JOURNAL OF APPLIED PHYSIOLOGY
Vol. 34, No. 2, February 1973. *Printed in U.S.A.*

Synchronization of the heart

L. HENK VAN DER TWEEL, FRITS L. MEIJLER, and FRANS J. L. VAN CAPELLE
Laboratory of Medical Physics and Department of Cardiology and Clinical Physiology, University of Amsterdam, Amsterdam, The Netherlands

VAN DER TWEEL, L. HENK, FRITS L. MEIJLER, AND FRANS J. L. VAN CAPELLE. *Synchronization of the heart.* J. Appl. Physiol. 34(2): 283–287. 1973.—The behavior of cardiac pacemaker cells bears a resemblance to that of relaxation oscillators. A characteristic property of relaxation oscillators is that they may be synchronized by an external signal, if the latter has a periodicity not differing too much from the spontaneous frequency of the oscillator. In the present study, it is shown that the isolated, perfused, and submerged rat heart can be synchronized by a sinusoidal electrical current through the heart. This synchronization is not to be confused with stimulation, which occurs at higher current densities. Synchronization was also found when the frequency of the synchronizing signal was in the neighborhood of a multiple of the spontaneous frequency of the heart. Synchronization was demonstrated for the SA node as well as for a lower pacemaker, presumably the AV node. The possible role which this type of synchronization may play in spontaneous impulse formation in pacemaker tissue is discussed.

pacemaker tissue; SA node; AV node; relaxation oscillator; impulse formation

AT PRESENT there is increasing interest in the nature of biological rhythms. For a number of them the concept of relaxation oscillation offers itself as a possible basis for the mechanisms involved. As early as 1926 Van der Pol stated that the rhythmic beating of the heart was a typical example of a relaxation oscillation (8, 9). Related phenomena also exist in respiration physiology (12). Studies were also undertaken for cases where a biological rhythm arises as a consequence of interactions between many units or cells, exhibiting properties of relaxation oscillations, each with an innate periodical activity (11).

In Fig. 1 the working principle of a typical relaxation oscillator is depicted. A condensor is gradually charged until a certain potential (threshold) has been reached. Then the neon tube is ignited, discharges the condensor, and the cycle repeats. It should be kept in mind that this is a very simple example of a relaxation oscillator, and that actual biological oscillators are much more complicated. The frequency of a relaxation oscillator is dependent on the energy supplied. Accordingly, it can be seen in Fig. 1 that a change in V will definitely change the period of the relaxation oscillator. If a periodic influence of a relatively small amplitude is applied on a relaxation oscillator and this signal has a periodicity not differing too much from the spontaneous frequency of the oscillator, the relaxation oscillator will change its periodicity to that of the superimposed signal. The frequency range in which this is possible depends on the relative amplitude of the synchronizing signal. An important feature is that, in principle, synchronization will also be possible at influencing frequencies which are multiples of the spontaneous frequency of the relaxation oscillator. The frequency of the synchronized relaxation oscillator will then be exactly, e.g., one-half, one-third, etc., of the influencing frequency, i.e., always near the frequency of the spontaneous rhythm. These properties are most typical for relaxation oscillators and are practically absent in oscillators based on resonance phenomena.

The present knowledge of the properties of cardiac pacemaker cells essentially confirms Van der Pol's original concept. Intracellular recordings show a time course of the diastolic transmembrane potential that is very suggestive of a relaxation oscillation (2). The membrane depolarizes slowly to a threshold at which a rapid depolarization occurs. Also a mathematical description of the behavior of excitable oscillatory membranes may be based on a generalization of Van der Pol's original equation for relaxation oscillations, and has been shown to be closely related to the Hodgkin-Huxley description (1).

From the above considerations, it seemed useful to decide whether the pacemaker of the heart can be synchronized in a way comparable to synchronization of a technical relaxation oscillator. This synchronization should be distinguishable from conventional stimulation of heart tissue. In the present paper we have attempted to demonstrate that the sinus node and the AV node of isolated rat hearts may indeed be synchronized by external "subthreshold" sinusoidal currents. Sinusoidal currents were preferred above other periodic variations because linear distortions do not change the time course of a sinusoid.

METHODS

Isolated hearts of white rats weighing approximately 250 g were perfused at 37 C and pH 7.35 using a modified Langendorff procedure (3), described previously (5). The animals were anesthetized with 20 mg sodium pentobarbital ip before the removal of the heart. The technique makes it possible to immerse the heart in a bath filled with perfusion fluid which acts as a volume conductor. The synchronizing sinusoidal current was applied to the immersion fluid via two circular electrodes (with a diameter of 1 cm), approximately 1.5 cm apart. This current was derived from an isolated transistorized current source, and a Hewlett-Packard waveform generator. The current was continuously monitored on one trace of the oscilloscope, by means of a small series resistance. The resulting signal was used as reference. All currents are presented as peak-to-peak values. The peak-to-peak current densities were in the order of 10–20 ma/cm^2. Since this study was concerned with synchronization of the heart and not with stimulation of the heart, we avoided the latter by positioning the electrodes and setting the current value in such a way that synchronization rather than stimulation occurred. The electrogram was recorded by means of bipolar ringshaped (1 mm) platinum electrodes stitched either to the right auricle, the left auricle, or the epicardial surface of the posterobasal region of the left ventricle. The interelectrode distance was approximately 2 mm. Bipolar leads were used to minimize artifacts, due to the synchronizing current. In addition a 12 db/oct high-pass filter (3 db at 18 cycles/sec) was used in the recording circuit. The complexes obtained in this way were satisfactory. Generally the electrical activity from the auricular tissue showed high frequency components and notches, which remained

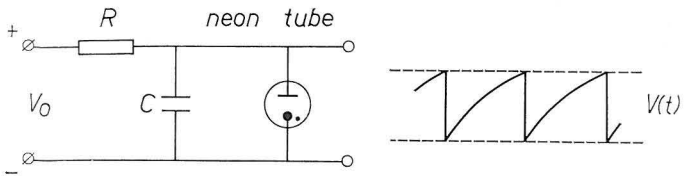

FIG. 1. Conventional neon-discharge relaxation oscillator. For details see text.

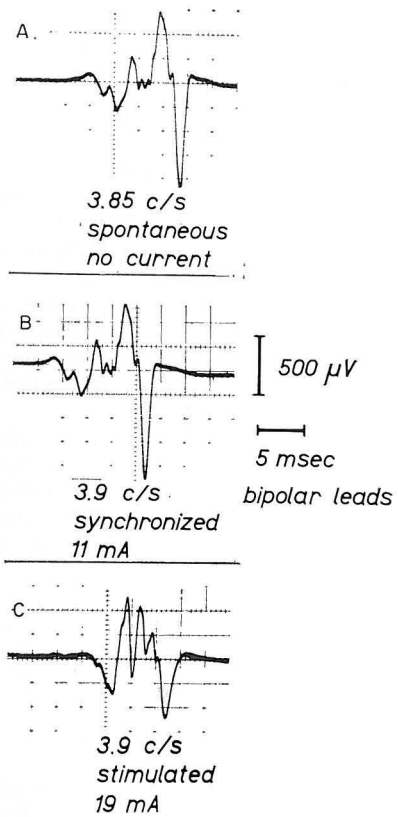

FIG. 2. Bipolar complexes from right auricle of an isolated rat heart. Synchronized complexes (*B*) are identical with the spontaneous ones (*A*). Complexes obtained during application of a higher current strength are profoundly altered and attributed to direct stimulation (*C*). Only one sweep is shown. Position of the complexes in *A* is arbitrary while the positions of the complexes in *B* and *C* are related to triggering of the sweep by synchronizing sine wave. Because of fast sweep speed the reference sine wave is not shown.

constant during the experiment. The ventricular electrical activity could be observed in the atrial electrogram and was used for measuring PQ times. For a further study of synchronization both auricles were removed without severing the AV node and bundle of His. This resulted in low heart rates. Removal of the atria does not impair the perfusion of the coronary system, particularly since in the setup used the coronary perfusion pressure is kept constant with an overflow pipe.

RESULTS

Figure 2*A* shows a bipolar complex derived from the right auricle of an isolated heart during spontaneous activity without synchronizing current. The heart rate is 3.85/sec which is in the normal range for this preparation at this temperature. A high sweep speed was used to allow detailed observation of the complex. Figure 2*B* was derived from the same leads, but now during application of a synchronizing current of 11 ma at 3.9 cycles/sec, which effected synchronization. Increase of the current strength displaced the complex with respect to the synchronizing sine wave, but the shape did not change. However, if the current strength was increased further, a differently shaped complex was seen (Fig. 2*C*), presumably caused by direct stimulation of the atrial tissue. The PR interval as well as the interval between right and left atrial complexes were identical with and without synchronizing current. This was no longer the case when current strength was increased sufficiently to produce stimulation. Synchronization occurs over a strictly limited frequency range, which depends on current strength, whereas the frequency range of direct stimulation with altered complexes is practically unrestricted. Figure 3 shows that synchronization does occur at a specific frequency but does not occur when the frequency of the sinusoidal current deviates slightly from that frequency. A number of sequential complexes were photographed. In this particular experiment the frequency of the synchronizing current was about twice that of the spontaneous heart rate (Fig. 3*B*) and was changed some 10% in the observations presented in Fig. 3, *A* and *C*. Figure 3*B* shows synchronization. The complexes are locked to the sine wave and therefore superimposed, however, their shapes are identical with those in Fig. 3, *A* and *C*, in which the time of occurrence of the complexes is not locked to the sine wave. Figure 4 shows the results obtained during application of a sine-wave current with a frequency nearly equal to that of the spontaneous heart rate (Fig. 4*A*) and a frequency of about four times that rate (Fig. 4*B*). Then the complexes occur only once in every four sine waves. In Fig. 4*C* the current has been increased to the extent that differently shaped complexes

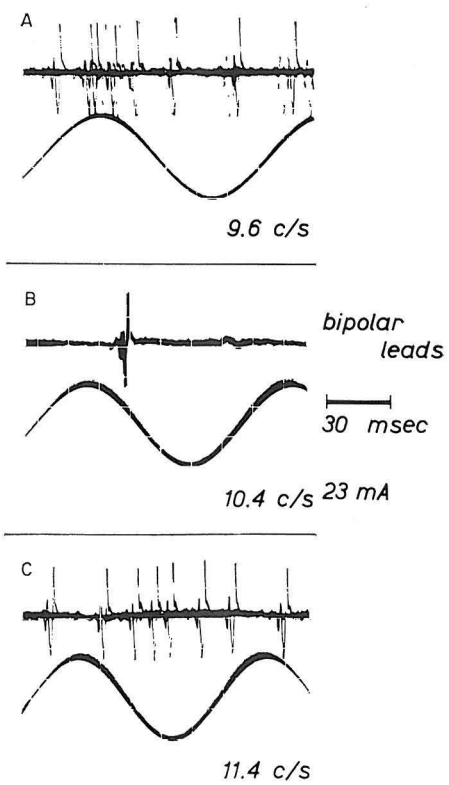

FIG. 3. Bipolar complexes from the left auricle of an isolated rat heart during application of a constant sinusoidal current with frequencies of 9.6, 10.4, and 11.4 cycles/sec. Although also at 9.6 and 11.4 cycles/sec the rhythm is influenced, synchronization only occurs at 10.4 cycles/sec, causing a heart rate of 5.2 cycles/sec (*B*). In all traces many sweeps were photographed. The speed of the sweep is much lower than in Fig. 2 and sine-wave current is therefore demonstrated in lower trace. Sweep is started by trigger delivered by waveform generator.

occur, attributable to direct tissue stimulation: now a 2:1 ratio between sine wave and stimulated atrial complexes exists.

In general, the phase in which the complexes were synchronized to the sine wave was found to be dependent on both frequency and amplitude of the synchronizing sine wave. The complexes tended to lock later in the sine wave as the frequency increased.

It was considered of interest to study whether sinusoidal but not yet synchronizing currents influence the variability of the spontaneous heart rhythm. Figure 5 shows that this indeed is the case. In these figures the time base is triggered by one complex and the following one is photographed. The variability is directly shown by the range over which the complexes are seen (Fig. 5, *B* and *C*). Synchronization (Fig. 5*D*) is then shown not so much by the coinciding of the complexes but also by the locking to the sine wave, which in this figure was not the signal that triggered the oscilloscope. It should be noted that the complexes in all recordings are identical. All of the phenomena described can be demonstrated with bipolar leads stitched to either the right or the left atrial appendage.

Furthermore we studied whether or not a presumably AV nodal focus can be synchronized too. The recordings shown in Fig. 6, obtained with a fast sweep, demonstrated that also for a ventricular preparation this is possible and the complexes obtained during synchronization (Fig. 6*B*) are unchanged. Of course the frequency of the applied current is much lower than for atrial synchronization because of the lower rate of nodal or ventricular foci, in this case about 1 cycle/sec. The current strength needed for synchronization was found to be much lower than during atrial synchronization. Figure 7, *A*, *C*, and *D*, shows ventricular synchronization, recorded with a slower time base. In each record a number of sweeps are superimposed at different synchronizing frequencies.

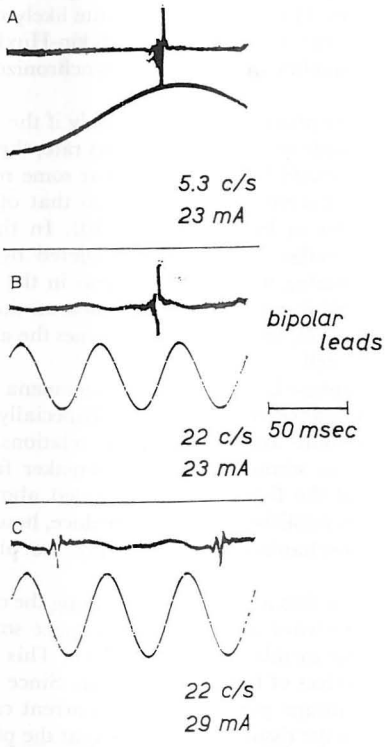

FIG. 4. Bipolar complexes from left auricle of an isolated rat heart during application of a sinusoidal current of 5.3 and 22 cycles/sec. During 22 cycles/sec at each fourth sine wave a complex occurs. Synchronization is effected in both instances (*A* and *B*). Many complexes are superimposed. *C* shows the effect of increased current strength at 22 cycles/sec. The form of the complexes changes and every second sine wave now causes depolarization. Sweep triggered as in Fig. 3.

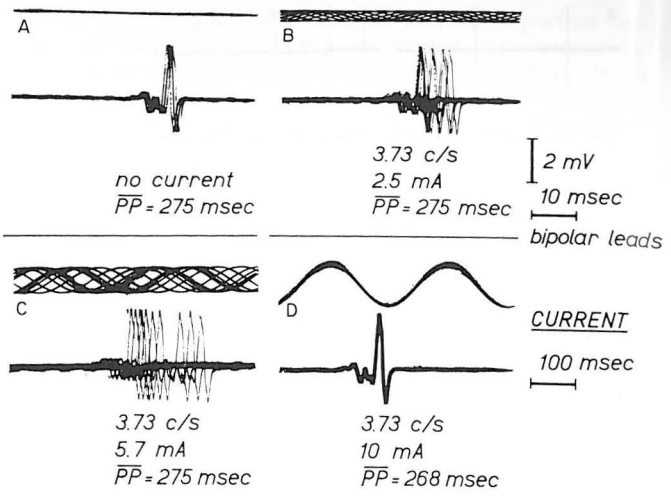

FIG. 5. Effect of increasing sinusoidal currents on P-wave rhythm from right auricle of an isolated rat heart. Time base of oscilloscope was triggered by an auricular complex and was further adjusted in such a way that the next complex was photographed. If this is done for a number of sweeps the spread in PP interval is visualized. Sweep speed used for the recording was expanded 10-fold with regard to the upper trace. *A:* spontaneous rhythm without current. The spread is small, showing the constancy of the preparation. *B* and *C:* with increasing current strength at a frequency nearly matching the spontaneous one, the spread increases. In upper tracings it can be seen that the period of sinusoidal current has no distinct relation to the period of sweep. *D:* when the current is further increased synchronization occurs. Now the sine waves and P waves are locked to each other.

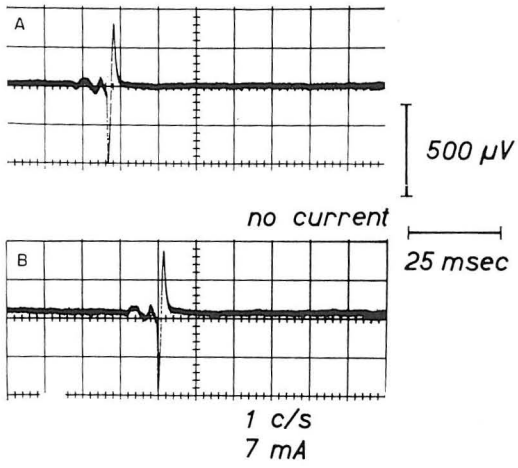

FIG. 6. Synchronization of a ventricular (or nodal) focus. *A* shows a spontaneous ventricular complex with no applied current at a high sweep velocity (RR interval 825 msec). *B* demonstrates the complex during synchronization. Sweep triggered as in Fig. 2.

Figure 7*B* shows that a change in sine-wave frequency from 1.2 to 1.6 cycles/sec disturbs the synchronization, whereas at 2.4 cycles/sec (about two times the spontaneous rhythm) synchronization has been restored. Synchronization was also present at 6.0 cycles/sec and could be demonstrated up to a ratio of 8:1 between the sine-wave frequency and the rate of the ventricles.

DISCUSSION

Considering the restrictions dictated by this experimental situation (only macroelectrode techniques were used), our findings point to the presence of synchronization, such as can be found in technical relaxation oscillators. Whereas Van der Pol's (9) model is

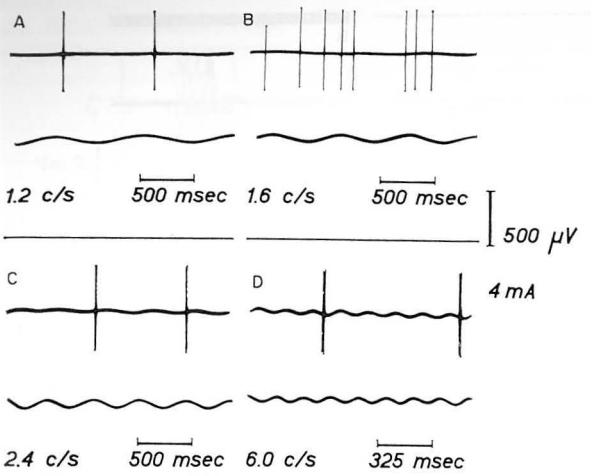

FIG. 7. *A*, *C*, and *D* show synchronization of a ventricular (or nodal) focus at a low sweep velocity and different related frequencies of the synchronizing current. *B* shows the lack of correspondence at an intermediate frequency. Sweep triggered as in Fig. 3.

supported by present knowledge of pacemaker cell activity, the analogy seems to hold even further, because a number of phenomena, well known from electronic relaxation oscillators, could quantitatively be demonstrated for a suspended isolated perfused complete heart. Those phenomena are: *a*) the range of the sine-wave frequencies at which synchronization occurs is limited; *b*) this (*a*) applies for sine-wave frequencies close to the spontaneous heart rate as well as for multiples of this frequency; *c*) the frequency span over which synchronization can be effected depends on the amplitude of the synchronizing current; *d*) if the synchronizing frequency is changed within synchronization range, the complexes occur at a different phase of the synchronizing sine wave; and *e*) a phase shift is generally also found if the amplitude of the synchronizing sine wave is changed.

There are indications that it is the normal pacemaker or the tissue very close to it that is synchronized by the externally applied sine wave. First of all the right atrial complexes do not change their (complicated) shape with application of the synchronizing current (Fig. 2). Moreover the PR interval as well as the interval between the right and the left auricular complexes were not affected when synchronization occurred but did change when stimulation occurred. Phenomena *a* and *b* are especially important arguments. As long as the synchronizing frequency is about the same as the spontaneous rate, the synchrony between sine wave and complexes is not necessarily a definite argument for the occurrence of synchronization as defined in this paper, since this would apply also for direct stimulation. However, comparable small changes in frequency of the applied current disturb the synchrony. This kind of selectivity is not found with conventional stimulation of the heart. Moreover, unaltered atrial complexes are found for each of two or more sine wave cycles at frequencies which are multiples of the spontaneous rhythm. For instance, at 4:1 synchronization (Fig. 4*B*) the tissue is certainly excitable for direct stimulation before the third cycle of the sinusoidal current. It would be hard to explain why the heart should "wait" during another sine wave period if direct stimulation was present.

The amplitude needed to synchronize a relaxation oscillator approaches zero for identical frequencies of the synchronizing input and the spontaneous rate, if both are constant. However, if in the present experiments the current strength is diminished below a certain value (still distinctly differing from zero), synchronization ceases. This is possibly analogous to the phenomenon found in conventional relaxation oscillators, viz., that the threshold of the synchronizing input is related to the constancy of the frequency of the free running oscillator. The less constant its period the higher the strength needed for synchronization.

If an applied current is not synchronizing, the complexes will fall in different phases of the sine wave. If this current indeed influences the pacemaker, this influence will be different at each beat. This may explain why the time spread, originally on the order of 0.5%, becomes larger for nonsynchronizing currents (Fig. 5).

Atrioventricular nodal rhythms behave essentially in the same way as the sinus rhythm. However, less current is needed to effect synchronization, and synchronization seems possible at higher frequency ratios between synchronizing current and spontaneous rhythm.

To come to a conclusion about the mechanism of the phenomena described, the results should be considered in relation with known electrophysiological properties of pacemaker tissue. Part of the externally applied current will also pass through the cell membranes at the site of the pacemaker, thereby alternately depolarizing and (hyper)polarizing these membranes to some degree. Therefore especially at multiple frequencies of the synchronizing sine wave, the net influence on the rate of diastolic depolarization will be negligible. Thus it seems to be the more or less instantaneous contribution of the current that makes the cell reach its threshold, just as it is the case with the oscillator of Fig. 1.

Present knowledge about the ionic mechanisms involved in pacemaker action in sinus nodal tissue is not sufficiently extensive to permit a detailed quantitative description in terms of ionic conductances. The pacemaker potentials of Purkinje fibers, however, have been described by Noble (6) in terms of the Hodgkin-Huxley equations. It is noteworthy that the Hodgkin-Huxley model has been shown by FitzHugh to belong to the same class of excitable-oscillatory systems as the Bonhoeffer-Van der Pol model (1), which is a generalization of Van der Pol's original equation for relaxation oscillations. Therefore it seems quite likely that the stable limit cycles which can occur in the Hodgkin-Huxley equations, including Noble's modification, could be synchronized by external periodic influences.

In the case of synchronization, particularly if the synchronizing frequency is a multiple of the resulting heart rate, the phenomenon of synchronization could be thought to bear some resemblance to the locking of the pacemaker frequency to that of subthreshold oscillations as found in Purkinje fibers (10). In the latter case, however, the pacemaker is eventually triggered by subthreshold oscillations of increasing amplitude, whereas in the present situation a small sinusoidal signal is superimposed on the spontaneous diastolic depolarization, and merely determines the actual moment of the firing of the cell.

In two recent papers (4, 7) interesting phenomena were reported which bear resemblance to our studies. Especially the work of Reid, where harmonic and subharmonic relations are reported between vagal nerve stimulation and pacemaker frequency, displays a number of the features also indicated above. From our results and the ones published we cannot deduce, however, whether (partly) similar mechanisms may underlay the phenomena reported.

It should be noted that a current depolarizing the cell membrane at one place has to leave the intracellular space somewhere else, hyperpolarizing the membrane at that place. This will generally not influence the effect of the depolarization. Since the variations added to the membrane potential by the current cannot be uniform through all of the tissue, it is possible that the phenomenon of synchronization may also be accompanied by a small pacemaker shift. Although the atrial complexes recorded during synchronization were completely superimposable on the spontaneous complexes, our method is not sensitive enough to decide on this point.

The present results indicate that small and slowly changing electrical currents can influence the pacemaker cells of an isolated

heart. There is evidence that the coupling between cardiac cells is of the low-resistance type and therefore it cannot be excluded that the transmembrane potentials of pacemaker cells will exert influences of the type just described on each other. It has been suggested indeed that cardiac pacemaker cells may synchronize each other by electrotonic interaction (11), which would present an alternative to the concept of an "earliest" cell which functions as cardiac pacemaker. The frequency of the heart beat would then be determined by a population of interacting pacemaker cells. Whether phenomena of this kind do play an important role in actual pacemaker activity requires further experimentation as well as more elaborate studies of systems of coupled oscillators.

This work was supported by the Netherlands Organization for Advancement of Pure Research (ZWO).

Present address of F. L. Meijler: Department of Cardiovascular Diseases, University Hospital, Utrecht, The Netherlands.

Received for publication 7 April 1972.

REFERENCES

1. FitzHugh, R. Impulses and physiological states in theoretical models of nerve membrane. *Biophysical J.* 1: 445–466, 1961.
2. Hoffman, B. F., and P. F. Cranefield. *Electrophysiology of the Heart.* New York: McGraw, 1960, p. 104–131.
3. Langendorff, O. Untersuchungen am überlebenden Säugetierherzen. *Arch. Ges. Physiol.* 61: 291–332, 1895.
4. Levy, M. N., P. J. Martin, T. Iano, and H. Zieske, Paradoxical effect of vagus nerve stimulation on heart rate in dogs. *Circulation Res.* 25: 303–314, 1969.
5. Meijler, F. L., F. van den Botgaard, L. H. van der Tweel, and D. Durrer. Postextrasystolic potentiation in the isolated rat heart. *Am. J. Physiol.* 202: 631–635, 1962.
6. Noble, D. A modification of the Hodgkin-Huxley equations applicable to Purkinje fibre action and pacemaker potentials. *J. Physiol., London* 160: 317–352, 1962.
7. Reid, J. V. O. The cardiac pacemaker: effect of regularly spaced nervous input. *Am. Heart J.* 78: 58–64, 1969.
8. Van der Pol, B. On "relaxation oscillations." *Phil. Mag.* 2 (Ser. 7): 978–992, 1926.
9. Van der Pol, B., and J. van der Mark. The heartbeat considered as a relaxation oscillation, and an electrical model of the heart. *Phil. Mag.* 6 (Ser. 7): 763–775, 1928.
10. Vassalle, M. Cardiac pacemaker potentials at different extra- and intracellular K concentrations. *Am. J. Physiol.* 208: 770–775, 1965.
11. Winfree, A. T. Biological rhythms and the behaviour of populations of coupled oscillators. *J. Theoret. Biol.* 16: 15–42, 1967.
12. Young, A. C. Neural control of respiration. In: *Physiology and Biophysics*, edited by T. C. Ruch and H. D. Patton. Philadelphia, Pa.: Saunders, 1966, p. 788–802