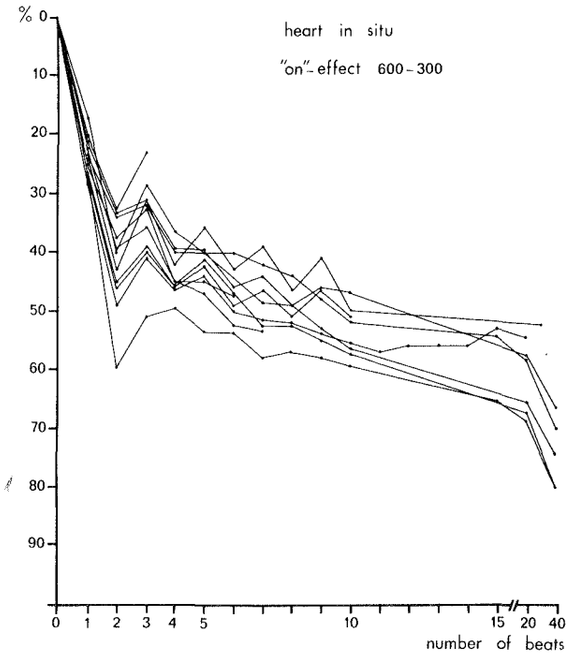

Fig. 17A. "On"effect for doubling the heart rate in 8 dogs (heart in situ). The refractory periods are expressed as a percent of the difference between the refractory periods during the steady state of the slow frequency (cycle length 600 msec) and during the steady state of the fast frequency (cycle length 300 msec).

shorter refractory periods occurred in even and odd beats respectively. This type of curve was also found in isolated perfused hearts, especially when the difference between slow and fast rate was large.
In general there existed a difference between the findings in situ and those obtained during perfusion of the isolated heart. Fig. 17 shows the "on" effects for the same doubling of the heart rate both in situ and during perfusion. To compare the results of several experiments, the difference between the steady state refractory period at fast and slow rate was taken as 100, and shortening in refractory period produced by subsequent beats of the fast rate is expressed as percentage of this difference. It is evident that the alternation between longer and shorter refractory periods in the isolated heart has more or less disappeared.

"On" effect at different sites in the myocardium

Experiments were carried out to determine whether a sudden increase in

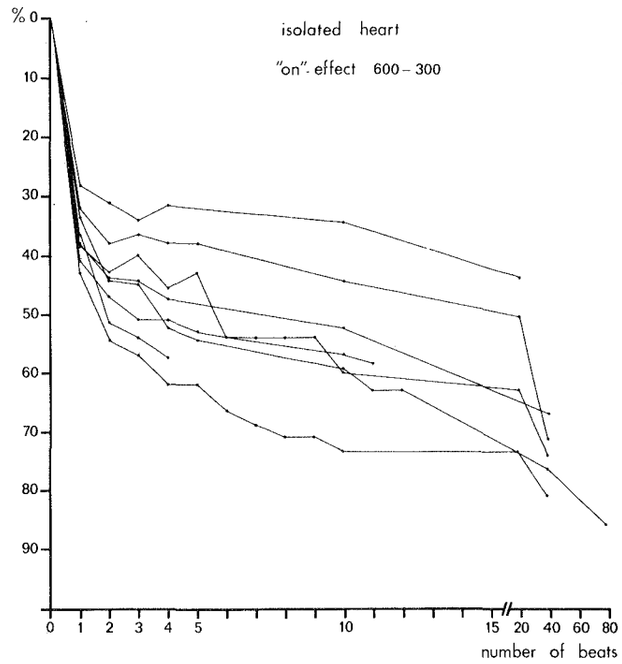

Fig. 17B. "On"effect for doubling the heart rate in 8 isolated hearts. Note that the alternation in refractory periods of subsequent beats is less than for the heart in situ.

heart rate would result in an increase in the differences in refractory periods at multiple sites, or whether the changes in refractoriness would occur in phase throughout the myocardium. For this purpose driving and testing stimuli were successively applied at several intramural electrode terminals of needles, inserted perpendicularly to the epicardial surface into the free wall of the left ventricle. Experiments were performed on the heart in situ.
Fig. 18 shows the results of one experiment in which the beat to beat variations in refractory period were measured at 13 different intramural sites when the driving frequency was increased. In steady state conditions, differences existed between the durations of the refractory period at different locations. In subepicardial layers the refractory period was slightly shorter than in subendocardial layers; in the middle layers of the ventricular wall both shorter and longer refractory periods were found. As is evident, this relationship persisted when the driving rate was doubled.
At this point it should be stated that we were unable to detect a systematic difference between the duration of the refractory period in subendocardial and subepicardial layers.
"Subepicardial" refractory periods could be both shorter and longer than

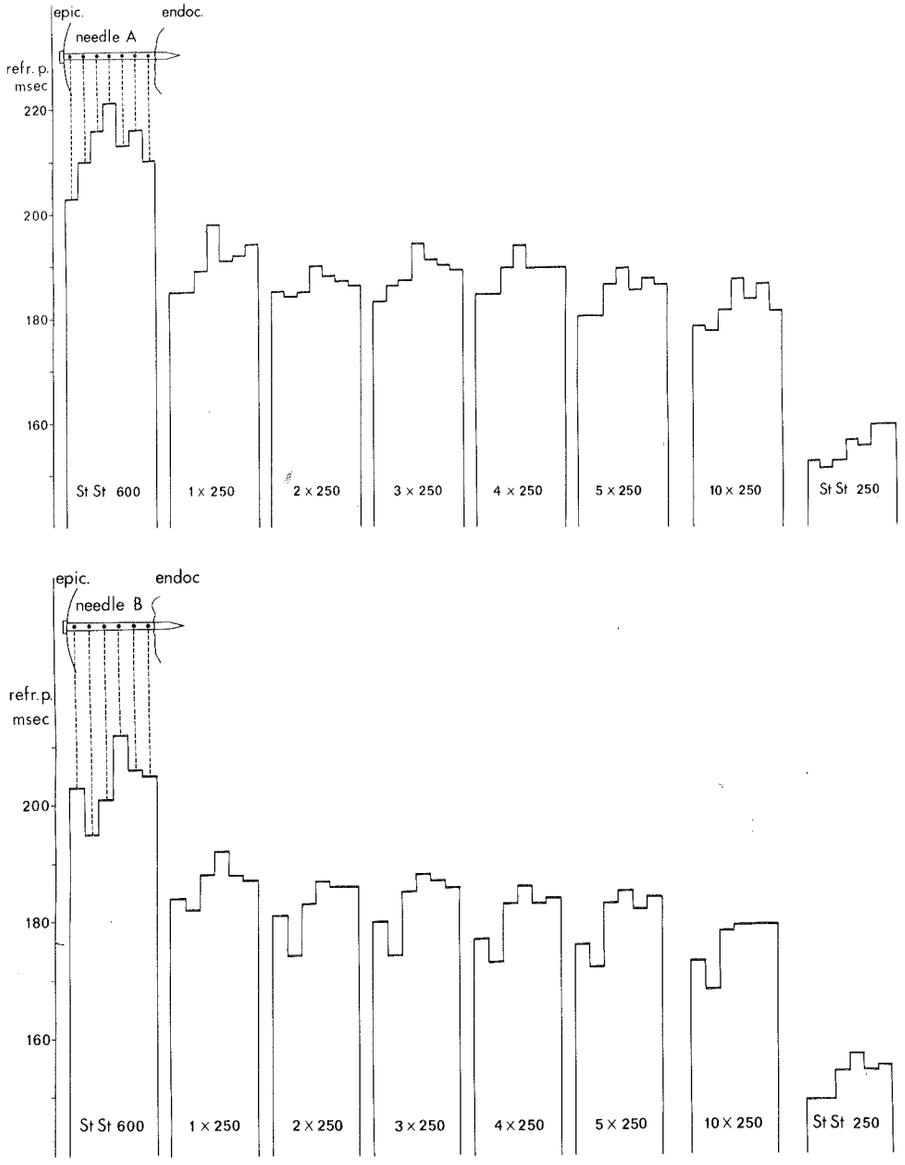

Fig. 18. "On"effect at different intramural sites, as determined at two needle electrodes (needle A and needle B) in the same heart. Cycle length of the slow rate 600 msec, cycle length of the fast rate 250 msec. St St = steady state.

"subendocardial" refractory periods. A controversy exists about this point in the literature. The results of Van Dam and Durrer [24] are in agreement with our findings; they found that in middle layers recovery of excitability may be more advanced than in inner and outer layers; sometimes the refractory period of the inner layers was shorter than in subepicardial layers, and sometimes longer. Moore, Preston and Moe [92] on the other hand stated that "action potentials recorded from epicardial cells were usually shorter than papillary muscle ("endocardial") action potentials". In our experiments the refractory period in subendocardial layers changed in a slightly different fashion from the refractory period in subepicardial layers during the first beats of a faster rate. When depicting the refractory period during the steady state of the slow drive and after the first three beats of the faster rate, three types of curves can be obtained. In all types, the refractory period is shortened by the first beat. In type A, the refractory period of the second beat is longer than that of the first beat and third beat. In type B, the refractory period of first, second and third beat are about equal. In type C, the refractory period of the second beat is shorter than that of the first and third beat. Subepicardial layers show either a type A or a type B curve; subendocardial layers either a type B or a type C curve. The middle layers show intermediate types of curves. In fig. 19 these different types of curves are visualized.

In table I the results are summarized. As a measure of temporal dispersion of refractory periods, the standard deviation was taken. As can be seen, no increase in standard deviation of the refractory periods occurs during the first beats of a new, faster heart rate, rather there is a decrease. Also indicated are the mean differences in refractory period in each situation, as compared with the steady state of the slower rate, and the standard deviation of these differences. If the refractory period at all sites would shorten by the same amount, this standard deviation should be zero. This is not the case. An attempt was made to see whether the amount of shortening depended on the value of the steady state refractory period, in other words, whether the relative shortening would be constant. When, at each site, the absolute amount of shortening was divided by the steady state refractory period, the values found were not constant. Although there seemed to be a tendency for the longer refractory periods to shorten more than the shorter refractory periods, this could not be decided with certainty. This was caused by the fact that subepicaridal and subendocardial sites behave differently when subjected to sudden increases in heart rate (see fig. 19). A comparison between subepicardial sites on the one hand and subendocardial sites on the other hand was felt to be useless, since the number of comparable sites would be too small.

There is one paper in the literature which deals with differences in refractory periods at multiple sites [50]. In this study, Han and Moe determined refractory periods at 12 epicardial sites, both during a regular frequency and after an early premature beat. In their experiments, basic and premature stimuli were applied to a central electrode, and, to determine refractory periods, test stimuli were passed through 12 electrodes success-

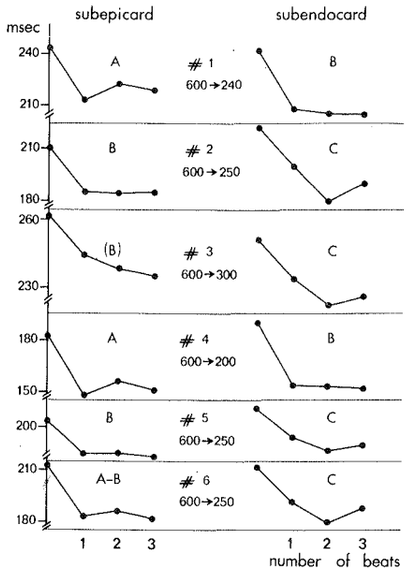

Fig. 19. Differences in the initial changes in refractory periods following an abrupt increase in heart rate at subepicardial sites and at subendocardial sites in 6 dogs. The R-R intervals of slow and fast rates are indicated at each curve. Further explanation in the text.

ively, arranged in 2 circles around the central electrode. Han and Moe found that an early premature beat, falling in the relative refractory period, greatly increased the temporal dispersion of recovery of excitability (which in their study was expressed as the difference between longest and shortest refractory period). A "late" premature beat caused only a moderate increase in temporal dispersion.

In order to compare our findings with those of Han and Moe, we made the same calculations from their data (fig. 2 of reference 50) as from ours. As can be seen in table II, there is a more than two-fold increase in the standard deviation of the refractory periods after one early premature beat in the study of Han and Moe. In our experiments, we see only a slight increase in the standard deviation of the refractory periods after an early premature beat. After "late" premature beats, the standard deviation decreases (first beat of the faster rate in experiments 17-3-'70 and 25-9-'68 in table I). Han and Moe wrote "It is likely that the degree of temporal dispersion of recovery of excitability should become greater and greater after each successive beat in a train of repetitive premature beats. Because of the danger of fibrillation, direct demonstration of this was not attempted" [50]. We therefore set out to determine refractory periods at different intramural sites, while applying a series of premature beats to the heart, each with the shortest possible preceding interval.

TABLE I

Sudden increase in heart rate

Experiment	Number of sites	R-R interval	Mean refractory period	Temporal dispersion (standard deviation)	Mean differences	Standard deviation of differences	Comments
25-9-'68	13	steady state 600-600	208	7.2			
		1 x 250	189	4.5	19	4.4	first beat of the new rate is a "late" premature* beat
		2 x 250	185	4.0	23	4.9	
		3 x 250	186	5.1	22	3.6	
		4 x 250	184	4.7	25	3.7	
		5 x 250	183	5.0	26	4.0	
		10 x 250	179	5.1	29	3.7	
		steady state 250-250	154	3.3	54	5.9	
17-3-'70	8	steady state 600-600	180	4.5			first beat of the new rate is a "late" premature* beat
		1 x 200	148	3.2	32	2.0	
		2 x 200	153	3.0	27	3.2	
		3 x 200	150	3.0	30	2.8	
18-4-'69	15	steady state 600-600	239	10.2			first beat of the new rate is an "early" premature* beat
		1 x 240	205	12.1	34	6.1	
		2 x 240	211	10.4	28	6.8	
		3 x 240	207	11.3	32	6.2	
		4 x 240	209	12.6	30	7.4	
		5 x 240	207	12.4	32	6.7	
		10 x 240	202	14.2	36	8.3	

* see text

TABLE II

REFRACTORY PERIODS AT MULTIPLE INTRAMURAL SITES

One early premature beat

Experiment	Number of sites	R-R interval	Mean refractory period	Temporal dispersion (standard deviation)	Mean differences	Standard deviation of differences	Comments
Han and Moe (fig. 2 of reference 50)	12	steady state 300-300 1 x 147	155 114	5.9 13.2	40	9.2	
18-4-'69	15	steady state 600-600 1 x 240	239 205	10.2 12.1	34	6.1	
18-11-'69	10	steady state 500-500 1 x 200	205 154	3.3 3.6	50	5.1	needle electrode parallel to epicardial surface
	8	steady state 500-500 1 x 210	210 164	11.2 14.2	45	9.9	needle electrode perpendicular to epicardial surface

Refractory periods at multiple sites following each of a series of premature beats with the shortest possible intervals

In the experiment of fig. 20 two needle electrodes were inserted into the free wall of the left ventricle, perpendicular to the epicardial surface, about 1 cm apart. Refractory periods were determined during the steady state and after each of a series of premature beats. The interval of the premature stimulus was selected after the refractory periods had been measured at all 12 testing sites, so that in each instance the earliest possible premature stimulus could be given. Basic stimuli, premature stimuli and test stimuli were applied successively to each of the 12 intramural electrode terminals.
Prior to each determination, the diastolic threshold for each site was measured and the intensity of the test stimulus set at 1.5 times that value. In this way slight variations in diastolic threshold during the course of the experiment could not influence the results. The first extra stimulus was given after an interval of 240 msec, the second extra stimulus 185 msec after the first, the interval of the third extra stimulus was 166 msec, and that of the fourth was 160 msec. Although subepicardial and subendocardial refractory periods do not change in the same way, it is evident from fig.

Fig. 20. Refractory periods at different intramural sites during steady state conditions (A, cycle length 600 msec), after 1 premature beat (B, preceding cycle length 240 msec), after 2 premature beats (C, preceding cycle 185 msec), after 3 premature beats (D, preceding cycle 166 msec), and after 4 premature beats (E, preceding cycle 160 msec). Note the alternation in the pattern of distribution of the refractory periods throughout the ventricular wall in the subsequent premature beats. Note that the range of variations in the durations of the refractory periods is about the same for every beat. Diagram shows stimulation pattern.

TABLE III

Series of early premature beats

Experiment	Number of sites	R-R interval	Mean refractory period	Temporal dispersion (standard deviation)	Mean differences	Standard deviation of differences	Comments
14-5-'70	12	steady state 600-600	232	6.0			basic stimuli, premature stimuli and test stimuli applied to the same electrode. EPB = early premature beat
		1 EPB (240)	180	5.8	53	9.7	
		2 EPB's (240-185)	159	6.0	73	4.2	
		3 EPB's (240-185-166)	154	5.7	78	10.3	
		4 EPB's (240-185-166-160)	147	6.1	85	6.0	
18-9-'70	15	steady state 600-600	189	5.1			same as above
		1 EPB (200)	160	4.8	29	5.9	
		2 EPB's (200-170)	134	3.4	55	4.2	
		3 EPB's (200-170-147)	127	3.0	62	5.1	
		4 EPB's (200-170-147-140)	131	3.3	57	5.3	
20-10-'70	15	steady state 500-500	198	8.3			basic and premature stimuli applied to one and the same electrode. Test stimuli successively applied to all other electrode terminals
		1 EPB (205)	157	7.8	42	12.7	
		2 EPB's (205-155)	120	8.8	79	6.6	
		3 EPB's (205-155-130)	123	5.9	76	10.4	
		4 EPB's (205-155-130-126)	115	9.0	84	10.5	
29-9-'70	21	steady state 450-450	196	5.6			same as above
		4 EPB's (208-159-145-142)	126	6.5	70	4.8	

20 that no increase in the range of variation of refractory periods occurs during this train of premature beats. As indicated in table III the standard deviation of the refractory periods remains about the same as for a basic beat (experiments 18-4-'70 and 14-5-'70).

Interestingly, the pattern of recovery of excitability throughout the ventricular wall is different for a basic beat (A) and the first premature beat (B). However, there is a similarity between the distribution of refractory periods throughout the ventricular wall for a basic beat (A) and the second (C) and fourth (E) extrasystoles. Also, the pattern of recovery after the first extrasystole (B) and third extrasystole (D) is similar. This alternation in the pattern of recovery of excitability throughout the ventricular wall is reflected in the standard deviation of the differences in refractory period (see table III, experiments 14-5-'70 and 20-10-'70). The differences in refractory period of the first (B) and third (D) premature beat, compared with the basic beat (A), show a large standard deviation, because for these beats the pattern of recovery is different from that of the basic beat. In the experiment described (and in the experiment of 18-9-'70, table III, in which the alternation in pattern of recovery is less clear) all stimuli were applied successively to each of the terminals. This implies that the premature stimuli did not always fall in the relative refractory period of each site. For every premature stimulus the shortest preceding interval was chosen at which the heart would follow the stimulus, regardless at which site the premature stimulus was applied. Since Han and Moe applied the premature stimulus at one central electrode, falling in the relative refractory period at that site and measured refractory periods at the other locations, by giving the test stimuli successively at the other electrode terminals, we did 2 experiments in which the same procedure was followed. Basic stimuli and premature stimuli (all falling in the relative refractory period of the preceding beat) were applied at an electrode terminal at the epicardial surface (point 10 of a needle electrode). Refractory periods were determined at the other terminals of the same needle, and at the terminals of other needles, inserted at distances of about 1 cm from the stimulating needle. All needles were inserted perpendicularly to the epicardial surface. By recording bipolar and unipolar leads of all terminals, and playing back the tape at low speed, the conduction times from the stimulus electrode to each testing site could be ascertined with an accuracy of 1 msec. By subtracting the conduction time from the shortest stimulus interval at which the test stimulus was succesful, the refractory period at each site was found.

The results of this type of experiment were very similar to the results shown in fig. 20. Either no increase, or only a slight increase in standard deviation of refractory periods occurred (experiment 20-10-'70 in table III), and the alternation in pattern of recovery was similar to the alternation in fig. 20.

To visualize the ranges of variation in refractory periods, the refractory periods at 21 intramural sites of a basic beat, and after the fourth premature beat are superimposed in fig. 21 (experiment 29-9-'70, table III).

In summary, it can be said that, even after a series of 4 premature beats, each falling in the relative refractory period of the preceding beat, the abso-

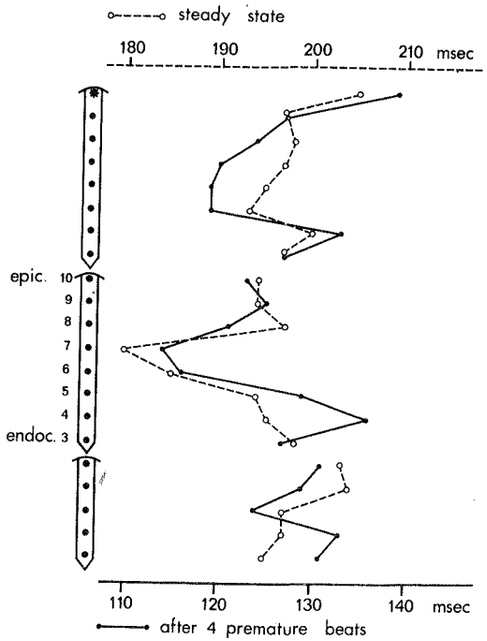

Fig. 21. Refractory periods at different intramural sites after a basic beat (open circles, dotted lines) and after the fourth of a series of premature beats (solid circles, solid lines). Basic stimuli and premature stimuli, all falling in the relative refractory period of the prededing beat, were applied to the subepicardial terminal of the uppermost needle electrode (asteriks). Test stimuli, used for the determination of refractory periods, were successively applied to the other terminals.

lute range of variations in refractory periods at multiple sites in the myocardium is of the same magnitude as for a basic beat. Certainly no increase in temporal dispersion of recovery of excitability as great as found by Han and Moe could be detected. However, even if the absolute range of differences in refractory period after a series of premature beats is of the same order of magnitude as during a basic beat, the relative differences are greater because the refractory periods have shortened considerably. Obviously, it is important to decide whether premature beats increase the degree of non-uniformity of recovery of excitability. If the degree of non-uniformity would be large enough after, for instance four early premature beats, a fifth premature beat might excite some elements, but would be unable to excite those parts which were still refractory. Thus, the possibility of re-excitation would be created, which might set the stage for fibrillation. It is therefore of interest to examine the changes in conduction occurring during a series of premature impulses.

Changes in functional refractory period

The results so far described apply only to changes in excitability, as measured by threshold determinations; they do not contain information about the recovery of conduction. The effect of changes in rhythm on the functional refractory period, i.e. the shortest interval between two propagated responses, was investigated. For this purpose, bipolar complexes were recorded from terminals located on the intramural electrode 2, 4, 6, 8, 10, and 12 mm from the point of stimulation. By playing back the tape at lower speed, activation times were measured with an accuracy of 1 msec (see page 22). Because of the technical impossibility of recording acceptable complexes from the electrode used for stimulation, the latency at the stimulus site itself could not be determined. Therefore the results do not indicate the changes in functional refractory period of the exact stimulus site. The shortest distance from the stimulus site, at which it was possible to distinguish between intrinsic deflection and stimulus artifact in bipolar records, was 2 mm. This may introduce a slight error, caused by possible differences in velocity or pathway of propagation between the responses, evoked by driving stimuli and early test stimuli. The first discernable fast deflection was taken as moment of activation [35, 36]. When test pulses with a strength of 1.5 times diastolic threshold were employed, the functional refractory period was not very different from the refractory period. The conduction time of a response, evoked by the earliest possible test pulse to the electrode located 2 mm from the stimulus site, was at most 5 msec longer than the conduction time of a basic response. After the activation front has passed the electrode terminal which is located 2 mm from the stimulus site, propagation along the intramural electrode occurs at normal diastolic velocity. The lines, connecting the activation times of the terminals of the intramural electrode used for stimulation, run parallel to each other for every test stimulus and after any number of beats of a new faster rate. This is also true when the heart has been subjected to a series of 4 extrasystoles with the shortest possible interval, after which the earliest succesful test pulse was applied at several electrodes of the same needle (see fig. 22). The moments of activation of the last extrasystole (preceding cycle length 160 msec) are indicated by solid dots; the moment of activation of the earliest possible test pulse (given at the end of the refractory period at each stimulus site) are indicated by open circles. It is evident that the response evoked by the earliest test pulse arrived later at the electrode 2 mm from the stimulus site, probably mainly because of a local delay at the site of stimulation. Propagation along the other terminals occurred at the same velocity as during the last extrasystole, which was nearly identical to the velocity of spread of activation of a basic impulse. Interestingly, sometimes the functional refractory period at a particular site was actually shorter than the refractory period at that site, when determined by stimulation at 1.5 times diastolic threshold. For example the refractory period at point 10 was 157 msec; when stimulating point 7 the earliest test pulse following the 4th extrasystole could be given after 145 msec. The excitatory wave thus evoked reached point 10 3 msec later than

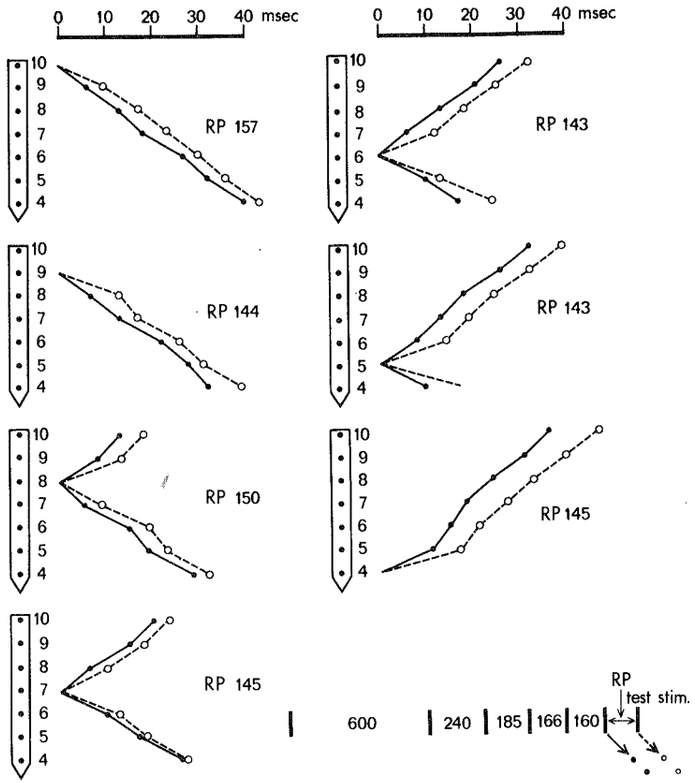

Fig. 22. Conduction times in msec along a needle electrode (same experiment as in fig. 20). Solid circles: conduction times of the fourth premature beat (preceding cycle 160 msec). Open circles: conduction times of the beat elicited by a test pulse in the relative refractory period (RP) of the fourth premature beat at each electrode terminal. Note that the impulse evoked in the relative refractory period at each site is conducted somewhat more slowly to the electrode terminals 2mm away, but that conduction velocities from that distance on are the same as for the prededing beat.

the 4th extrasystole, in other words the functional refractory period of point 10 was 148 msec, 9 msec shorter than its refractory period. Two explanations are possible:

1. Fluctuations in the duration of the refractory period during the course of the experiment may have occurred.
2. The stimulating efficacy of an excitatory wave is greater than that of an electrical stimulus of 1.5 times diastolic threshold.

In fig. 23 bipolar complexes are shown taken during the series of premature beats in which the largest differences occurred in activation times of the last extrasystole and the preceding ones. The interval of the last extrasystole was equal to the refractory period at that site. As can be seen, conduction

is delayed in the area close to the site of stimulation: terminal 5 is activated 6 msec later, and terminal 7 10 msec later than during the preceding beat. Conduction along the other terminals proceeded at normal diastolic velocity. The shape of the bipolar complexes recorded between terminals 5 and 6, and between 6 and 7, is different from the shape of the preceding complexes: they are broader and slightly more notched. This indicates that this impulse was conducted more slowly, and more irregularly in the vicinity of the site of stimulation than the preceding impulses. In areas further away than about 4 mm from the site of stimulation, the spread of activation is identical to that of a basic beat.

Van Dam, Durrer and van der Tweel [26] concluded that the delay of one early premature beat is caused by local delay at the site of stimulation, and that from a distance of 1 mm onward from the stimulus site, the rate of conduction of the extrasystole always had the same value.

Our results indicate that a series of extrasystoles does not cause an increase in dispersion of recovery of excitability, and also that the propagation of premature beats is not greatly different from that of basic beats.

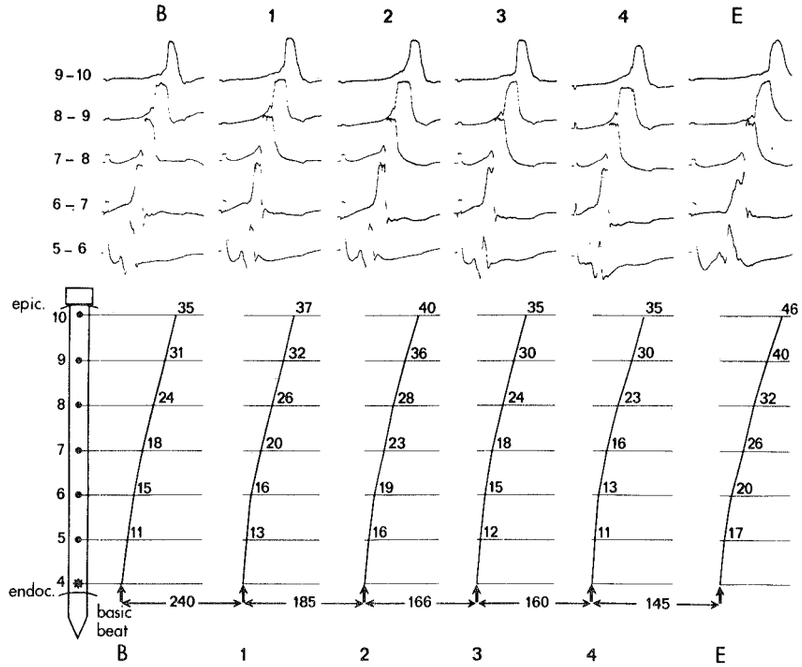

Fig. 23. Bipolar complexes recorded between consecutive electrode terminals, while stimulating terminal 4 of a needle electrode. The basic beat B is the last of a long series of beats with an interval of 600 msec. The next beats are separated by the intervals indicated between them. The diagram shows the conduction times in msec (t = 0 is the stimulus artefact). Note the different configuration of complexes 5-6 and 6-7, evoked by the test stimulus E, falling in the relative refractory period of the fourth premature beat.

Beat to beat changes in refractory period following a sudden decrease in heart rate ("off"- effect)

To determine the lengthening in refractory period effected by one long cycle, or any number of repetitive long cycles, introduced during a steady state fast rate, essentially the same method as previously described was used. The beat with the long preceding cycle (or the last beat of a series of successive long intervals) was followed by a test pulse. In each instance the shortest interval preceding a succesful test pulse was determined. The initial part of the "off" effect is not the exact mirror image of the "on" effect. The first long cycle has the largest effect (about 30% of total lengthening). After the first beat of a slow frequency the rate of change in refractory period is diminished, and each subsequent beat lengthens the refractory period by approximately the same amount. No alternation in duration of refractory periods in subsequent beats occurs (see fig. 24 and fig. 25).

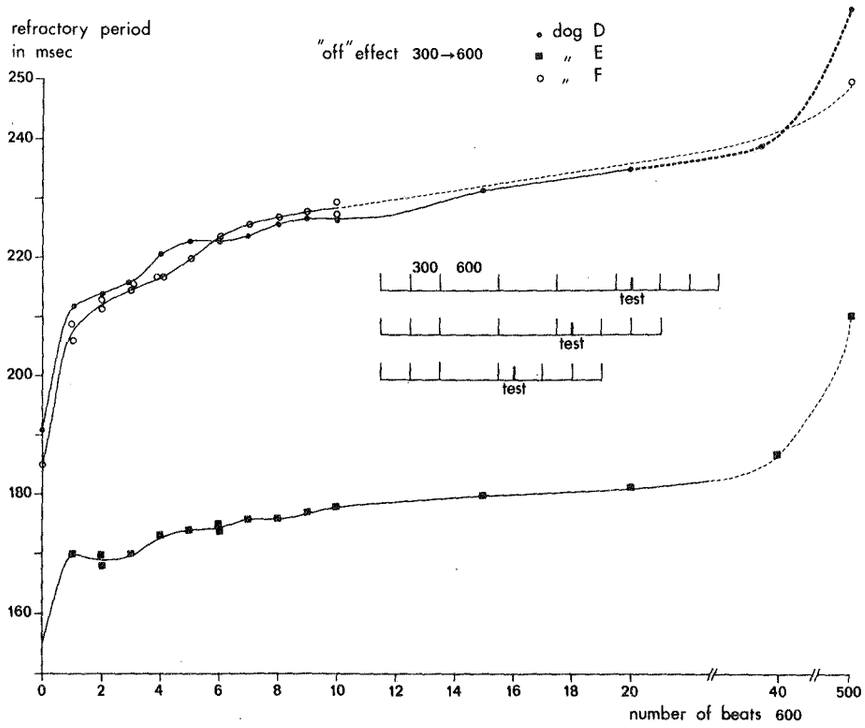

Fig. 24. "Off"effect: immediate changes in refractory period when the driving rate is suddenly reduced from a cycle length of 300 msec to one of 600 msec in three dogs (same as in fig. 14). Inset: stimulation pattern.

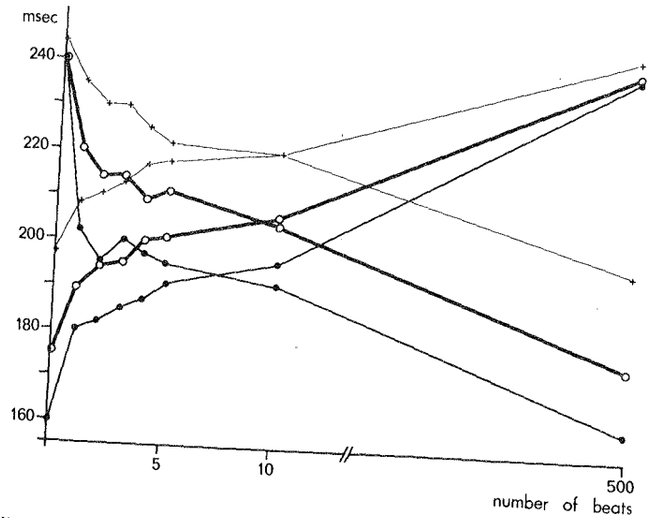

Fig. 25. "On"- and "off"effects for different changes in frequency. The basic driving rate had an interval of 800 msec, and the steady state refractory period was 240 msec. The "on"effect for the transition of the basic rate to a rate with an interval of 400 msec is indicated by the symbol +, and so is the "off"effect, when from a steady state frequency with a 400 msec interval the driving rate is suddenly reduced to an interval of 800 msec. Open circles: "on" effect from 800 msec to 300 msec interval, and "off"effect from 300 msec to 800 msec interval. Solid circles: "on"effect from 800 msec to 250 msec interval, and "off"effect from 250 msec to 800 msec interval.

"Off" effect at multiple sites

In the experiment of fig. 26, the refractory periods at 18 intramural sites were determined, both in steady state conditions during rapid heart rates (cycle length 300 msec and 200 msec), and after one and two long cycles (900 msec). The sudden introduction of one or two long cycles during a rapid rate does not change the sequence of recovery of excitability throughout the ventricular wall. If there is an increase in the temporal dispersion of the refractory periods, it is a matter of a few msec only. The results of 2 experiments are summarized in table IV.

TABLE IV

REFRACTORY PERIODS AT MULTIPLE INTRAMURAL SITES

Sudden decrease in heart rate

Experiment	Number of sites	R-R interval	Mean refractory period	Temporal dispersion (standard deviation)	Mean differences	Standard deviation of differences	Comments
24-8-'70	18	steady state	139	5.6			
		200-200					
		1 x 900	165	5.5	25	4.7	
		2 x 900	167	4.7	27	5.9	
	18	steady state	163	4.2			
		300-300					
		1 x 900	180	4.8	17	2.9	
		2 x 900	184	4.8	21	2.7	
20-10-'70	14	steady state	123	4.3			
		200-200					
		1 x 900	137	3.0	14	2.9	
		2 x 900	138	3.0	15	2.9	

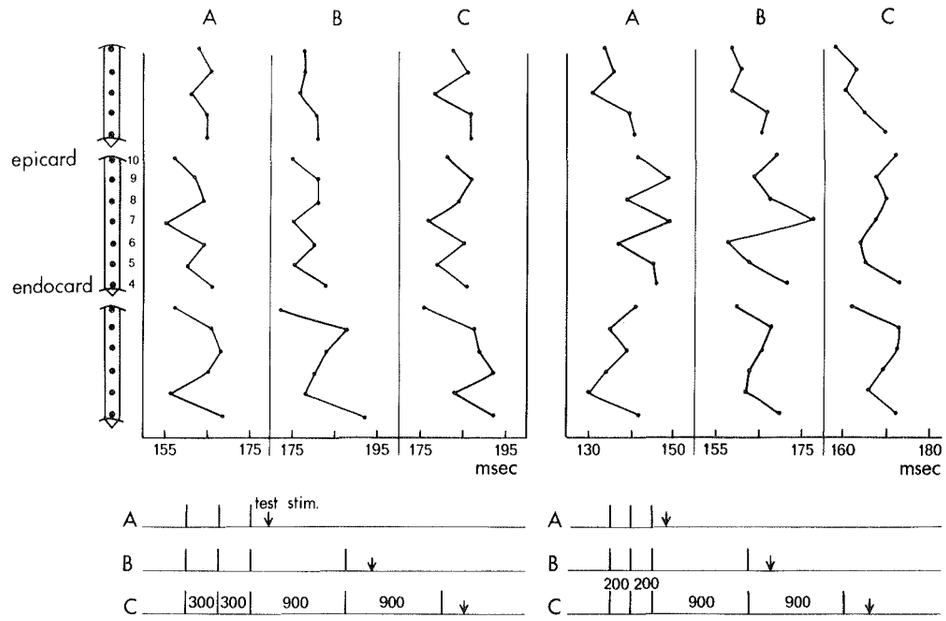

Fig. 26. Refractory periods at different intramural sites during a steady state fast heart rate (cycle lengths 300 msec, and 200 msec) and after one and two long cycles (interval 900 msec).

RESULTS - THE BUNDLE BRANCHES

Determination of the refractory period of the bundle branches is of course possible only when the Purkinje tissue is selectively stimulated, and not simultaneously with the underlying septal myocardium. It is known that thresholds determined by intracellular stimulation through micro-electrodes, and by extracellular stimulation through surface electrodes, are lower for Purkinje fibers than for myocardial fibers [37, 83]. In our experiments, bipolar leads of terminals located on the bundle branch close to the site of stimulation showed two components: a Purkinje spike, which preceded a larger deflection caused by the depolarization of the underlying myocardium. When the stimulus intensity was increased beyond a certain level, an abrupt decrease in latency between stimulus artifact and muscle deflection occurred, indicating that simultaneous excitation of Purkinje and muscle fibers took place. In some experiments the intensity necessary to stimulate the myocardium directly was more than 10 times the diastolic threshold for Purkinje fibers. In all experiments it was possible to use test pulses of

1.5 times diastolic threshold strength, to determine the refractory period of the Purkinje fibers, without simultaneously stimulating the myocardium directly. Careful monitoring of Purkinje spikes and myocardial deflections provided the necessary control during the experiment; the results were always checked later by playing back the tape recorded complexes.

Steady state refractory periods

The duration of the steady state refractory period of the specialized conduction system is also inversely related to heart rate. In addition, during steady heart rates the refractory period of the bundle branches is longer than that of the ventricular myocardium. This is shown in fig. 27 based on determinations in one isolated heart. At slow rates the difference in duration between refractory periods of bundle branches and myocardium is about 60 msec; when the heart is driven at increasingly faster rates, this difference gradually decreases and may even disappear at the shortest attainable cycle length. These findings agree with those of Moore, Preston and Moe [92], and with those of van Dam, Hoffman and Stuckey [27].

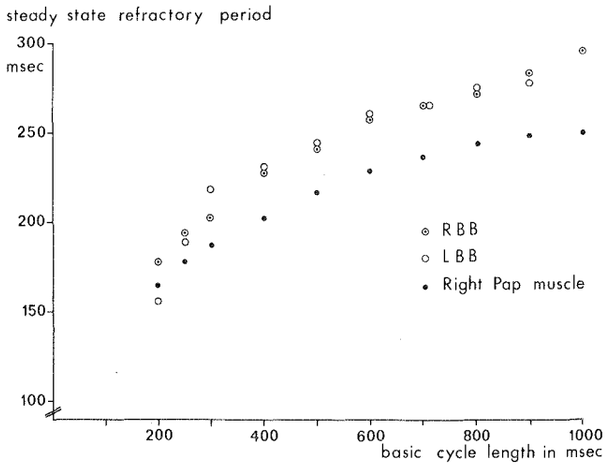

Fig. 27. Steady state relationship between basic cycle length of driving frequency and the duration of the refractory periods of right bundle branch (RBB), left bundle branch (LBB) and right papillary muscle.

Alternation in refractory period following increase in heart rate

Following an abrupt increase in heart rate, the refractory period of the bundle branches changes in a consistently and strikingly different pattern from that observed for ventricular myocardium. Thus, the first beat of a fast rate results in a pronounced shortening to nearly the steady state

level, which will be reached eventually, whereas after the second beat a marked lengthening of up to 40 msec occurs. In subsequent beats the duration of the refractory period alternates between shorter (odd beats) and longer values (even beats).
This oscillation gradually damps out during a large number of beats and in most experiments is detectable for more than 20 beats (fig. 28 and 29).
In one experiment a slight alternation in refractory period was still present in the steady state of the fast rate: when determining the steady state refractory period by giving a test pulse after every tenth basic beat, a value of 155 msec was found; when the test pulse was applied after every eleventh basic stimulus, the refractory period was 150 msec. Evidently an alternation of 5 msec was still present.
In other experiments, however, the steady state refractory period had indeed a stable value. In one case for instance the test pulse was applied after every 10th, 11th, 12th and 13th beat of the fast rate when the heart was in a steady state, and the refractory periods found were 171, 171, 170 and 171 msec respectively.

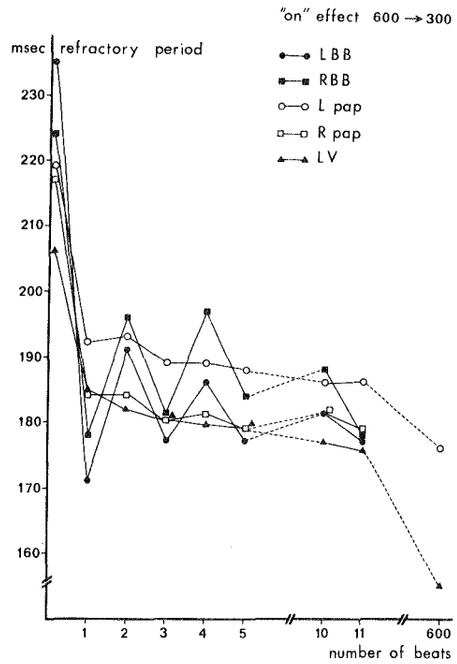

Fig. 28. "On"effect for the transition of a basic cycle length of 600 msec to one of 300 msec, determined at the left bundle branch (LBB), right bundle branch (RBB), left papillary muscle (Lpap), right papillary muscle (Rpap) and the free wall of the left ventricle (LV) in an isolated dog's heart. Note the large alternation in the refractory periods of the bundle branches in subsequent beats of the new, faster rate.

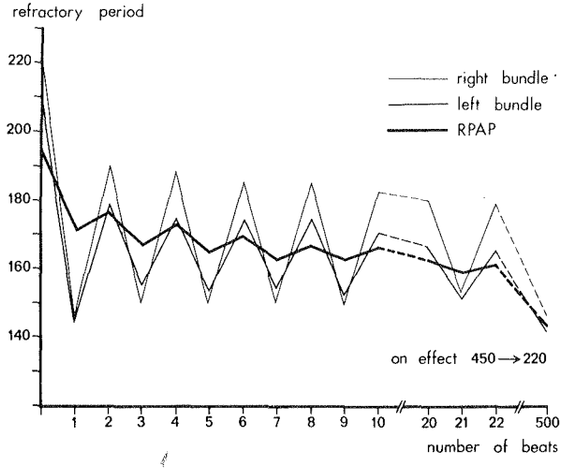

Fig. 29. "On"effect (cycle lengths 450 msec and 220 msec) for right bundle branch. left bundle branch and right papillary muscle (RPAP) in another isolated heart as in fig. 28. Note that the alternation in refractory periods of the bundle branches is still present after 20 beats of the new rate. Note that the refractory period of the bundle branches is alternatively longer and shorter than the refractory period of the myocardium.

Apart from individual variations in absolute values for the duration of the refractory period, this pattern of change in refractory period was consistently observed in all 7 isolated hearts, both during perfusion with modified Tyrode solution (5 hearts) and during perfusion with heparinized blood (2 hearts). One chance observation, made during an in situ experiment on a heart with a chronic myocardial infarction, suggests that the same pattern of change in refractory period of the Purkinje fibers, following an increase in heart rate, exists in vivo. While determining the refractory periods in subsequent terminals of a needle, inserted perpendicularly to the epicardial surface, it was found that the refractory period, determined at electrode terminal number 5, showed a typical "Purkinje behaviour" upon a sudden increase in heart rate. The steady state refractory period was 248 msec, about 40 msec longer than the refractory periods at the other electrode terminals; after the first beat of a new rate of 250 msec basic interval the refractory period shortened to 180 msec, now being equal to the refractory periods at the other terminals; after the second beat it lengthened again to 245 msec, and after the third beat it shortened to 212 msec. An unipolar recording of this needle during spontaneous activity showed a small Purkinje spike at terminal number 5 prior to the beginning of the left ventricular cavity; it also showed that terminal number 5 was located in the innermost layer of the ventricular wall, because the next terminals were located in the ventricular cavity, showing a typical cavity potential. Stimuli delivered through terminal number 5 might well have stimulated this peripheral strand of Purkinje fibers selectively.

During the first beats of a newly initiated faster rate, an interesting discrepancy exists between myocardium and bundle branches as far as the durations of their refractory periods is concerned. Up to now it has been widely accepted that the refractory period of Purkinje fibers outlasts that of myocardial fibers [56, 92], and no experimental evidence has to our knowledge been produced to challenge this concept. In the light of our findings it appears that this holds true for steady state conditions only and that sudden changes in heart rate result in an imbalance in the recovery of excitability in the two tissues. Thus after the first beat of a faster rate, the bundle branches recover earlier than the myocardium. After the second beat the situation is more like the steady state condition, the myocardium now being the first to recover.

"Off" effect

Fig. 30 shows the changes in refractory period of both bundle branches, when, departing from a steady state rapid heart rate (cycle length 300 msec) the driving rate is reduced to a cycle length of 600 msec. The first beat of the slower rate effects a considerable lengthening of the refractory period. In the following beats, an alternation occurs, which rapidly diminishes, and which is less outspoken than the alternation following the transition from a slow to a rapid rate.

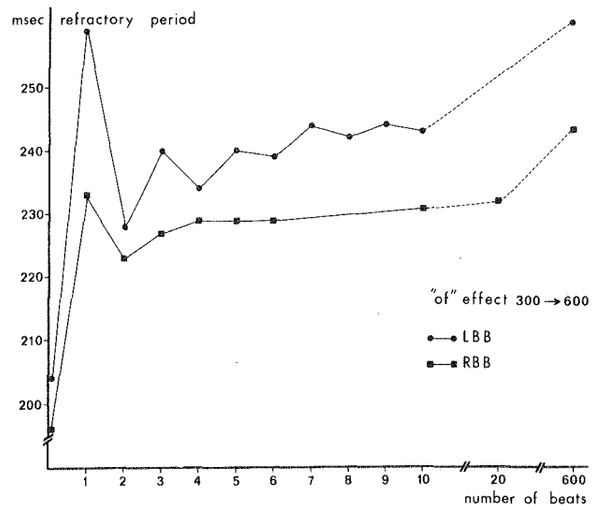

Fig. 30. Lengthening of the refractory periods of left- and right bundle branches (LBB and RBB) after the driving rate was suddenly decreased from an interval of 300 msec to an interval of 600 msec.

RESULTS - CONDUCTION OF PREMATURE IMPULSES

As was the case for the myocardium, the use of stimuli of 1.5 times diastolic threshold strength to determine the refractory period, did not cause large variations in stimulus-response latency in the Purkinje tissue, provided recordings were taken in the close vicinity of the site of stimulation. The functional refractory period, which is the shortest interval between two propagated impulses, was generally equal to, or at most 5 msec longer than, the refractory period, defined as the shortest interval between two succesful stimuli. This again demonstrates the usefulness of this way of determining the refractory period, because not only data are obtained concerning the recovery of excitability, but also about the recovery of conduction. However, in areas further removed from the stimulus site, the interval between two propagated impulses could greatly differ from the interval close to the site of stimulation. Based on the relationship between the duration of the refractory periods of bundle branches and myocardium, predictions could be made concerning the conduction of early premature impulses. Thus, the earliest possible premature beat, initiated in one of the bundle branches during a steady state rhythm, should be conducted without difficulty throughout the myocardium because the myocardium has a shorter refractory period than the bundle branches. A second early premature beat, however, can be initiated in the bundle branches at a moment when the myocardium is still in its refractory state. This impulse might reach the myocardium when its level of excitability is reduced and therefore it can be expected to be conducted more slowly through the myocardium. On the other hand, the earliest extrasystole evoked in the myocardium will have difficulty in invading retrogradely the bundle branches due to the longer refractory period of the bundle branches during a steady state rhythm. A second myocardial premature impulse however, will find the bundle branch system recovered and retrograde conduction into the bundle should not be hampered. To test these predictions the moment of activation of selected sites was measured for several types of premature beats. Recordings were made of the right and left bundle branches, and of intramural myocardial sites close to the Purkinje-papillary junction, i.c. the base of the right papillary muscle and the base of the left posterior papillary muscle. Also a site in the free anterior wall of the left ventricle was chosen.
Fig. 31 A and B illustrates this situation for a typical experiment. During regular driving with a basic cycle length of 450 msec, the refractory period of the right bundle branch is 220 msec and that of the right papillary muscle 195 msec (fig. A). The activation times following the driving stimulus applied to the right bundle branch are represented by open circles and those following a test pulse delivered after 220 msec by dots; some difference in latency occurs and this is apparent in the activation of the left bundle and in the activation of the myocardial sites. We suggest that the delay at the myocardial sites is caused by the fact that more peripheral Purkinje fibers have a longer refractory period than fibers in the bundle branches itself [94]. Following this beat, the refractory period of the right bundle branch was

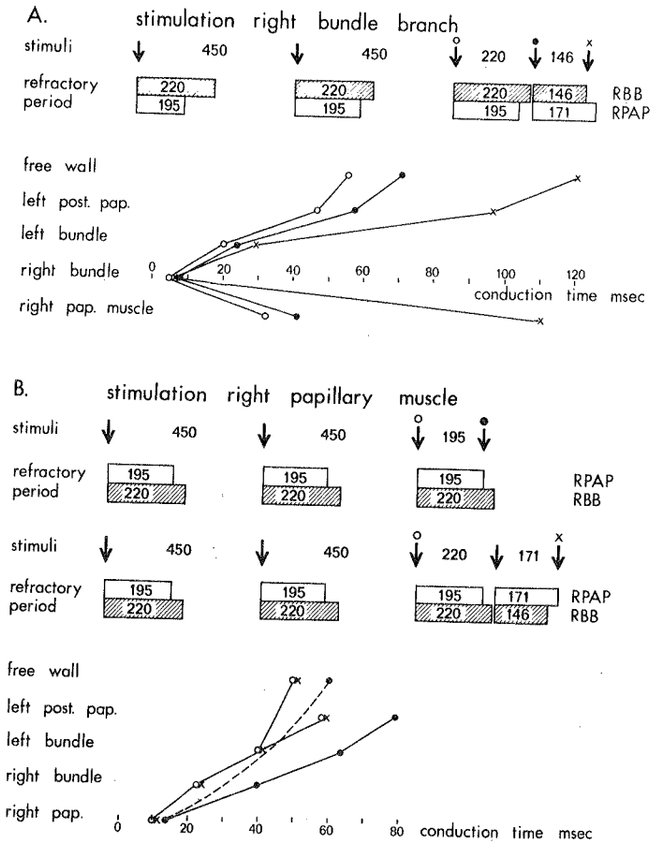

Fig. 31A. Propagation of premature impulses initiated by early stimuli applied to the right bundle branch (RBB). In the upper part, the stimulation pattern is indicated, together with the duration of the refractory periods of the right bundle branch and the right papillary muscle (RPAP). In the lower part, conduction times in msec for different beats are indicated. Note the long delay of the second premature beat in right- and left papillary muscles (crosses). Free wall: free wall of left ventricle. Left post pap: left posterior papillary muscle.

Fig. 31 B. Propagation of premature impulses initiated by early stimuli applied to the right papillary muscle. Note that the conduction of the first premature beat is only moderately delayed in the right bundle branch (solid dots). The dotted line indicates that the free wall of the left ventricle received its excitation by way of muscle conduction.

146 msec. The propagation of the premature beat elicited after this interval is represented by crosses in fig. A. Following a relatively short latency at the stimulus site, conduction along the left bundle branch is slightly delayed; activation of the myocardial sites, however, is retarded for 40 msec

at the left papillary muscle, and even more in the right papillary muscle (70 msec). This may partially be explained by the changes in refractory period as determined on the right papillary muscle, where a refractory period of 171 msec was found following the first premature beat, which is 25 msec longer than that of the right bundle branch. Thus, it appears that changes in recovery of conduction in bundle branches and myocardium follow more or less the same pattern as found by means of threshold determinations. While driving on the right papillary muscle with a steady state frequency of 450 msec basic interval (fig. B, open circles), the earliest possible response to a stimulus of 1.5 times diastolic threshold strength could be evoked after 195 msec. Again, a 25 msec difference exists between the duration of the refractory period of papillary muscle and right bundle branch, that of the bundle branch being longer. However, in the right bundle branch the delay of the premature beat, given after 195 msec to the right papillary muscle, is only 18 msec. Evidently, retrograde conduction is easier than antegrade conduction across the Purkinje-papillary junction. This seemingly paradoxical phenomenon has been observed by others [2, 82]. Mendez et al. [82] suggested this could be due to the geometrical arrangement; the Purkinje-muscle junction being a funnel-shaped system in which the terminal Purkinje fibers would form the narrow portion, while the conical part would be composed of a progressively increasing number of muscle fibers. Propagation in a muscle-Purkinje direction would take place with a larger margin of safety than in the physiological direction. In our experiment, conduction of the premature beat initiated in the right papillary muscle after 195 msec takes place partly through myocardium and partly via the bundle branch system: the anterior wall of the left ventricle was excited earlier than the left bundle branch and was presumably activated by way of muscle conduction via the interventricular septum; the posterior papillary muscle was excited later than the left bundle branch and received its excitation probably in the normal way.

After an interval of 220 msec, the refractory period of the papillary muscle was 171 msec, that of the right bundle branch 146 msec. Therefore, the second premature beat initiated in the papillary muscle should be conducted without delay over the bundle branches. Indeed, no difference existed compared with a basic beat (Fig. 31 B). In 5 other experiments in which the activation times in similar conditions were measured, essentially the same results were obtained. In three experiments, an interesting phenomenon was found. In those experiments, the right papillary muscle was stimulated at a regular rate, and premature stimuli were applied after every 10th beat at progressively shorter intervals. (Fig. 32 shows a schematic representation of the position of the electrodes during these experiments.) Fig. 33 shows recordings from one experiment. When the interval preceding the premature stimulus was shortened from 230 msec to 195 msec, the latency between premature stimulus and response of the specialized fibers in the right bundle branch (P) increased, in such a way that the P-P interval of basic and premature beat remained constant. The Purkinje spike (P) preceded the myocardial complex when the interval of the premature stimulus was 230 msec. At shorter intervals, the activation of the specialized fibers

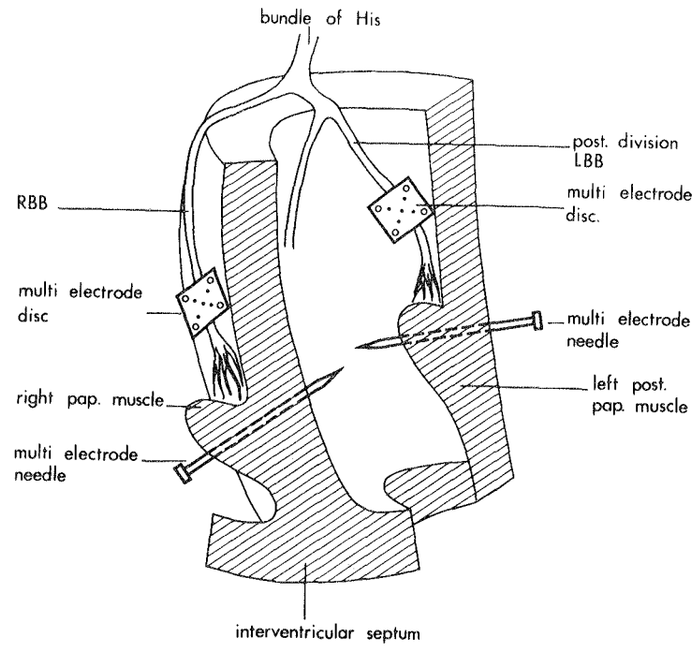

Fig. 32. Schematic representation of the position of the electrodes, used for stimulation and recording in the experiments of Figs. 31, 33, 34 and 35. Disc electrodes, carrying 5 electrode terminals, are placed on the right bundle branch (RBB) and the posterior division of the left bundle branch (LBB), about 1 cm from the Purkinje-muscle junction at the right papillary muscle and the left posterior papillary muscle. Intramural needle electrodes, carrying 10 electrode terminals, are inserted into the papillary muscles.

coincided with the activation of the underlying septal myocardium, and at still shorter intervals, it occurred later. Obviously, retrograde conduction of the premature impulse into the right bundle branch was delayed by as many msec as the premature stimulus was given earlier, which in this experiment amounted to 35 msec. Similar figures were found in other experiments. Most likely, this delay occurred in those elements having the longest refractory period, probably the peripheral Purkinje fibers at the muscle-Purkinje junction. This delay might be a very local phenomenon.

In fig. 34 it can be seen that, when the interval of the premature stimulus was shortened from 195 to 190 msec, an abrupt increase in the stimulus-Purkinje spike interval occurred. At the shortest possible preceding interval (170 msec), the interval between stimulus artefact and Purkinje spike was 170 msec. It is very unlikely that a local delay may be that long. A possible explanation is that retrograde activation of the right bundle branch by the premature impulse failed. The premature impulse was conducted exclusively via the myocardium to the left bundle branch, which conducted the impulse (possibly after a local delay) retrogradely to the common bundle

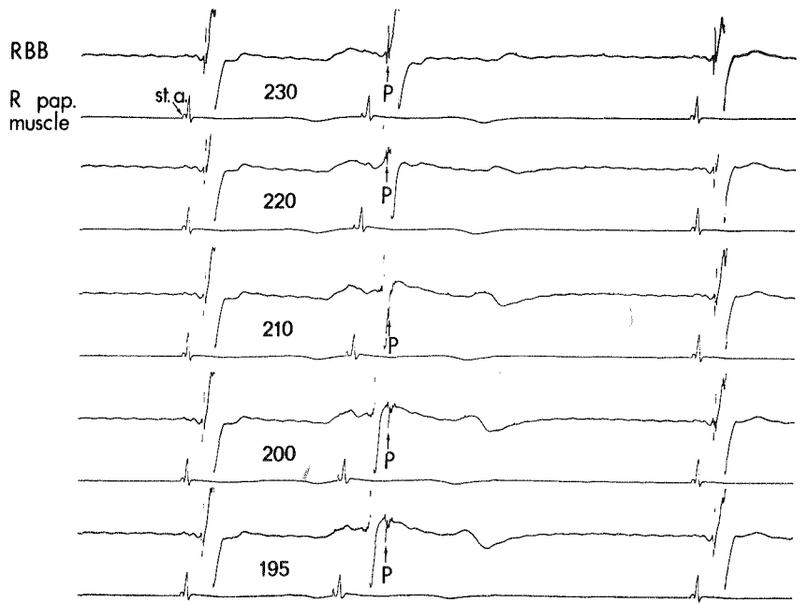

Fig. 33. Bipolar recordings from the right bundle branch (RBB) and the right papillary muscle (Rpap muscle). Stimuli were applied to the right papillary muscle (St.a. = stimulus artefact), and after every tenth basic beat, a premature stimulus was applied, at progressively shorter intervals. Note that when the stimulus interval is shortened from 230 msec to 195 msec the interval between the Purkinje spikes (P) of the right bundle branch elicited by the basic stimulus and the premature stimulus remains constant.

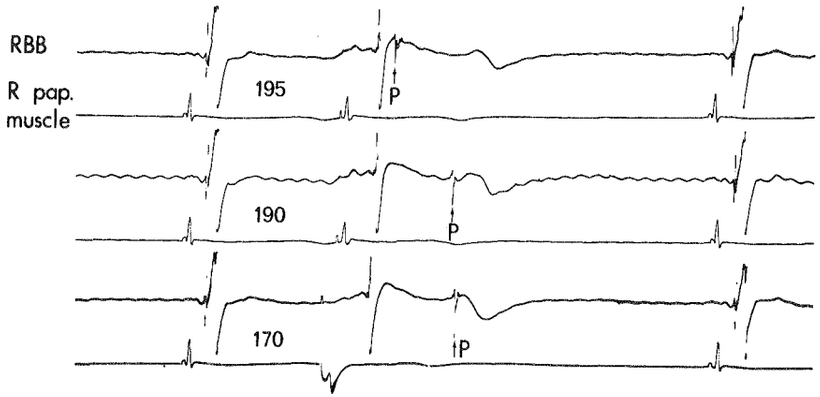

Fig. 34. Same experiment as in fig. 33. When the premature stimulus is given after 190 msec, an abrupt prolongation of the stimulus- Purkinje spike (P) interval occurs.

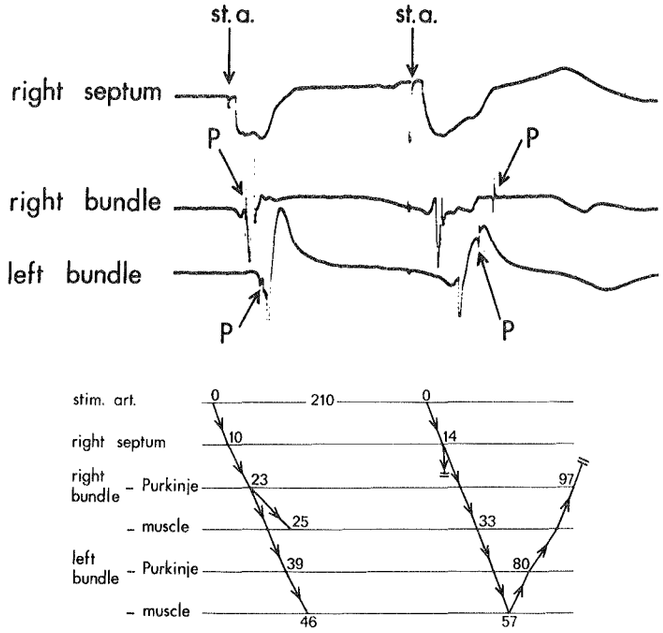

Fig. 35. Bipolar leads taken from the right side of the interventricular septum, right bundle branch and left bundle branch in an isolated heart. Stimuli were applied to the right septal surface (St.a. = stimulus artefact). The last of a series of basal stimuli (interval 600 msec) is followed by a premature stimulus after 210 msec. In the recordings of the bundle branches, the Purkinje spike (P) prededes the myocardial complex during a basic beat, and activity in the right bundle branch precedes activity in the left bundle branch. During the premature beat, the Purkinje spikes occur later than the myocardial complexes, and activity in the left bundle precedes activity in the right bundle branch. The diagram, which is not on the same scale as the recording, indicates the sequence of activation in msec. For further explanation see text.

of His. There, excitation crossed over to the right bundle branch. The impulse travelled in an antegrade direction to the recording electrode on the right bundle branch, reaching it after 170 msec. The AV node could not have played a role since it was destroyed. After one early premature beat, the refractory period of the right papillary muscle may be expected to be shorter than 170 msec, and one wonders why re-excitation of the right papillary muscle did not occur. Possibly, the longer refractory period of the peripheral Purkinje fibers prevented the impulse to reach the muscle, or the excitatory wave had a low safety factor.

In another experiment, support for this hypothesis was obtained (see fig. 35). During a basic beat, Purkinje activity in the right bundle branch preceded myocardial activity, and the impulse was conducted over the right bundle to the left bundle, which carried the excitatory wave to the left myo-

cardium. The premature impulse, applied to the right septal surface, did not invade the right bundle branch retrogradely, but was conducted by way of muscle conduction to the left ventricle, were it arrived after about 50 msec. Then, the left bundle was activated (80 msec after the stimulus artefact), and the right bundle was reached via the bundle of His, 97 msec after the stimulus artefact. Thus, although the possibility of a self-sustaining circus movement seemed present, the circle was not closed, as again no re-excitation of the myocardium on the right side took place.
In summary, premature stimuli are able to cause large differences in the moments of activation of myocardial fibers and Purkinje fibers located closely together. Both tissues can be brought out of phase in two different ways. Either by applying a premature stimulus during a regular rate to the myocardium, or by giving two successive premature stimuli to one of the bundle branches. Neither procedure, although upsetting the balance between the two tissues to a large extent, does result in an arrhythmia.

CHAPTER IV

DISCUSSION

Our results show that upon a sudden change in frequency the refractory periods of both myocardium and conducting system change in a characteristic way and reach a new steady state value only after a considerable number of beats.
This is in contrast to the earlier results of Mendez et al [80] who concluded that the duration of the refractory period is determined only by the length of the immediately preceding cycle. Shortly after part of our work had been published [65], the same group of workers published a study in which they did find a cumulative effect of cycle length on the refractory period of cardiac tissues [51]. They stated that in their earlier work premature beats and test stimuli were given after a series of 4 or 5 basic beats. Also, differences of 10 msec were not thought to be significant. In our study differences of 1 to 2 msec were reproducible. Certainly differences of 10 msec cannot be neglected. From our results it is clear that after a disturbance of a steady state rhythm a considerable number of basic beats has to be awaited before the heart is in a state of equilibrium again (see p. 34). Possibly in the study of Mendez et al [80], measurements of refractory periods were done with the heart not in a state of equilibrium.

Ionic mechanisms associated with the cardiac action potential

In the present state of knowledge no adequate explanation can be given for the complex time course of the adaptation of the refractory period to a new frequency.
Although a thorough discussion about the ionic mechanism involved in the different phases of the cardiac action potential is beyond the scope of this work, a brief outline of the factors responsible for depolarization and repolarization cannot be avoided.
Generally speaking, the level of the membrane potential is determined by the movements of ions across the cell membrane. Net inward current, caused either by positive ions moving into the cell, or by negative ions leaving the cell, will cause depolarization. Net outward current will cause repolarization. The movements of a particular ion are determined by the driving force on the ion (its electrochemical gradient), and by the ease with which the ion can penetrate the cell membrane (the permeability). The electrochemical gradient consists of an electrical and a concentration gradient. K^+ ions, for instance, have a tendency to leave the cell because the K-concentration inside the cell is much higher than outside the cell. In the resting state ("diastole") the inside of the cell is negative with respect to the outside, and this electrical gradient tends to keep K^+ inside the cell. Therefore, in the resting state net K current is practically zero, although

the permeability for K is high. As a measure for permeability usually the chord conductance g is used [127].

$$(g_{Na} = \frac{I_{Na}}{(E - E_{Na})}$$

where

g_{Na} = the chord conductance for Na^+ ions,
I_{Na} = the current carried by Na^+ ions,
E = the membrane potential, and
E_{Na} = the equilibrium potential for Na, according to the Nernst formula.)

Information about changes in conductances brought about by changes in membrane potential has been obtained largely by means of the so called voltage clamp technique. The membrane potential is changed abruptly, and kept at a constant level by a feedback mechanism. The current, necessary to keep the membrane potential constant is measured, and is equal to the ionic current. By changing the ionic concentrations in the perfusion fluid, the components of the ionic current can be elucidated.

There is a considerable amount of evidence that depolarization of a cardiac cell occurs as a result of a sudden increase in membrane permeability to Na^+ ions. The resulting rapid influx of Na^+ ions down their electrochemical gradient carries the depolarizing charge across the membrane (for references see 112, 20, 69).

Following the upstroke of the action potential there is an initial phase of rapid repolarization to about the 0 mV level, at which the membrane potential stays a considerable time. During this plateau there is a balance between inward current and outward current. The movements of at least 4 ions across the cell membrane play a role in the maintenance of the plateau.

Sodium: Following the rapid increase in permeability to Na^+ ions, resulting in the upstroke of the action potential, there is a rapid decrease in Na-permeability, called inactivation. However, inactivation is not complete, the conductance for Na^+ ions (gNa) remains at a level higher than in diastole, thus accounting for some inward current during the first 100 msec of the plateau [102, 69].

Calcium: In addition to the Na-inward current, there is an appreciable amount of Ca-inward current during the plateau, which upon depolarization is activated with a delay of 10 to 20 msec [102].

Chloride: Directly after the upstroke of the action potential there is an inward movement of Cl-ions. This outward current is partly responsible for the rapid repolarization from the peak of the action potential to the plateau level [102, 31].

Potassium: Immediately following the upstroke the K-conductance g_K decreases to a level below the resting conductance. This is called anomalous rectification [29]. During the plateau there seem to be two different ways in which the K-conductance changes. In Purkinje fibers, starting from a low level, g_K rises progressively. Because of this so called delayed rectification, the outward current carried by K-ions will eventually overcome the inward current carried by other ions, and repolarization will be initiated [95, 77]. Recent studies indicate that there are three separate slow outward

currents, not all of them K currents [96]. However, outward current carried by K^+ ions is the most important.

In ventricular muscle fibers, another mechanism is operative. The conductance for K^+ ions does not rise during the plateau, but a progressive decline in g_{Na} leads to a point where the inward current is smaller than the outward current, thus initiating repolarization [47]. Whether this mechanism is an exclusive property of muscle fibers is not clear. In Purkinje fibers, Deck and Trautwein [30] could confirm the decrease in g_K following a depolarization, but were unable to find a subsequent increase in g_K. They thought that repolarization was initiated by a decrease in g_{Na}. Following repolarization, an increase in g_K was observed.

Although a model for repolarization, in which the movements of all ions are accounted for, is lacking, there seems to be no doubt that an outward current, domineering over the inward current, and carried largely by K^+ ions is responsible for the initiation and completion of repolarization, at least in Purkinje fibers. There is evidence that an increase in the K^+ concentration on the outside of the cell membrane produces earlier repolarization. Weidmann [123] injected a K-rich solution into the coronary arteries of a turtle heart just after the onset of activity and observed a shortening of the same action potential. He suggested that normally repolarization could result from an accumulation of K^+ ions outside the membrane. Although the driving force for K would be reduced, an increased outflow of positive charge could occur if the increased K concentration on the outside of the cell would lead to an increased permeability of the membrane to K, or to a decreased permeability for Na. Weidmann pointed out that if the K^+ ions leaving the cell during the plateau would be evenly distributed over the extracellular space, the expected rise in K-concentration of about 0.6 mM would not be sufficient to cause repolarization. In order to shorten the action potential at least 10 mM of extra K had to be applied. If, however, the K^+ ions escaping the cell during the action potential would meet another ionic barrier close to it and accumulate to a high concentration in a small space, the K concentration outside the cell membrane might rise sufficiently.

Carmeliet produced evidence that a raised extracellular K concentration results in an increase in the permeability for K [14]. The membrane potential of Purkinje fibers is the same at an extracellular K-concentration of zero and of 13.4 mM. The exchange of K^+ ions at zero K-concentration was less than 30 % of that when the K-concentraiton was 13.5 mM. The often quoted experiments of Wilde and associates [125] seemed to provide evidence that an increased K-efflux exists during repolarization. Small muscle strips were loaded with radioactive K 42 , and then the preparation was perfused with non isotopic fluid. The perfusate was spread evenly on a strip of filter paper passing at constant speed through the perfusion chamber. Radio-assay revealed that K 42 efflux showed a peak coinciding with repolarization. Later experiments by Pak et al [98], however, showed that this peak was an mechanical artefact caused by contraction, since efflux profiles of isotopes which remained extracellular, such as albumine J 131, showed similar peaks. Still, if the K leaving the cell during activity would be confined largely to

a small space, the amount of K ions reaching the vast extracellular space might be so small as to escape detection. Such a small space might be the transverse tubule system.
Adrian and Freygang [1] proposed that the residual depolarization seen in skeletal muscle after a train of impulses (the afterpotential) was due to accumulation of K in the tubular system. Howell and Jenden [63] were able to destroy the transverse tubular system selectively by treating frog muscles with a Ringer solution containing 400 mM glycerol for one hour and then placing the muscles in plain Ringer solution. Gage and Eisenberg [45] found that after destroying the transverse tubular system in this way the membrane remained excitable and normal action potentials, without contractions, could be evoked. After a series of rapid impulses the afterpotential, present in control preparations, had disappeared. Efforts to produce electrical-mechanical uncoupling in heart muscle by treating the heart with glycerol solution have proved to be unsuccesful [42]. Moreover, at least in Purkinje cells of the cat heart, the transverse tubule system is very scarce [69].
Whether accumulation of K^+ ions outside the cell membrane is the factor responsible for normal repolarization in the heart remains an unsettled question. According to Mc Allister and Noble [77] "only a small change in i_K due to K-accumulation in the space can occur during depolarization of the magnitude and duration of the normal action potential... the largest time dependent change in i_K is due to delayed rectification". Of course, the mechanism of delayed rectification remains unclear.
However, accumulation of K^+ ions may play a role in the changes in action potential duration which occur when the heart rate is suddenly increased.

Effects of an increase in heart rate

In the theoretical model of Noble [95], developed for Purkinje fibers, the K conductance (g_K) rises during the action potential to a certain value at which repolarization is initiated. After the end of the action potential g_K decreases slowly. The first action potential of a new, faster rate will be shortened because g_K is still high when this action potential starts, due to the short preceding diastole, and so less time is needed to reach the value of g_K required to initiate repolarization. Noble's computed action potentials after a sudden increase in frequency also show an alternation in the duration of successive action potentials: the second action potential is longer than the first and third action potentials. The diastole preceding the second action potential is longer than the diastole preceding the first action potential. Consequently, g_K will be lower at the start of the second action potential, therefore it will take more time to reach the level at which repolarization starts, and the second action potential will be longer than the first one. This alternation goes on for an unspecified number of beats; the even action potentials are longer than the odd ones. The behaviour of these computed action potentials is strikingly similar to the behaviour of the refractory period of the bundle branches after a sudden increase in frequency in our study.

However, for myocardium the situation is different. Usually the refractory period of the second beat of a new, faster rate is shorter than that of the first beat, and also shorter than that of the third beat; the odd action potentials are longer than the even ones. Evidently a g_K which rises during the action potential and decreases in diastole cannot explain the changes in refractory period following a sudden increase in rate in the myocardium. It is of interest to note that according to Giebisch and Weidmann [47] g_K does not rise during the plateau of the action potential of ventricular myocardium, but repolarization is brought about by a decrease in g_{Na}.
Peper and Trautwein [99] found in Purkinje fibers that following a depolarizing voltage clamp to levels more negative than -30 mV, an inward current is activated. This inward current is reduced when the repetition rate of the depolarizing steps is increased. Since the inward current, of which it was not possible to determine by which ions it was carried, would slow down repolarization, its reduction at rapid rates of stimulation would be a factor in the shortening of the action potential at rapid heart rates.
As for the long period of adaption to a new steady state, it can be argued that shifts in ionic gradients may play a role.
After each activation the cell gains a small amount of Na^+ and loses a small amount of K^+. For the ionic gradients to be restored, an active, energy consuming mechanism is needed because the ions have to be "pumped" against both an electrical and a concentration gradient. The active outward movement of Na^+ and the inward movement of K^+ are at least partially linked, although not on a 1 : 1 basis. There is much evidence that adenosine-triphosphate is the source of energy for this "pumping" mechanism [69, 12]. Significantly, it has been shown that in erythrocytes the K^+ ion is able to stimulate membrane ATP-ase only at the outer surface of the membrane, whereas Na^+ can only stimulate the enzyme at the inner surface [48]. It is very likely that accumulation of Na^+ at the inside of the membrane, and accumulation of K^+ at the outside of the membrane, would result in an increase of the activity of the membrane ATP-ase, in other words in an increase of the pumping rate. When the heart rate is suddenly augmented, the pumping rate has to increase in order to prevent an increase in intracellular Na^+ and a loss of intracellular K^+. It has been shown that there is a transient net loss of intracellular K^+ (2 to 3% of the intracellular K^+) following an abrupt increase in frequency [103, 70]. This net loss of K^+ is probably linked to a net gain of intracellular Na^+. A relationship between intracellular Na concentration and heart rate has been demonstrated. At high rates the intracellular concentration is much higher than at slow rates, the variations being from 7.5 to 19 meq/l. [19]. It would seem that, following an abrupt increase in frequency, the pumping mechanism needs time before its pumping rate has sufficiently increased to keep the ionic gradients at a steady state. Langer [69] has called this adaptation the "lag phenomenon" of the sodium-pump.
As already mentioned, accumulation of K^+ ions outside the membrane leads to a shortening of the action potential. An increase in the intracellular Na^+ concentration has the same effect [16].
In a general way, the course of events following a sudden increase in heart

rate may be described as follows: In the suddenly shortened diastoles the sodium-pump is unable to completely restore the ionic gradients following each activation. Therefore, in the initial stage of a new frequency, Na^+ ions accumulate inside the cell, K^+ ions outside the cell. This results in a shortening of the action potential, and also in an increase in the activity of the sodium-pump. Eventually a steady state will be reached when the pump, working at a faster rate, is able to transport all the Na which has entered the cell during an action potential back to the extracellular milieu, and all the K which has left the cell into the intracellular space, although it has to do it in a now much shorter diastole. However, the Na concentration in the cell is now higher, and the K concentration lower, than at the slow rate and consequently the action potential is shortened. When the frequency is suddenly changed from a fast frequency to a slow frequency, the pumping rate will be high initially, and will decrease gradually together with a normalization of the ionic gradients, and with a gradual prolongation of the action potential.
In 1955, Carmeliet and Lacquet [16] already suggested that the shorter duration of the action potential at higher rates was due to changes in ionic concentrations in the immediate neighbourhood of the cell membrane. Langer [69] formulated the concept of the "lag phenomenon" of the sodium-pump. The relative insufficiency of the sodium-pump following a sudden increase in heart rate was used by Viersma [117] to explain the decrease in maximal rate of depolarization which occurs at high rates. The rise in intracellular Na, resulting from the insufficiency of the pump, leads to a decrease in driving force on the Na ion, and consequently, the speed with which Na ions enter the cell during the upstroke of the action potential is decreased. This speed determines the maximal rate of depolarization. It is known that cardiac glycosides inhibit the Na-K activated membrane ATP-ase [49]. Viersma was able to show that at fast heart rates the maximal rate of depolarization was less in the presence of therapeutic concentrations of ouabain than in the control situation. It would be of interest to study the changes in refractory period following changes in frequency under the influence of cardiac glycosides, since it may throw some light on the influence of the relative insufficienty of the sodium-pump on the changes in refractory period.

It is doubtful whether the important changes in refractory period of the very first beats of a new rate can be explained solely on the basis of the shifts in ionic gradients. Until more is known about not only the behaviour of g_K, g_{Na}, g_{Ca} and g_{Cl}, but also why these conductances change, a full explanation of these phenomena seems impossible.

The relationship between refractory period and conduction of premature impulses

There are many factors deciding whether at a given instant an impulse will be propagated, and if so at which speed. Apart from the duration of the refractory period in the different portions of the heart, a group of factors, which together determine the "strength" of the excitatory wave has to be considered. An important parameter of the excitatory efficacy of an impulse is the maximal rate of depolarization of the elements which will provide excitatory current to the cells in the pathway ahead. The maximal dV/dt is to a large extent determined by the level of the membrane potential just prior to the moment of excitation, and thus bears a relationship to the refractory period. On the other hand, it is determined by the ratio of intra- and extracellular Na concentrations. Since this ratio is changed by an increase in frequency, the Na concentration in the cell becoming higher, a decrease in maximal rate of depolarization may be expected following an increase in heart rate, because the driving force on the Na ion is diminished. This has been shown to occur in atrial muscle, and is accompanied by a decrease in conduction velocity [117]. Furthermore, the shape of the wave front is of importance. A large wave front, in which many cells fire synchronously, will provide a strong excitatory current to adjacent cells. If only a few cells are simultaneously excited, a large part of the charge which otherwise would be used to depolarize these cells, will leak away to neighbouring cells. The maximal dV/dt of the cells participating in the small wave front will be smaller, and the excitatory current will be divided over more adjacent quiescent cells than would be the case if the front were large and homogeneous. This "area factor" plays a role in structures like the AV node and the Purkinje-papillary junction. Thus, the direction of the atrial wave front is a factor determining the succesful passage through the AV node. Driving the atrium on the interatrial septum at a rapid rate causes 2 : 1 AV block, while stimuli delivered at the crista terminalis at the same frequency are all conducted through the AV node; in both instances the level of excitability of the AV nodal elements is the same. Atrial activity arising from the interatrial septum probably invades the AV node in an irregular fashion, causing a fractionated activation, and block can be observed in fibers which have a normal level of excitability, due to the diminished stimulating efficacy of the approaching wave front [64]. Propagation across the Purkinje-papillary junction is easier in the muscle-Purkinje direction than in the physiological Purkinje-muscle direction. This was found in our experiments, and in those of others [82, 2]. Mendez et al [82] suggested that it could be due to the geometrical arrangement: the Purkinje-muscle junction being a funnel-shaped system in which the terminal Purkinje fibers would form the narrow portion, while the conical part would be composed of a progressively increasing number of muscle fibers. Propagation in the muscle-Purkinje direction would take place with a larger margin of safety than in the physiological direction.
Thus, although determinations of refractory periods alone will not provide all the information needed to fully understand all the aspects of conduction of impulses in the heart, they can at least lead to some considerations about the conduction of premature impulses, and the possible significance for the occurrence of arrhythmias such as ventricular fibrillation.
The area which, in steady state conditions, has the longest refractory period distal to the AV node, is the peripheral part of the Purkinje system [94].

Early premature impulses descending along the specialized conducting system can be delayed, or even blocked in the distal Purkinje fibers. This means that the muscle mass is protected against premature impulses originating in or above the specialized conducting system. Either the premature impulse is delayed and reaches the myocardium when it has fully recovered, or the impulse is blocked. As we showed, following one premature beat, the refractory period of central Purkinje fibers is often shorter than that of papillary muscle fibers. Therefore, a second premature beat can be evoked in the bundle branches at a moment when the myocardium has not yet completely repolarized. We have also seen that this second premature beat is considerably delayed during its propagation to the ventricular muscle mass. Unfortunately, we do not know whether the delay took place in the distal Purkinje fibers or in the myocardium itself. Neither do we know whether the distal Purkinje fibers have a shorter refractory period following an early premature beat than the myocardial cells. If the second early premature beat were delayed in the distal Purkinje fibers, the myocardium probably would be excited when it had recovered completely its excitability, and propagation through the myocardium would proceed in a regular, uniform fashion. If, on the other hand, the myocardium was excited prior to complete recovery, i.e. in its "vulnerable period", the stage might be set for the initiation of ventricular fibrillation.

The "vulnerable period"

At this point a discussion of the meaning of the "vulnerable period" is in order. As early as 1920, De Boer [6] observed that induction shocks applied near the end of the systole could induce fibrillation in frog's hearts which were in a bad condition. Similar findings were reported on the mammalian heart [126]. Wiggers and Wégria [124] coined the name "vulnerable period", during which alone stimuli are effective in inducing ventricular fibrillation. This period roughly corresponds to the T wave of the electrocardiogram. Brooks and associates [9] related the vulnerable period to the so-called "dip" phenomenon, observed in strength-interval curves determined by bipolar stimulation. In the normal heart, thresholds for fibrillation were much higher than for extrasystoles, but, for instance vagal stimulation decreased the threshold for fibrillation towards that for extrasystoles. Van Dam et al [25] showed that the "dip" phenomenon (a period of hyperexcitability during the relative refractory period) was true only for anodal stimulation. Hoffman and Cranefield [56, 58] conclude that fibrillation evoked by strong stimuli during the vulnerable period results from the effects of anodal current. Van Dam was unable to induce fibrillation by applying strong anodal currents during the "dip" via a small electrode. He concluded that a large area had to be stimulated for fibrillation to occur [23]. Opinions about the necessary intensity of the stimuli differ: Hoffman and Cranefield [58] state that the fibrillation threshold is about 20 times as great as for diastolic stimulation; others give a figure of several 100 times the diastolic threshold [89]. At any rate, stimuli which can initiate fibrillation in the normal heart are many

times the safety factor of a normal action potential. Moreover, fibrillation is probably the result of anodal excitation so that the question arises whether the vulnerable period might be an artifact related to the technic. If the vulnerable period were to have a physiological meaning, spontaneous early premature beats falling in the relative refractory period of a preceding beat should be able to initiate fibrillation. The report by Preston, Mc Fadden and Moe [101] suggests that this is possible. In hearts of infant goats and swine, the AV conduction system recovers excitability before the ventricular myocardium. An early premature atrial response, arriving before the completion of the T wave was able to initiate ventricular fibrillation: a later premature atrial impulse caused aberrant ventricular conduction without fibrillation. Moore and Swain [93] studied the properties of a substance U-0882, which prolongs the action potential and refractory period of ventricular muscle but does not influence conduction in the His-Purkinje system; after administration of this substance, a premature atrial beat may reach the ventricles early in the T wave and initiate ventricular fibrillation in anaesthetized adult dogs.

For a long time, clinical studies have emphasized the role of early premature beats in the initiation of fibrillation. In cardiac patients, there may be a relation between the occurrence of ventricular extrasystoles interrupting the preceding T wave and fibrillation ("R on T phenomenon", [109, 108]). Interruption of the T wave was observed experimentally during the phase of chaotic rhythm preceding ventricular fibrillation which was induced by adrenaline given after the administration of amarine [40] . If an early extrasystole, originating spontaneously, can induce fibrillation the most likely explanation is that the premature beat is propagated in an irregular fashion due to the fact that it encounters fibers with different levels of excitability. In areas with a long refractory period the extrasystole would be blocked, in areas with a shorter refractory period the impulse would be propagated. Propagation in partially refractory tissue occurs at a subnormal speed [28] ; conduction may be decremental in some regions, in other regions conduction velocity may increase as the wave front reaches tissue in a more advanced state of recovery. In any case, spread of excitation is irregular and the area which was originally bypassed may be re-excited by a wave front travelling slowly along a tortuous route. As a result of re-entrant activity, differences in refractoriness will increase since different areas in the heart have been excited at various preceding cycle lengths. The re-entrant activity may again invade the heart, and fractionation of conduction velocity and refractory periods will increase, eventually involving the whole heart which is then fibrillating. The concept that non-uniformity of refractory periods may play a key role in the initiation of ventricular fibrillation is widely accepted [87, 90, 9, 88, 58].

The physiological basis for re-entry has been given by Hoffman and associates [58, 54, 55]. When the membrane potential is low at the moment of excitation the newly evoked action potential will be conducted with decrement. A decreased membrane potential may be the result of incomplete repolarization, or of the slow depolarization which is present in automatic fibers [107]. Decremental conduction has been defined as a mode of conduction where the

action potential, because of a low amplitude and a slow rate of rise becomes progressively less effective as a stimulus to cells in the pathway ahead [56]. Thus, conduction velocity diminishes progressively and complete failure of conduction may be the result. If the appropriate anatomical arrangement exists, the area of block may be invaded in a retrograde fashion by activity which, via another route, bypassed the region of block. Local re-entry may occur. The pathways involved may be very small, i.e. a few millimeters. According to Hoffman, the most likely areas for these phenomena are the peripheral Purkinje branches and the attached ventricular muscle [54, 55]. The same mechanism could exist in the myocardium itself, provided there are local differences in refractory period. However, there are few studies in which re-entrant activity occurring on the basis of differences in refractory periods has been proven. Wallace and Mignone [118] created differences in effective refractory period of the order of 80 msec between areas 2 mm apart in the wall of the left ventricle of dogs by cooling of the epicardium. Early premature supraventricular impulses were conducted from endocard to epicard with a delay of as much as 90-150 msec; non stimulated extrasystoles occurred which were propagated from epicardium to endocardium as could be ascertained by the inverted polarity of a bipolar lead of the subendocardial layer, and therefore probably originated from the intramural portion of the wall which was cooled. Very frequently, runs of tachycardia, degenerating in ventricular fibrillation followed the first non-stimulated beat. Watanabe and Dreifus [119] recorded transmembrane potentials in the hypothermic dog heart in situ simultaneously with two micro-electrodes; they found differences in action potential duration, as a cause of which an early premature systole fully activated one fiber, but only incompletely depolarized another fiber. Rapid development of asynchrony between the fibers followed, suggesting a slow irregular spread of excitation and local block, resulting in ventricular fibrillation. These authors defined the vulnerable period as "the period during which fiber excitability differs in various portions of the ventricle" [119, 120]. Thus, local temperature differences may create local differences in refractory period and, if a premature impulse is either induced or develops spontaneously, re-entry and ventricular fibrillation may occur. Incidentally, the same factor which may lead to ventricular fibrillation initiated by a premature impulse (non-uniform recovery of excitability) has been thought to account for the necessary premature impulse. When adjacent fibers repolarize at different rates, the potential difference may be great enough to cause re-excitation of the fiber which recovers earlier [58, 22]. Moore, Morse and Price [91] showed that in a Purkinje-papillary muscle preparation under the influence of adrenaline a marked difference was created between the duration of a transmembrane action potential of the Purkinje fiber and of the papillary muscle, the former being longer. Often they observed two responses in the papillary muscle, where only one action potential occurred in the Purkinje fiber; the coupled beat could well have resulted from re-excitation due to the delay in repolarization of the Purkinje fiber. A decrease in the extracellular calcium concentration results in a delay in repolarization in fibers of the sino-atrial node before there is any change in the adjacent atrial muscle. Repetitive firing of the atria may be

observed following each sinus nodal action potential [76]. Mendez et al [81], however, raised serious objections against the idea that coupled extrasystoles could be generated by the differences in potential between adjacent cells which repolarize at different times. This group of investigators demonstrated that the intracellular connections at the Purkinje-papillary junction offer a relatively low resistance to current flow [81, 82]. "During repolarization the current flowing between neighbouring elements with intrinsically different repolarization times should therefore minimize the disparity in action potential duration on either side of the junctional site; a continuously graded change in duration would result" [81]. The important question arises whether asynchrony in recovery of excitability occurs in a normal heart. Han and Moe 50 determined refractory periods at 12 different points within a radius of 4 mm from a primary site of stimulation, in a variety of conditions known to favour the occurrence of ventricular fibrillation. Temporal dispersion of recovery of excitability increased during sympathetic nerve stimulation, toxic doses of ouabain, ischemia, while these factors also caused a general shortening of the refractory period. An increase in the duration of the refractory period, together with an increase in temporal dispersion of recovery was found during the administration of chloroform, and high doses of quinidine, and during hypothermia. Also, these authors found that an early premature beat caused a shortening of the refractory period and an increase in temporal dispersion of recovery. Temporal dispersion was minimal after a basic beat (the difference between longest and shortest refractory period being 19 msec), but increased after one premature beat (the difference between longest and shortest refractory period being now 37 msec). A "late" premature beat resulted in a moderate dispersion of recovery. The authors conclude: "It is likely that the degree of non-uniformity of recovery of excitability should become greater and greater after each successive beat in a train of repetitive premature beats. Because of the danger of fibrillation, direct demonstration of this was not attempted." Since Han and Moe applied the premature stimulus (S2) to a central electrode, the possibility exists that differences in conduction time to the various testing sites might account for the differences in refractory period at these sites, since the preceding cycle length might not be the same for all testing sites. Their figures, however, show that the S2-R2 intervals for all 12 testing sites are equal. In the study of Han and Moe, about 5 minutes were needed to assess the refractory period at all 12 sites. In our experiments, 30 to 40 minutes were needed to measure the refractory periods at 12 intramural sites, partly because of the necessity to let the heart stabilize for a sufficient number of beats following a rhythm disturbance. A possible explanation for the difference between the results of Han and Moe and our results could be, that in their study test pulses were given too quickly in succession, without allowing the heart to reach a steady state after each interruption of the basic driving rate. Possibly the number of regular beats between each pair of premature and testing stimuli was not always the same, which might account for the finding of an increased temporal dispersion of excitability following one premature beat. Our results do not lend support to the concept that early premature beats cause an in-

crease in the differences between refractory periods at different intramural sites, thereby increasing the likelihood of the occurrence of re-entry and fibrillation. Neither during a regular rhythm, nor during the first beats of a new, faster rate, nor during a series of early premature beats, did the standard deviation of the refractory periods of different intramural sites differ more than about 10 msec. Moreover, no differences between the standard deviations of the refractory periods during a steady state rhythm and those following premature beats was found. The difference between shortest and longest refractory period could sometimes be as much as 40 msec, but this figure is not representative for the temporal dispersion, since it is determined only by the findings at 2 sites. Furthermore, it may be that our findings are an exaggeration of the real situation, since in the course of the experiment the duration of the refractory period may change somewhat, due to slight variations in temperature or level of anaesthesia. Moreover, we observed only slight abberations in the conduction of impulses elicited in the relative refractory period, which were confined to the area immediately surrounding the stimulating electrode. The existing local differences in refractory period were not large enough to cause substantial irregularities in conduction. Thus, even when the myocardium is excited in its relative refractory period, possibly by a second early premature beat originating in the specialized conducting system, there would be no reason for the excitatory wave to proceed in an irregular way, and there would be no reason for re-entry to occur. In our experiments we subjected the normal heart to complex stimulation patterns, either stimulating the specialized conducting system or myocardium, but never observed as a result multiple responses or fibrillation.

SUMMARY

The refractory period of cardiac fibers is a function of heart rate; it is short at rapid rates and long when the heart beats slowly. This is an established fact, as is evident from the review of the literature in chapter I. Less agreement exists concerning the question whether upon a sudden change in heart rate, the refractory period changes immediately to a new, stable value, or changes gradually. Few data are available about the beat to beat changes in refractory period following a sudden change in frequency. In this study of transient changes in refractory period following alterations in rate and rhythm, different types of multi-electrodes for stimulation and recording of both the ventricular myocardium and the main branches of the specialized conducting system, were used. The diameter of the electrode terminals was 0.1 mm, and through it, square wave cathodal current pulses of variable strength and a duration of 1 to 2 msec were passed to stimulate the heart. The electrical activity was recorded in the close vicinity of the stimulation electrode (2 mm), and from areas further away. A stimulator developed in the laboratory of Medical Physics enabled us to deliver various complicated stimulation patterns. Because a total atrioventricular block was made, the spontaneous activity of the ventricles had a slow rate (cycle length was usually longer than 1 second) and did not interfere with the stimulation pattern. The details of the experimental procedure, as well as the operating techniques, and methods to perfuse the isolated heart, are described in chapter II.

In chapter III the results are presented.

First of all, it was established that for the ventricular myocardium several hundred beats of a new heart rate are needed before the refractory period has reached a new equilibrium. This is true both for a sudden increase, and for a sudden decrease in heart rate. In the first 20 beats, about 50% of the total change in duration of the refractory period occurs.

When the heart rate is suddenly increased, the refractory period is shortened considerably (20 to 30% of total shortening) by the first beat of the new rate. Usually, the second beat accounts for a further shortening to 30 to 50% of total shortening. Subsequent beats have a much smaller effect on the refractory period, and an alternation in the duration of the refractory period in successive beats occurs. Thus, the refracory period of the third beat is longer than that of the second and fourth beat. Gradually, the alternation disappears. The alternation is more marked in the heart in situ than in the isolated heart. In some hearts, especially when the difference between slow and fast rate is large, the alternation starts already after the first beat: the refractory period of the second beat is longer than that of the first and third beat. This type of change occurs particularly in subepicardial layers, subendocardial layers display the type of alternation mentioned first. The "amplitude" of the alternation is discrete: the difference in duration of the refractory periods in successive beats is never more than 10 msec.

When strength-interval curves were made after each of the first 5 beats of a new, faster rate, it was found that the final phase of recovery occur some-

what more slowly than during the steady state of the slow rate.
The effect of short episodes of successive rapid beats, introduced during a steady state slow heart rate, on the refractory period after the original rate has been restored, was studied. The influence of one premature beat on the refractory period of subsequent basic beats can be detected for at most 5 beats. After, for instance, 10 rapid beats, about 20 basic beats are needed to restore the refractory period to the steady state level. Thus, the heart has a "memory" which extends over many previous cycles. It is important to realize that previous changes in rhythm may influence the duration of the refractory period at a given moment.
When, departing from a steady state fast heart rate, the driving rate is suddenly decreased, the refractory period lengthens. About 25 to 30% of total lengthening is accomplished by the first beat of the slower rate. Subsequent beats have a very small effect on the refractory period, which gradually lengthens over several hundred beats until the steady state value for the slow frequency is reached. No alternation between the refractory periods of subsequent beats occurs.
Determinations of the "functional refractory period" (the shortest interval between two propagated responses) showed that the recovery of the ability to conduct impulses occurs parallel to the recovery of excitability, provided test pulses of low intensity (1.5 times diastolic threshold) are used. The interval between moment of stimulation, and arrival of the excitatory wave at the electrode terminal located 2 mm from the stimulus site, was compared for basic beats and for impulses evoked by the earliest possible test pulse. Usually, the difference was only a few msec. The largest difference found was 7 msec. Conduction of the impulse elicited at the end of the "refractory period" (shortest interval between driving stimulus and test stimulus with an intensity of 1.5 times diastolic threshold) along electrode terminals located further than 2 mm away from the site of stimulation, occurred at the same velocity as during basic beats. Thus, the delay of impulses elicited during the relative refractory period, is confined to an area close to the site of stimulation. Experiments in which refractory periods were determined at many (up to 21) intramural sites, showed that the changes in refractory periods following sudden changes in frequency, occur in phase throughout the myocardium. Although local differences in refractory period exist, no increase in temporal dispersion of refractory periods was found when the heart rate was suddenly increased or decreased. Also, no increase in the range of variation was found when a series of 4 early premature beats (each preceded by the shortest possible cycle length) was applied to the heart. Furthermore, no important irregularities in the conduction of these successive premature beats were found.
The refractory period of left and right bundle branches was measured in isolated hearts, perfused according to the Langendorff technique. Both an artificial perfusion fluid and heparinized blood obtained from a second dog, were used to perfuse the heart.
In steady states, the refractory period of the bundle branches is longer than that of the myocardium. At slow rates the difference in duration is most marked. At very rapid rates both refractory periods are shortened

and approach a common value. After a sudden increase in heart rate, the refractory periods of bundle branches and myocardium are out of phase. In the bundle branches, shortening of the refractory period after the first beat greatly exceeds that in myocardium: the refractory period of the bundle branches becomes even shorter than the myocardial refractory period. After the second beat the refractory period of the bundle branches lengthens again, by as much as 40 msec, and is now longer than the refractory period of the myocardium. The alternation between shorter and longer refractory periods in odd and even beats continues for more than 20 beats, gradually decreasing in size. The difference in adaptation of the refractory periods of both tissues to sudden rate changes is reflected in the conduction of premature impulses. An early premature beat, originating during a steady state frequency in the bundle branches, reaches the myocardium when it has recovered its excitability and is conducted without difficulty. A second early premature beat, however, meets refractory myocardium and is delayed by up to 70 msec.
In the discussion (chapter IV), a short review of the ionic mechanisms associated with the cardiac action potential is given. Although different authors do not agree about every aspect, it can be said, that repolarization is initiated when outward current (positive ions leaving the cell) dominates inward current. This may be caused by a voltage and time dependent increase in the permeability of the cell membrane for K^+ ions, or by a decrease in the permeability for Na^+ ions. An increase in the permeability for K^+ ions may be caused by an accumulation of K^+ ions at the outside of the cell membrane. The role of the so called sodium-pump (an energy consuming mechanism which restores the ionic gradients across the cell membrane after each action potential) in the long term changes in refractory period after sudden changes in heart rate, is discussed. Following an abrupt increase in heart rate, this ionic "pump" is unable to transport all the K^+ ions which left the cell during activity back into the cell, and all the Na^+ ions which entered the cell back to the extracellular space, because of the suddenly shortened diastole. This relative insufficiency of the "pump" causes an accumulation of K^+ ions outside the cell and an accumulation of Na^+ ions inside the cell. Both factors shorten the action potential, and thus the refractory period. Also, they may lead to an increased "pumping" rate. Eventually, due to the increased "pumping" rate, a further accumulation of ions is prevented, and a steady state is reached, in which the Na^+ content of the cell is higher than during the slow rate, and the K^+ content is lower. When the heart rate is suddenly decreased, the "pump", initially working at a high rate, slowly restores the ionic gradients, and gradually decreases its activity. Together with the normalization of the ionic gradients, the action potential lengthens. No adequate explanation of the alternation in refractory period, observed during the initial stages of a new, faster heart rate, could be given.
Finally, the relationships between duration of refractory periods and conduction, and their possible role in the initiation of arrhythmias, are discussed. The concept of re-entry plays a central role in the attempts to understand the initiation of certain arrhythmias, such as fibrillation. If a

premature impulse propagates through tissue in which substantial differences exist in the duration of the refractory period of neighbouring elements, propagation may fail in those areas which have the longest refractory period. These areas would then be available for re-excitation, provided the appropriate anatomical conditons exist. In addition to non uniform recovery of excitability, low conduction velocity and short refractory periods favour the occurrence of re-entry. We found that large differences in the duration of refractory periods of myocardial fibers and fibers of the specialized conducting system may occur. An early premature beat, elicited during a regular frequency in the right papillary muscle, may fail to propagate retrogradely into the right bundle branch, because the refractory period of the specialized conducting system is longer than that of myocardium. The premature impulse is conducted through the myocardium to the left bundle branch, which has recovered its excitability by the time of its arrival. The impulse is conducted retrogradely over the left bundle, and reaches the right bundle via the common bundle of His. The stage might be set for re-excitation of the right papillary muscle, and once the circle would be completed, this sequence might be repeated, leading to ventricular tachycardia. Although on occasion we found that right papillary muscle and right bundle branch were out of phase by as much as 170 msec, we never saw a completed circuit leading to re-excitation. Apparently, in the normal dog's heart, the refractory periods and conduction velocities of the different elements, and the length of the pathway the impulse has to travel, are so delicately balanced as to prevent the establishment of a circus movement.
In the experiments of Han and Moe [50], it was found that in ventricular myocardium there was a significant increase in the range of variation of refractory periods at different sites after the introduction of an early premature beat. It was supposed that the degree of non-uniformity in recovery of excitability should increase with each beat of a series of repetitive premature beats, and that eventually multiple re-entries would degenerate into ventricular fibrillation. In contrast, we concluded from our experiments, that no such increase in temporal dispersion of refractory periods occurs. The range of variation remained about the same during of a series of 4 early premature beats. There was only a small increase in conduction time of the premature impulses, which was localized in the immediate vicinity of the site of stimulation. Conditions, necessary for re-entry to occur, could not be created by inducing multiple premature beats, nor by other changes in rate or rhythm. The normal dog's heart seems very well protected indeed against arrhythmias.

SAMENVATTING

De duur van de refractaire periode van hartcellen hangt af van de hartfrequentie. Hoe sneller het hart kopt, hoe korter de refractaire periode. Uit het litteratuuroverzicht blijkt, dat er weinig gegevens zijn over de wijze waarop de refractaire periode verandert na veranderingen van de hartfrequentie. Volgens sommige onderzoekers heeft de duur van de refractaire periode direct na de eerste slag van een nieuwe frequentie reeds een stabiele waarde, horende bij die frequentie. Andere gegevens wijzen erop, dat na een plotselinge frequentieverandering een zekere aanpassingsperiode bestaat, gedurende welke de refractaire periode geleidelijk verandert.
In dit onderzoek werden refractaire perioden bepaald van het ventrikelmyocard, en van de bundeltakken van het specifieke geleidingsysteem van het hondehart, vóór, en na diverse frequentieveranderingen. Naaldelectroden, die 10 platina electroden bevatten met een diameter van 0,1 mm, werden gebruikt om electrische prikkels aan het myocard toe te dienen, en om de locale electrische activiteit op diverse plaatsen in het hart af te leiden. Schijfvormige electroden, die meerdere electrode uiteinden bevatten, werden gebruikt voor prikkelen en afleiden van het specifieke geleidingsysteem. Met behulp van een prikkelaar, ontworpen en vervaardigd in het laboratorium voor Medische Fysica, konden ingewikkelde prikkelpatronen, nodig om de refractaire periode in tal van situaties te meten, aan het hart aangeboden worden. De gebruikte prikkels waren kathodale stroompulsen, met een duur van 1 of 2 msec, en met een regelbare intensiteit. De intervallen tussen de prikkels konden zodanig worden gevarieerd, dat de duur van de refractaire periode met een nauwkeurigheid van 1 msec kon worden bepaald. De details van de experimentele procedure zijn beschreven in het tweede hoofdstuk.
In het derde hoofdstuk worden de resultaten vermeld.
Allereerst werd vastgesteld, dat het hart na een frequentieverandering gedurende enkele honderden slagen moet kloppen, alvorens de refractaire periode een stabiele duur heeft. Ongeveer 50% van de totale verandering in duur van de refractaire periode vindt plaats in de eerste 20 slagen van een nieuwe hartfrequentie.
Tevens kon worden gemeten hoe de refractaire periode van slag tot slag verandert na een frequentie verandering. Zo bleek, dat 20-30% van de verkorting die de refractaire periode uiteindelijk ondergaat, veroorzaakt wordt door de eerste slag van een nieuwe hogere frequentie. De tweede slag bewerkstelligt gewoonlijk een verdere verkorting tot 30% of 50%. De invloed van volgende slagen op de duur van de refractaire periode is veel geringer, terwijl bovendien een alternans optreedt in de duur van opeenvolgende refractaire perioden. De refractaire periode van de derde slag is langer dan die van de tweede en vierde slag. Deze alternans, die zich in het hart in situ duidelijker manifesteert dan in het geïsoleerde hart, verdwijnt geleidelijk na een twintigtal slagen. In sommige gevallen, vooral wanneer het verschil tussen lage en hoge frequentie groot is, begint de alternans reeds na de eerste slag. De refractaire periode van de tweede slag is dan langer dan die van de eerste en van de derde slag. Dit type alternans werd vooral

gezien in subepicardiale lagen. Subendocardiale lagen vertoonden het eerder genoemde type. De "amplitude" van de alternans is gering; het verschil in duur van opeenvolgende refractaire perioden was nooit groter dan 10 msec. Drempelwaarde-interval curven, gemaakt na elk van de eerste vijf slagen van een nieuwe, hogere hartfrequentie, lieten zien, dat tijdens deze overgangsperiode de laatste fase van het herstel van de prikkelbaarheid iets trager verloopt dan tijdens de evenwichtstoestand. Het effect van één, of meerdere extrasystolen, toegediend tijdens een langzame basisfrequentie, op de duur van de refractaire periode van de erop volgende basisslagen, werd onderzocht. De invloed van één extrasystole strekt zich uit tot ten hoogste vijf erop volgende basisslagen. Na een serie van, bijvoorbeeld, tien slagen van een hoge frequentie, zijn ongeveer twintig basisslagen nodig om de refractaire periode naar haar uitgangswaarde te doen terugkeren. Het hart heeft dus een "geheugen", dat zich over vele voorafgaande cycli uitstrekt. Het is van belang te beseffen dat voorafgaande frequentieveranderingen de duur van de refractaire periode op een bepaald moment kunnen beïnvloeden. Wanneer, uitgaande van een hoge hartfrequentie, de frequentie plotseling verlaagd wordt, neemt de duur van de refractaire periode toe. De eerste slag van de nieuwe frequentie veroorzaakt 25% tot 30% van de totale verlenging. Tijdens de volgende slagen neemt de duur van de refractaire periode zeer geleidelijk toe, tot na enige honderden slagen een stabiele waarde bereikt wordt. Een alternans doet zich hierbij niet, of nauwelijks, voor.

De "functionele refractaire periode", gedefinieerd als het kortste interval tussen twee voortgeleide impulsen, bleek slechts enkele msec langer dan de "refractaire periode", gedefinieerd als het kortste interval tussen twee succesvolle prikkels. Een activatiefront, opgewekt aan het eind van de "refractaire periode", door een prikkel met een sterkte van anderhalf maal de diastolische drempelwaarde, ondervindt een geringe vertraging in de onmiddellijke nabijheid van de prikkelelectrode. Hierdoor arriveert dit front enkele msec later, hooguit 7 msec later, bij een electrode, 2 mm verwijderd van de prikkelelectrode, dan het activatiefront opgewekt door de laatste basisprikkel. De impulsgeleiding langs electroden, verder dan 2 mm van de prikkelelectrode verwijderd, vindt altijd met dezelfde snelheid plaats.

Experimenten, waarin de refractaire perioden op meerdere plaatsen in het myocard bepaald werden, wezen uit, dat de veranderingen in refractaire perioden na frequentieveranderingen in het gehele myocard min of meer in fase verlopen. Hoewel er locale verschillen in duur van de refractaire periode bestaan, en hoewel, bijvoorbeeld, subepicardiale lagen en subendocardiale lagen hun refractaire perioden niet op precies dezelfde wijze veranderen, nemen de verschillen in refractaire perioden niet toe na een plotselinge stijging, of daling, van de hartfrequentie. Dit geldt ook na een serie van vier, zo vroeg mogelijk na elkaar toegediende, extrasystolen. Evenmin deden zich belangrijke afwijkingen in de voortgeleiding van extrasystolen door het myocard voor.

De refractaire perioden van rechter- en linker bundeltak werden bepaald in het geïsoleerde, volgens Langendorff doorstroomde, hart. Als perfusievloeistof werd zowel een gewijzigde Tyrode oplossing gebruikt, als gehepariniseerd bloed, afkomstig van een tweede hond. Tijdens "steady states" is de refractaire periode van vezels van het specifieke geleidingsysteem langer dan die van myocardvezels. Het verschil in duur is het grootst bij lage hartfrequenties. Bij zeer hoge frequenties zijn beide refractaire perioden verkort, en naderen een gemeenschappelijke waarde. Tijdens de eerste slagen van een nieuwe, hogere hartfrequentie zijn de refractaire perioden van myocard en specifiek geleidingsysteem uit fase. De verkorting van de refractaire periode van de bundeltakken overtreft verre de verkorting in het myocard. Hierdoor wordt de refractaire periode van de bundeltakken zelfs korter dan die van het myocard. Na de tweede slag is de refractaire periode van de bundeltakken 40 tot 50 msec langer dan na de eerste slag, en nu weer langer dan de refractaire periode van het myocard. De alternans tussen kortere en langere refractaire perioden in oneven en even slagen zet zich voort tot na de twintigste slag, en dempt geleidelijk uit. Het verschil in aanpassing van de refractaire perioden van beide weefsels aan een nieuwe frequentie, komt tot uiting in de voortgeleiding van bepaalde extrasystolen. Zo zal een vroege extrasystole, opgewekt in een van de bundeltakken, het myocard bereiken na afloop van zijn refractaire periode, en zonder moeite worden voortgeleid. Een tweede vroege extrasystole stuit op refractair myocard en ondergaat een vertraging in de orde van 70 msec.

In de discussie (vierde hoofdstuk) wordt een kort overzicht gegeven van de rol die diverse ionen spelen bij het tot stand komen van de actiepotentiaal. Hoewel er verschil van mening bestaat over verschillende details, kan gesteld worden dat repolarisatie begint wanneer de uitwaardse stroom over de celmembraan, veroorzaakt door het uit de cel treden van positieve ionen, overheerst over de inwaardse stroom. Het herstel van de prikkelbaarheid valt samen met de repolarisatiefase. De toename van de uitwaardse ionenstroom kan veroorzaakt worden door een tijd- en voltage afhankelijke toename in de permeabiliteit van de celmembraan voor K^+ ionen, of door een afname in de permeabiliteit voor Na^+ ionen. Een toename in de permeabiliteit voor K^+ ionen kan veroorzaakt worden door een ophoping van K^+ ionen aan de buitenzijde van de celmembraan.

De rol van de zgn. Natrium-pomp bij de aanpassing van de refractaire periode aan een nieuwe hartfrequentie wordt besproken. De Natrium-pomp is een energie verbruikend mechanisme, dat de ionenconcentratiegradiënten na elke actiepotentiaal herstelt. Na een plotselinge stijging van de hartfrequentie kan deze pomp niet alle K^+ ionen die de cel verlaten hebben, en alle Na^+ ionen die de cel zijn binnengedrongen, verwerken, als gevolg van de ineens veel kortere diastole. Hierdoor hopen K^+ ionen zich op aan de buitenzijde van de celmembraan, en neemt de intracellulaire Na^+-concentratie toe. Beide factoren verkorten de actiepotentiaal, en daarmee de refractaire periode. Bovendien leiden zij mogelijk tot een activatie van het pompmechanisme, dat, sneller werkend, een verdere ophoping van ionen aan weerszijde van de celmembraan voorkomt. In de "steady state" veranderen de ionengradiënten niet meer, al is de Na^+-concentratie in de cel hoger dan tijdens de lage frequentie, en de K^+-concentratie lager. Wordt de frequentie plotseling verlaagd, dan zal de pomp, aanvankelijk snel werkend, de ionencon-

centratiegradiënten normaliseren, wat gepaard gaat met een verlenging van de actiepotentiaal en van de refractaire periode. Deze verklaring is hypothetisch. Een verklaring voor de alternans in de duur van de refractaire periode van opeenvolgende slagen tijdens de eerste fase van een nieuwe, hogere hartfrequentie, werd niet gevonden.
Tenslotte wordt de samenhang besproken tussen refractaire periode en impulsgeleiding, en beider betekenis voor het ontstaan van ritmestoornissen. Het begrip "re-entry" speelt een grote rol in de pogingen om het ontstaan van fibrilleren te begrijpen. Wanneer een vroegtijdige impuls zich uitbreidt in weefsel, waarin de refractaire periode niet in alle elementen dezelfde is, kan geleidingsblock ontstaan in de cellen met de langste refractaire periode. Die cellen zijn dan beschikbaar voor "re-entry". D.w.z., zij kunnen later geactiveerd worden vanuit het door de vroegtijdige impuls wèl geactiveerde gebied, mits de anatomische verhoudingen gunstig zijn, en de impuls opnieuw doorgeven aan elementen die inmiddels van de vorige activatie hersteld zijn. Een lage geleidingsnelheid en een korte refractaire periode bevorderen het optreden van "re-entry". Wij vonden dat grote verschillen in duur van de refractaire perioden van myocard en specifiek geleidingsysteem kunnen optreden. Een vroege extrasystole, opgewekt door vroegtijdige prikkeling van de rechter papillairspier tijdens een regelmatig ritme, kan, als gevolg van de langere refractaire periode van de rechterbundel, deze niet retrograad activeren. De impuls wordt door het myocard voortgeleid naar de linkerbundel, waarvan inmiddels de refractaire periode beëindigd is. De impuls wordt retrograad door de linker bundel voortgeleid, en bereikt, via de bundel van His, de rechter bundel. Wanneer de rechter papillairspier opnieuw, vanuit de rechter bundel, geactiveerd zou worden, was een complete cirkelbeweging ontstaan, die zich voortdurend zou kunnen voortzetten. Dit zagen wij nooit gebeuren, hoewel het verschil in activatiemoment van rechter papillairspier en rechterbundel soms 170 msec bedroeg. Blijkbaar zijn in het normale hondehart refractaire perioden, en geleidingsnelheden van de diverse structuren, alsook de afstand welke de impuls moet afleggen, dermate met elkaar in evenwicht, dat, zich zelf onderhoudende, cirkelbewegingen niet kunnen ontstaan.
Han en Moe [50] vonden dat de verschillen in refractaire perioden, op meerdere plaatsen in het myocard bepaald, na een vroege extrasystole aanzienlijk groter zijn dan na een basisslag. Zij veronderstelden dat de mate van ongelijkheid in refractaire perioden op diverse plaatsen zou toenemen na elke slag van een reeks, zo vroeg mogelijk na elkaar toegediende, extrasystolen. Uiteindelijk zouden dan meerdere "re-entry" circuits kunnen ontstaan, wat tenslotte tot ventrikelfibrilleren zou kunnen leiden. Wij konden uit onze experimenten niet concluderen dat de verschillen in duur van de refractaire perioden, op meerdere plaatsen in het myocard gemeten, groter werden na een reeks van vroege extrasystolen. De gemiddelde refractaire periode werd korter, de onderlinge verschillen bleven gelijk. Slechts geringe veranderingen in de voortgeleiding van de extrasystolen werden waargenomen, die zich beperkten tot de onmiddellijke omgeving van de prikkelelectrode. Omstandigheden waarin "re-entry" mogelijk zou zijn, konden door ons door geen enkele frequentie-, of ritmeverandering, worden geschapen. Het normale hondehart lijkt tegen het optreden van ritmestoornissen zeer goed beschermd.

ADDENDUM

A fourfold current stimulator for cardio-physiological investigation

A. van Oosterom, M.Sc., J. Strackee, Ph.D., and F.J.L. van Capelle, M.Sc.

A pulse generator will be described which has been designed to generate a rather large number of stimulation patterns, which are, or will be, of interest in cardiophysiology. Current stimulation has been chosen in order to make the strength of the stimulus independent of electrode resistance.

Circuit description

The main design of the apparatus will be discussed with the help of the block diagram in fig. 1. A standard crystal oscillator (time mark generator) delivers trigger impulses to a counting circuit, consisting of dekatron counting

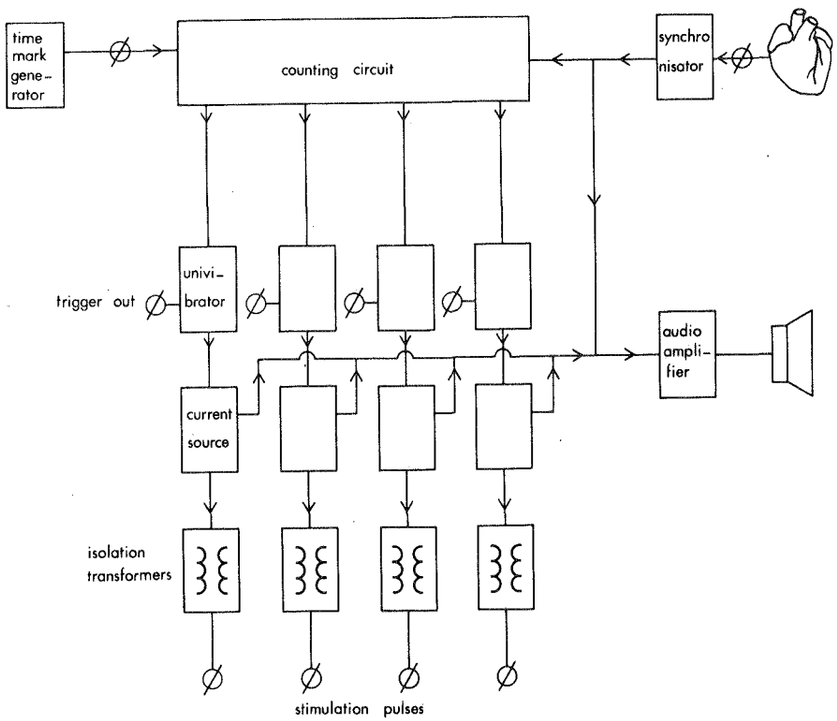

Fig. 1.

tubes and gate circuits. By this circuit, four univibrators are controlled. In each of these, the duration of the generated impulses can be varied between 10 microsec and 12.5 msec. Each univibrator drives a current source after which the combined output current can be applied to one electrode, or, alternatively, each current pulse to different electrodes.
The current is passed through a transformer, thus providing floating output.
In addition to the time generator, signals derived from the electrical activity of the heart can also control the counting circuit. Thus, the impulses can be applied to the heart after any desired interval following a spontaneous heart beat.
The univibrator impulses and, if desired, the signals derived from the activity of the heart, are fed into an audio amplifier. In this way, the stimulation pattern can be heard, so that unwanted irregularities can easily be detected during the experiment.

Pulse patterns

Although many variations are possible, basically three types of stimulation pattern can be obtained:

A) Adding extra pulses to a regular train of basic pulses.
Up till three extra pulses (E_1, E_2, E_3) can be applied once after a preset number of basic pulses. Timing, strength, and duration of each of these extra pulses, can be varied independently. (See fig. 2A.)

B) Repetition of one of the extra pulses.
The interval between last basic pulse and E_1, the interval between E_1 and E_2, and the intervals between the repetitive E_2 pulses can be made identical. E_3 can be given after a desired number of E_2 pulses after a variable interval. In this way, the heart can suddenly be driven at a new

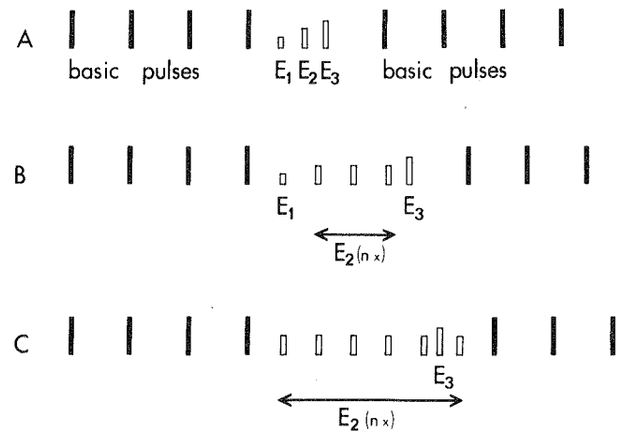

Fig. 2.

rate, and after n beats at the new rate, an extra pulse can be applied. (See fig. 2B.)

C) Interpolation of an extra pulse during a train of repetitive extra pulses. (See fig. 2C.)

The intervals between any kind of pulses can be varied from 1 msec to 2000 msec, in steps of 1, or more, msec.

During the time extra pulses are applied, the basic pulses can be dealt with in three different ways:

1) The timing for the basic pulses is interrupted; their output is suppressed. The interval between the last extra pulse and the first basic pulse is the same as the interval between basic pulses.
2) The timing for the basic pulses continues; their output is suppressed. This implies that the interval between last extra pulse and first basic pulse is not necessarily the same as the interval between basic pulses.
3) The basic pulses are continuously applied.

Technical aspects

Fig. 3 shows a simplified diagram of the counting circuit. The crystal oscillator is a Tektronix time mark generator (type 180 A). The duration of the interval between pulses is arrived at by counting the trigger impulses from the time mark generator in dekatron counting tubes. Of each tube, one of the cathodes is selected by a decimal switch and its signal, combined with the signals of the respective cathodes of other dekatron tubes, applied to an "AND gate". If the preset number of trigger impulses is reached, the "AND gate" produces a step voltage, which will fire a reset circuit, consisting of two univibrators. This resetting is effected by lowering the potential of the zero cathodes of the counting tubes to - 150 V by a 100 microsec signal from the first univibrator. During the reset impulse, the potential between anode and the zero cathode is higher than between anode and the other cathodes, and therefore the discharge will move to the zero cathodes. Since the signal on the zero cathodes of the counting tubes is used as a carry in order to trigger the next counting stage, the positive voltage at the end of each reset would put the next counting tube in the "one" position. To overcome this problem, the second univibrator, having a longer duration (200 microsec), is started simultaneously with the reset impulse. The signal from this extra univibrator is used to suppress (in a gate circuit) the driving of the next counting stages.

To generate the duration of the output current impulses, univibrators of the phantastron type are used, providing linear control of the duration.

The current source used is formed by a NPN transistor, acting as a cathode resistance in a valve circuit, the anode of which provides the output current. This produces an output impedance of approximately μR_C, in which μ is the amplification factor of the valve, and R_C the collector to base impedance of the transistor.

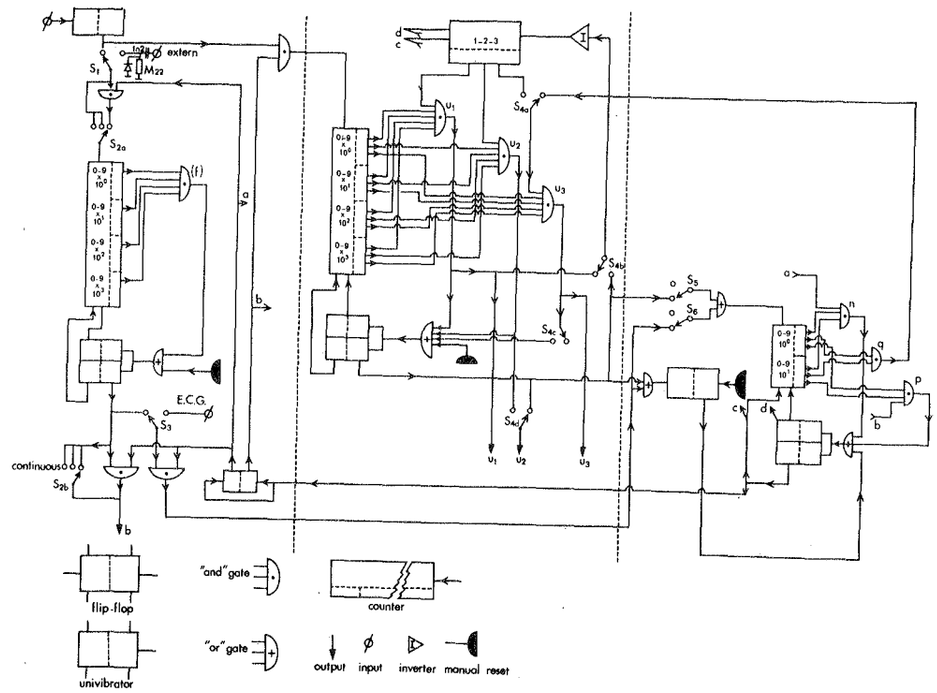

Fig. 3.

REFERENCES

1. ADRIAN, R.H. AND FREYGANG, W.H.: The potassium and chloride conductance of frog muscle membrane. J. Physiol. (London) 163:61, 1962.
2. ALANIS, J., BENITEZ, D. AND PILAR, G.: A functional discontinuity between the Purkinje and ventricular muscle cells. Acta Physiol. Latino-Am. 11:171, 1961.
3. ALANIS, J. AND BENITEZ, D.: Transitional potentials and the propagation of impulses through different cardiac cells. In: Electrophysiology and ultrastructure of the heart. Edited by T. Sano, V. Mizuhira and K. Matsuda. Grune & Stratton, 1967, p. 153.
4. BAZETT, H.C.: Analysis of time relations of electrocardiograms. Heart 7:353, 1920.
5. BIGGER Jr, J.T., BASSET, A.L. AND HOFFMAN, B.F.: Electrophysiological effects of diphenylhydantoin on canine Purkinje fibers. Circulation Res. 22:221, 1968.
6. DE BOER, S.: Eine neue Theorie ueber das Entstehen von Kammerwuehlen. Pflügers Arch. Ges. Physiol. 178:1, 1920.
7. BRADY, A.J. AND WOODBURY, J.W.: Effects of stimulus rate and external ion concentrations on ventricular transmembrane potentials. Am. J. Physiol. 187:588, 1956.
8. BRADY, A.J. AND WOODBURY, J.W.: The sodium-potassium hypothesis as the basis of electrical activity in frog ventricle. J. Physiol. (London) 154:384, 1960.
9. BROOKS, C.McC., HOFFMAN, B.F., SUCKLING, E.E. AND ORIAS, O.: Excitability of the heart. Grune & Stratton, New York 1955.
10. BURDON-SANDERSON, J. AND PAGE, F.J.M.: On the time course of the excitatory process in the ventricle of the heart of the frog. J. Physiol. (London) 2:384, 1879-1880.
11. BURDON-SANDERSON, J. AND PAGE, F.J.M.: On the electrical phenomena of the excitatory process in the heart of the frog and the tortoise, as investigated photographically. J. Physiol. (London) 4:24, 1883.
12. CALDWELL, P.C.: Factors governing movement and distribution of inorganic ions in nerve and muscle. Physiol. Reviews 48:1, 1968.
13. CARLSON, A.J.: On the mechanism of the refractory period in the heart. Am. J. Physiol. 18:71, 1907.
14. CARMELIET, E.E.: Chloride and potassium permeability in cardiac Purkinje fibers. Thesis. Editions ARSCIA S.A., Bruxelles, Presses Académiques Européennes S.C., 1961.
15. CARMELIET, E.E.: Influence du rythme sur la durée du potential d'action ventriculaire cardiaque. Arch. int. physiol. 63:222, 1955.
16. CARMELIET, E.E. AND LACQUET, L.: Durée du potential d'action ventriculaire de grenouille en fonction de la fréquence. Influence des variations ioniques de K^+ et de Na^+. Arch. int. physiol. 66:1, 1958.
17. CARMELIET, E.E. AND BOULPAEP, E.: L'adaptation de la durée du potential d'action cardiaque au changement de la fréquence des excitants. Arch. int. physiol. 66:87, 1958.
18. CHILDERS, R.W., MERIDETH, J. AND MOE, G.K.: Supernormality in Bachmann's bundle. Circulation Res. 22:363, 1968.
19. CONN, H.L. AND WOOD, J.C.: Sodium exchange and distribution in the isolated heart of the normal dog. Am. J. Physiol. 197:631, 1959.
20. CORABOEUF, E.: Some considerations about the cardiac action potential plateau. J. Electrocardiology. 2:91, 1969.
21. CRANEFIELD, P.F., HOFFMAN, B.F. AND SIEBENS, A.A.: Anodal excitation of cardiac muscle. Am. J. Physiol. 190:383, 1957.
22. DAGGETT, W.M. AND WALLACE, A.G.: Vagal and sympathetic influences on ectopic impulse formation. In: Mechanisms and therapy of cardiac arrhythmias. Edited by: L.S. Dreifus, W. Likoff, and J.H. Moyer. Grune & Stratton, New York, 1966, p. 64.
23. VAN DAM, R.Th.: Experimenteel onderzoek naar het prikkelbaarheidsverloop van de hartspier. Thesis. Klein Offsetdrukkerij Poortpers. Amsterdam, 1960.
24. VAN DAM, R.Th. AND DURRER, D.: Experimental study on the intramural distribution of the excitability cycle and on the form of the epicardial T wave in the dog heart in situ. Am. Heart J. 61:537, 1961.
25. VAN DAM, R.Th., DURRER, D., STRACKEE, J. AND VAN DER TWEEL, L.H.: The excitability cycle of the dog's left ventricle determined by anodal, cathodal and bipolar stimulation. Circulation Res. 4:196, 1956.
26. VAN DAM, R.Th., DURRER, D. AND VAN DER TWEEL, L.H.: Origin and propagation of extrasystoles resulting from stimulation during diastole and during the refractory period of the dog's heart. Acta Physiol. Pharmacol. Neerlandica 9:431, 1960.
27. VAN DAM, R.Th., HOFFMAN, B.F. AND STUCKEY, J.H.: Recovery of excitability and of impulse propagation in the in situ canine conduction system. Am. J. Cardiol. 14:184, 1964.
28. VAN DAM, R.Th., MOORE, E.N. AND HOFFMAN, B.F.: Initiation and conduction of impulses in partially depolarized cardiac fibers. Am. J. Physiol. 204:1133, 1963.
29. DECK, K.A. AND TRAUTWEIN, W.: Ionic currents in cardiac excitation. Pflügers Arch. 280:63, 1964.
30. DECK, D.A. AND TRAUTWEIN, W.: Voltage clamp in heart cells. In: Electrophysiology of the heart. Edited by: B. Taccardi and G. Marchetti. Pergamon Press, 1965, p. 97.
31. DUDEL, J., PEPER, K., RüDEL, R. AND TRAUTWEIN, W.: The dynamic chloride component of membrane current in Purkinje fibers. Pflügers Arch. 295:197, 1967.
32. DURRER, D.: Experimenteel onderzoek naar het verloop van het activatieproces in de hartspier. Thesis. Scheltema & Holkema, Amsterdam, 1952.
33. DURRER, D.: The spread of the electrical impulse through the myocardium. 4th Inter-American Congress of Cardiology, Buenos Aires, 1952.
34. DURRER, D. Electrical aspects of human cardiac activity: a clinical-physiological approach to excitation and stimulation. Cardiovascular Res. 2:1, 1968.
35. DURRER, D., AND VAN DER TWEEL, L.H.: Spread of activation in the left ventricular wall of the dog (I). Am. Heart J. 46:683, 1953.
36. DURRER, D. AND VAN DER TWEEL, L.H.: Spread of activation in the left ventricular wall of the dog (II). Am. Heart J. 47:192, 1954.
37. DURRER, D. AND VAN DER TWEEL, L.H.: Excitation of the left ventricular wall of the dog and goat. Ann. N.Y. Acad. Sci. 65:779, 1956.
38. EDMANDS, R.E., GREENSPAN, K. AND FISCH, C.: Effect of cycle length alteration upon the configuration of the canine ventricular action potential. Circulation Res. 19:602, 1966.
39. EINTHOVEN, W.: Ein neues Galvanometer. Ann. Phys. (ser. 4) 12:1059, 1903.
40. FASTIER, F.N. AND SMIRK, F.H.: Some properties of amarine with special reference to its use in conjunction with adrenaline for the production of idioventricular rhythm. J. Physiol. (London). 107:318, 1948.
41. FISCH, C., EDMANDS, R.E. AND GREENSPAN, K.: The effect of change in cycle length on the ventricular action potential in man. Am J. Cardiol. 21:525, 1968.
42. FRANK, H.L.L., DAEMS, W.Th. AND HEMKER, H.C.: Reversibele ontkoppeling van excitatie en contractie in het geïsoleerde geperfundeerde rattehart. 11e Federatieve Vergadering van Medisch-Biologische Verenigingen.
43. FREUD, G.E. AND DURRER, D.: Transmembrane potential in the isolated human heart. In preparation.
44. FRIDERICIA, L.S.: Die Systolendauer im Elektrokardiogram bei normalen Menschen und bei Herzkranken. Acta Med. Scand. 53:469, 1920.
45. GAGE, P.W. AND EISENBERG, R.S. Action potentials without contractions in frog skelettal muscle fibers with disrupted transverse tubules. Science 158:1702, 1967.
46. GIBBS, C.L. AND JOHNSON, E.A.: Effect of changes in frequency of stimulation upon rabbit ventricular action potential. Circulation Res. 9:165, 1961.
47. GIEBISCH, G. AND WEIDMANN, S.: Membrane currents in mammalian ventricular heart muscle using a "voltage-clamp" technique. Helvetica physiol. et pharmacol. Acta. 25:189, 1967.
48. GLYNN, J.M.: Activation of adenosinetriphosphatase activity in a cell membrane by external potassium and internal sodium. L. Physiol. (London) 160:18 P, 1962.
49. GLYNN, J.M.: The action of cardioglycosides on ion movements. Pharmacol. Reviews. 16:381, 1964.
50. HAN, J. AND MOE, G.K.: Nonuniform recovery of excitability of ventricular muscle. Circulation Res. 14:44, 1964.

51. HAN, J. AND MOE, G.K.: Cumulative effects of cycle length on refractory periods of cardiac tissues. Am J. Physiol. 217:106, 1969.
52. HOFF, H.E.: The history of the refractory period. A neglected contribution of Felice Fontana. Yale J. Biology and Medicine. 14:635, 1941-1942.
53. HOFF, H.E. AND NAHUM, L.H.: The supernormal period in the mammalian ventricle. Am. J. Physiol. 124:591, 1938.
54. HOFFMAN, B.F.: The genesis of cardiac arrhythmias. Progress Cardiovasc. Dis. 8:319, 1966.
55. HOFFMAN, B.F.: The possible mode of action of antiarrhythmic agents. In: The myocardial cell. Edited by: S.A. Briller and H.L. Conn Jr. New York, Harper and Row, 1969.
56. HOFFMAN, B.F. AND CRANEFIELD, P.F.: Electrophysiology of the heart. McGraw-Hill, New York, 1960.
57. HOFFMAN, B.F., CRANEFIELD, P.F., STUCKEY, J.H. AND BAGDONAS, A.A.: Electrical activity during the P-R interval. Circulation Res. 8:1200, 1960.
58. HOFFMAN, B.F. AND CRANEFIELD, P.F.: Physiological basis of cardiac arrhythmias. Am. J. Medicine. 37:670, 1964.
59. HOFFMANN, B.F., KAO, C.Y. AND SUCKLING, E.E.: Refractoriness in cardiac muscle. Am. J. Physiol. 190:473, 1957.
60. HOFFMAN, B.F. AND SINGER, D.H.: Appraisal of the effects of catecholamines on cardiac electrical activity. Ann. N.Y. Acad. Sci. 139:914, 1967.
61. HOFFMAN, B.F. AND SINGER, D.H.: Effects of digitalis on electrical activity of cardiac fibers. Progress Cardiovasc. Dis. 7:226, 1964.
62. HOFFMAN, B.F. AND SUCKLING, E.E.: Effect of heart rate on cardiac membrane potentials and the unipolar electrogram. Am. J. Physiol. 179:123, 1954.
63. HOWELL, J.W. AND JENDEN, D.J.: T-tubules of skelettal muscle: morphological alterations which interrupt excitation-contraction coupling. Fed. Proc. 26:553, 1967.
64. JANSE, M.J.: Influence of the direction of the atrial wave front on AV nodal transmission in isolated rabbit hearts. Circulation Res. 25:439, 1969.
65. JANSE, M.J., VAN DER STEEN, A.B.M., VAN DAM, R.Th. AND DURRER, D.: Refractory period of the dog's ventricular myocardium following sudden changes in frequency. Circulation Res. 24:251, 1969.
66. JENERICK, H.P. AND GERARD, R.W.: Membrane potential and threshold of single muscle fibers. J. Cellular Comp. Physiol. 42:79, 1953.
67. KAO, C.Y. AND HOFFMAN, B.F.: Graded and decremented response in heart muscle. 194:187, 1958.
68. LANGENDORFF, O.: Untersuchungen am überlebenden Säugetierherzen. Pflügers Arch. 61:291, 1895.
69. LANGER, G.A.: Ion fluxes in cardiac excitation and contraction and their relation to myocardial contractility. Physiol. Reviews. 48:708, 1968.
70. LANGER, G.A. AND BRADY, A.J.: Potassium in dog ventricular muscle: kinetic studies of distribution and effects of varying frequency of contraction and potassium concentration of perfusate. Circulation Res. 18:164, 1966.
71. LEWARTOWSKI, B., CZARNECKA, M., AND ZIELINSKI, A.: Adaptation pattern of cellular action potentials of rabbit atria to the changes in rate and rhythm of stimulation. Acta Physiol. Acad. Sci. Hungaricae. 36:335, 1969.
72. LING, G. AND GERARD, R.W.: The normal membrane potential of frog sartorius fibers. J. Cell. Comp. Physiol. 34:383, 1949.
73. LOWN, B., FAKHRO, A.M., HOAD Jr, W.B. AND THORN, G.W.: The coronary care unit. JAMA 199:156, 1967.
74. MAREY, E.J.: Des excitations électriques du coeur. Physiologie expérimentale. Travaux du laboratoire de M. Marey. Paris, Masson. Vol II, 63, 1876.
75. MATSUDA, K., KAMIYAMA, A. AND HOSHI, T.: Configuration of the transmembrane action potential of the Purkinje-ventricular fiber junction and its analysis. In: Electrophysiology and ultrastructure of the heart. Edited by: T. Sano, V. Mizuhira and K. Matsuda. Grune & Stratton, p. 177, 1967.
76. MATSUMURA, M. AND TAKAORI, S.: The effects of drugs on the cardiac membrane potentials in the heart. II. Auricular muscle fibers and the sinoatrial node. Jap. J. Pharmacol. 8:143, 1959.
77. McALLISTER, R.E. AND NOBLE, D.: The time and voltage dependence of the slow outward current in cardiac Purkinje fibers. J. Physiol. (London) 186:632, 1966.
78. McWILLIAM, J.A.: On the rhythm of the mammalian heart. J. Physiol. (London) 9:167, 1888.
79. MELTZER, L.E. AND KITCHELL, J.B.: The incidence of arrhythmias associated with acute myocardial infarction. Progress Cardiovasc. Dis. 9:50, 1966.
80. MENDEZ, C., GRUHZIT, C.C. AND MOE, G.K.: Influence of cycle length upon refractory period of auricles, ventricles, and A-V node in the dog. Am. J. Physiol. 184:287, 1956.
81. MENDEZ, C., MUELLER, W.J., MERIDETH, J. AND MOE, G.K.: Interaction of transmembrane potentials in canine Purkinje fiber-muscle junctions. Circulation Res. 24:361, 1969.
82. MENDEZ, C., MUELLER, W.J. AND URGUIAGA, X.: Propagation of impulses across the Purkinje fiber-muscle junctions in the dog heart. Circulation Res. 26:135, 1970.
83. MERIDETH, J., MENDEZ, C., MUELLER, W.J. AND MOE, G.K.: Electrical excitability of atrioventricular nodal cells. Circulation Res. 23:69, 1968.
84. MEIJLER, F.L.: Over de mechanische activiteit van het geïsoleerde volgens Langendorff doorstroomde zoogdierhart. Thesis. Amsterdam, 1960.
85. MINES, G.R.: On functional analysis by the action of electrolytes. J. Physiol. (London) 46:188, 1913.
86. MINES, G.R.: On dynamic equilibrium in the heart. J. Physiol. (London) 46:349, 1913.
87. MOE, G.K.: On the multiple wavelet hypothesis of atrial fibrillation. Arch. int. Pharmacodyn. 140:183, 1962.
88. MOE, G.K., ABILDSKOV, J.A. AND HAN, J.: Factors responsible for the initiation and maintenance of ventricular fibrillation. In: Sudden cardiac death. Edited by: B. Surawicz and E.D. Pellegrino. Grune & Stratton, 1964.
89. MOE, G.K., HARRIS, A.S. AND WIGGERS, C.J.: Analysis of the initiation of fibrillation by electrocardiographic studies. Am. J. Physiol. 134:473, 1942.
90. MOE, G.K., RHEINBOLDT, W.C. AND ABILDSKOV, J.A.: A computer model of atrial fibrillation. Am. Heart J. 67:200, 1964.
91. MOORE, E.N., MORSE, H.T. AND PRICE, H.L.: Cardiac arrhythmias produced by catecholamines in anesthetized dogs. Circulation Res. 15:77, 1964.
92. MOORE, E.N., PRESTON, J.B. AND MOE, G.K.: Durations of transmembrane action potentials and funcional refractory periods of canine false tendon and ventricular myocardium. Circulation Res. 17:259, 1965.
93. MOORE, J.J. AND SWAIN, H.H.: Sensitization to ventricular fibrillation. I. Sensitization by a substituted propriophenone U-0882. J. Pharmacol. 129:243, 1960.
94. MYERBURG, R.J., STEWART, J.W. AND HOFFMAN, B.F.: Electrophysiological properties of the canine peripheral AV conducting system. Circulation Res. 26:361, 1970.
95. NOBLE, D.: A modification of the Hodgkin-Huxley equations applicable to Purkinje fiber action and pacemaker potential. J. Physiol. (London) 160:317, 1962.
96. NOBLE, D. AND TSIEN, R.W.: Reconstruction of the repolarization process in cardiac Purkinje fibers based on voltage clamp measurements of membrane current. J. Physiol. (London) 200:233, 1969.
97. NOLASCO, J.B. AND DAHLEN, R.W.: A graphic method for the study of alternation in cardiac action potentials. J. Appl. Physiol. 25:191, 1968.
98. PAK, J.P., WALKER, J.L., GREENE, E.A., CHUN KYN LOH, AND LORBER, V.: Measurement of tracer efflux during cardiac cycle. Am. J. Physiol. 211:1455, 1966.
99. PEPER, K. AND TRAUTWEIN, W.: A membrane current related to the plateau of the action potentials of Purkinje fibers. Pflügers Arch. 303:108, 1968.
100. POHL, R.: Untersuchungen über die Erholung des Herzmuskelelementes nach einer und mehreren Reizungen. Zeitschr. für die gesamte Experimentelle Medizin 70:590, 1930.
101. PRESTON, J.B., McFADDEN, S. AND MOE, G.K.: Atrioventricular transmission

in young mammals. Am. J. Physiol. 197:236, 1959.
102. REUTER, H.: Slow inactivation of currents in cardiac Purkinje fibers. J. Physiol. (London) 197:233, 1968.
103. SARNOFF, S.J., GILMORE, J.P., MITCHELL, J.H. AND REMENSNYDER, J.P.: Potassium changes in the heart during homeometric autoregulation and acetylstrophantidin. Am. J. Med. 34:440, 1963.
104. SCHOO, L. AND BAKKER, R.R.: A stimulator for cardiological research. In preparation.
105. SCHULTZ, W.H.: The effect of chloralhydrate upon the properties of heart muscle. Am. J. Physiol. 16:483, 1906.
106. SIEBENS, A.A., HOFFMAN, B.F., GILBERT, J.L. AND SUCKLING, E.E.: Effect of rate on excitability of dog's ventricle. Am. J. Physiol. 166:610, 1951.
107. SINGER, D.H., LAZZARA, R. AND HOFFMAN, B.F.: Interrelationships between automaticity and conduction in Purkinje fibers. Circulation Res. 21:537, 1967.
108. SMIRK, F.H.: R waves interrupting T waves. Brit. Heart J. 11:23, 1949.
109. SMIRK, F.H. AND PALMER, D.G.: A myocardial syndrome with special reference to the occurrence of sudden death and of premature systoles interrupting antecedent T waves. Am. J. Cardiol. 6:620, 1960.
110. STRAUSS, H.C., BIGGER Jr., J.T., BASSET, A.L. AND HOFFMAN, B.F.: Action of diphenylhydantoin on the electrical properties of isolated rabbit and canine atria. Circulation Res. 23:463, 1968.
111. TEIGER, D., SCHEIDER, F. AND FARAH, A.: The effects of sodium ion and rate of stimulation on the refractory period of isolated rabbit atrial muscle. J. Pharmac. exptl. ther. 155:58, 1967.
112. TRAUTWEIN, W.: Generation and conduction of impulses in the heart as affected by drugs. Pharmacol. Reviews 15:277, 1963.
113. TRAUTWEIN, W. AND DUDEL, J.: Aktionspotential und Mechanogram des Warmblüterherzmuskels als Funktion der Schlagfrequenz. Pflügers Arch. 260:24, 1954.
114. TRAUTWEIN, W., KASSEBAUM, D.G., NELSON, R.M. AND HECHT, H.: Electrophysiological study of human heart muscle. Circulation Res. 10:306, 1962.
115. TRAUTWEIN, W.,AND ZINK, K.: Ueber Membran- und Aktionspotentiale einzelner Myocardfasern des Kalt- und Warmblüterherzens. Pflügers Arch. 256:68, 1952.
116. TRENDELENBURG, W.: Ueberdie Zeitliche Beziehungen der Refraktärphase des Herzens zu einem Aktionsstrom. Pflügers Arch. 144:39, 1912.
117. VIERSMA, J.W.: Hartfrequentie en impulsgeleiding in het atrium. Thesis. Amsterdam. 1969.
118. WALLACE, A.G. AND MIGNONE, R.J.: Physiologic evidence concerning the reentry hypothesis for ectopic beats. Am. Heart J. 72:60, 1966.
119. WATANABE, Y. AND DREIFUS, L.S.: Mechanism of ventricular fibrillation. Jap. Heart J. 7:110, 1966.
120. WATANABE, Y. AND DREIFUS, L.S.: Newer concepts in the genesis of cardiac arrhythmias. Am. Heart J. 76 :114, 1968.
121. WEIDMANN, S.: The effect of the cardiac membrane potential on the rapid availability of the sodium carrying system. J. Physiol. (London) 127:213, 1955.
122. WEIDMANN, S.: Effects of calcium ions and local anaesthetics on electrical properties of Purkinje fibers. J. Physiol. (London) 129:568, 1955.
123. WEIDMANN, S.: Shortening of the cardiac action potential due to a brief injection of KCl following the onset of activity. J. Physiol. (London) 132:157, 1956.
124. WIGGERS, C.J. AND WEGRIA, R.: Ventricular fibrillation due to single localized induction and condensor shocks applied during the vulnerable phase of ventricular systole. Am. J. Physiol. 128:500, 1940.
125. WILDE, W.S.: The pulsatile nature of the release of potassium from heart muscle during systole. In: The electrophysiology of the heart. Edited by: H. Hecht. Ann. N.Y. Acad. Sci. 65:693, 1957.
126. WILLIAMS, H.B., KING, B.G., FERRIS, L.P. AND SPENCE, P.W.: Susceptibility of heart to electric shock in different phases of the cardiac cycle. Proc. Soc. Exp. Biol. & Med. 31:873, 1933.
127. WOODBURY, J.W.: Cellular electrophysiology. In: Handbook of Physiology, section 2, vol. I. Edited by: W.F. Hamilton and Ph. Dow. American Physiological Society. Washington, 1962, p. 237.
128. WOODBURY, L.A., HECHT, H.H. AND CRISTOPHERSON, A.R.: Membrane resting and action potentials of single cardiac muscle fibers of the frog ventricle. Am. J. Physiol. 164:307, 1951.