

Directional Difference of Conduction Velocity in the Cardiac Ventricular Syncytium Studied by Microelectrodes

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In the theory of electrocardiography the direction of myocardial fibers has been almost ignored. In order to evaluate whether this is proper or not, directional difference of conduction velocity in the ventricular syncytium was examined by capillary microelectrodes. Conduction velocity in the direction of myocardial fibers was found to be usually several times larger than that vertical to them. This difference is significantly large and cannot be ignored even though rapidity of conduction through Purkinje fibers is considered. This statement became more applicable in various abnormal conditions. Thus it is concluded that the direction of myocardial fibers should be given more attention in discussion of propagation of excitation waves in the cardiac ventricle.

IN THE THEORY of electrocardiography much importance has been attached to the role of Purkinje fibers in the propagation of ventricular excitation, relatively little to the role of myocardial fibers. The muscle fibers are assumed to be capable of conducting excitation readily and equally. This assumption may arise from the belief that the conduction velocity of Purkinje fibers is extremely fast when compared with the differences in the conduction velocity throughout the myocardium, which depends on the syncytial arrangement of heart muscle. However, nothing seems to have been reported as to whether such belief is correct or not or whether there are differences in rate of conduction in different directions. The purpose of this study is to examine this question by the intracellular microelectrode method.

To separate the conduction velocity of myocardial fibers from that of Purkinje fibers is difficult. To isolate one myocardial fiber is technically difficult, and if accomplished, the intervention might alter the normal electric property of the cardiac syncytium. Reported values¹ for conduction velocity of the isolated papillary muscle, obtained by the microelectrode method, might be the result of Purkinje fibers and of myocardial fibers. One approach to this problem lies in the histologically known scantiness of Purkinje fibers in the epicardial side of the ventricle. However, to study the

conduction velocity on the epicardial side of the ventricle in the spontaneously beating heart *in situ*, as was performed by Schaefer and Trautwein,² would not clarify this problem, since it is possible that the excitation wave reaches the epicardial side from the inside more rapidly through Purkinje fibers. We therefore decided to isolate small cardiac muscle strips from the external (epicardial) side and from the internal (endocardial) side of the ventricle, to stimulate them electrically, and to compare both results.

METHODS

Dogs varying greatly in age and weight were anesthetized by intravenous injection of thiopental sodium. Under artificial respiration their hearts were removed. From the external or internal surface of their ventricles a small strip of cardiac muscle was excised. This was mounted in a muscle chamber which contained aerated Tyrode solution maintained at 37 to 38 C. The apparatus is similar to that described by Draper and Weidmann.³

Two or three glass capillary microelectrodes, the external tip diameter of which was about 0.5 μ , were inserted simultaneously into ventricular cells. Electrodes having an electric resistance of about 10 M Ω were selected for use in this study. Sometimes, when three microelectrodes were used, chiefly when the internal surface of the ventricle was examined, one of them was employed for intracellular stimulation. In other cases a thin silver wire of 100 μ in diameter, insulated except for the tip, was used for extracellular stimulation. The stimulus current consisted of square pulses of 60 per minute. Strength of current was selected by increasing either magnitude or duration of stimulation until there was no further shortening of the latency of action potentials obtained by the

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microelectrode close to the stimulating electrode. Stimulation slightly stronger than this was employed. Stimulation was synchronized with the sweep circuit of the cathode ray oscilloscope of a 3 channel type using an electronic switch. The details of the microelectrode, a cathode follower preamplifier, a D-C amplifier and other recording systems were reported elsewhere.⁴

As cardiac muscle strips representative of the external and internal surfaces of the ventricle, the epicardial and endocardial sides were examined. Under a binocular microscope, one microelectrode was inserted as close as possible (usually about 300 μ) to the stimulating electrode. Another microelectrode was inserted at variable distances from the stimulating electrode, both horizontally and vertically to the course of ventricular fibers. When effects of drugs and temperature were examined, both horizontal and vertical sets were inserted at the same time. In such cases all three microelectrodes were used for recording.

The interval between the steepest rise of two action potentials was measured for conduction time in horizontal or vertical direction to the ventricular fibers. From measurements of the distance between the microelectrode close to the stimulating electrode and that at a distance from it, made by an ocular micrometer scale in the binocular microscope, conduction velocity was calculated.

RESULTS

In preliminary experiments conduction velocity was found to be constant at least for five hours after isolation, provided that the preparations were held in good condition, i.e., in a fresh Tyrode solution continuously saturated with oxygen. Therefore, efforts were made to finish the experiments within this time. In the experiments with external (subepicardial) strips, intracellular stimulation by the microelectrode gave almost the same values as extracellular stimulation by the silver wire. Therefore the technically easy extracellular stimulation was employed. But in experiments with internal (subendocardial) strips, extracellular stimulation often gave apparently contradictory results. Thus action potentials obtained by the microelectrode close to the stimulating electrode often appeared later than those at the remote electrode. Changing to intracellular stimulation then usually resulted in disappearance of these contradictory phenomenon. It was assumed that the extracellular stimulation involved other points than those where the stimulating elec-

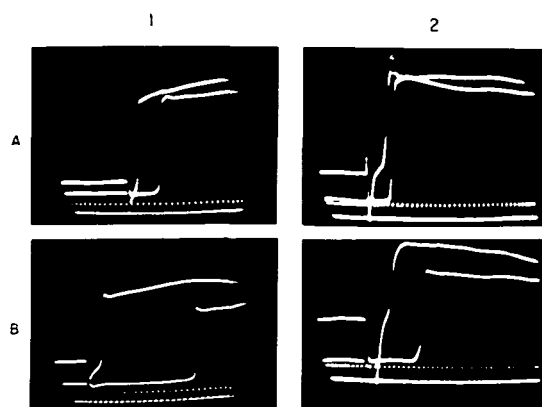


FIG. 1. Difference of conduction velocity in directions parallel to myocardial fibers (in *A*) and vertical to them (in *B*) in myocardial strips isolated from the epicardial side of the dog ventricle (in 1) and from its endocardial side (in 2). The distance between the two recording microelectrodes was 5 mm. in all cases. Time marks at 2 msec. intervals.

trode was placed, and Purkinje fibers were suspected because of the absence of this phenomenon in the external strips. Therefore intracellular stimulation was felt necessary when internal strips were examined. Because of difficulty in inserting three microelectrodes at the same time, one of which was employed for intracellular stimulation, we were unable to obtain many results by such stimulation. Although the results obtained by extracellular stimulation with internal strips were much more numerous, they were used only for comparative purposes and are not included in this report.

In strips of cardiac muscle fibers from the external surface of the dog ventricle the conduction velocity parallel to ventricular fibers (i.e., horizontally) was usually two to five times larger than that vertical to them, as is shown in figure 1. When the stimulating electrode and the microelectrode close to it were kept fixed and another microelectrode was shifted to variable distances, the relation between conduction time and distance was usually found to be linear, as is shown in figure 2A. Occasionally, however, similar values for conduction velocity were obtained both in parallel and vertical directions. In such cases it was often found that parallel conduction velocity changed suddenly in some posi-

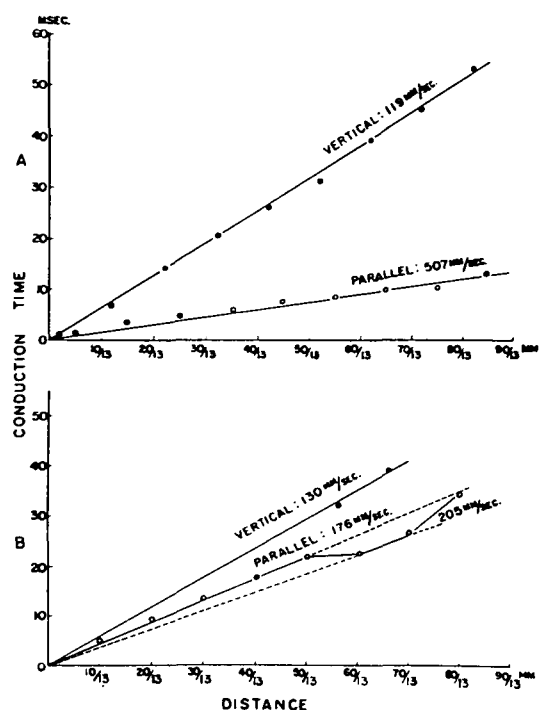


FIG. 2. Two examples to show the relation between the distance of the two recording microelectrodes and the conduction time measured by them. Open and closed circles denote values obtained when the two microelectrodes were placed in direction parallel and vertical to myocardial fibers, respectively. The denominator 13 in the abscissas means that 13 units of the ocular micrometer scale equaled 1 mm. Each figure was obtained from measurements in one preparation.

tions as the remote microelectrode was shifted, as if it were inserted then in a different ventricular fiber (fig. 2B). All the results of 94 measurements in 8 dogs are summarized in figure 3A. This shows that the conduction velocity parallel to ventricular fibers ranged widely from 73 to 709 mm./sec., except for one of 961 mm./sec., whereas that vertical to them varied only from 80 to 199 mm./sec., which corresponds to the lower limits of the former. Although the cardiac muscle strips were obtained from the epicardial side of various parts of the ventricle, no regional differences could be established.

With internal strips the conduction velocity was generally much greater than that with external strips. The relation between parallel and vertical conduction velocity was more complicated here, but the former usually

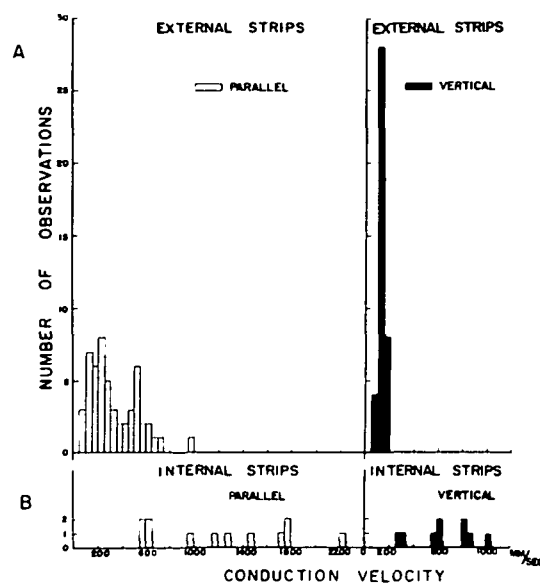


FIG. 3. Difference of conduction velocity depending upon the direction of fibers in myocardial strips and the side from which they were isolated. *Abscissas* denote conduction velocity in millimeters per second in directions parallel to myocardial fibers (on the left) and vertical to them (on the right) in myocardial strips isolated from the epicardial side of the dog ventricle (in A) and from its endocardial side (in B). *Ordinates* denote the number of observations in which this conduction velocity was noted, grouped in the range of 50 mm./sec.

showed larger values, often exceeding 1,000 mm./sec. The results of 23 measurements in 6 dogs are summarized in figure 3B. Although only a few results could be obtained, for the reason mentioned, it is evident that both parallel and vertical conduction vary widely and the former shows much larger values than the latter.

Effects of Temperature on Conduction Velocity. The effects of temperature change on the conduction velocity of the ventricular syncytium were examined by heating or cooling the perfusing Tyrode solution over the range of 15 C. to 42 C. Within this range the conduction velocity increased linearly with the rise of temperature (fig. 4). This was the same in both parallel and vertical directions and with both external and internal strips. However, the rate of increase was greater with the parallel conduction velocity as affected by the rise of temperature, as is shown in figure 4.

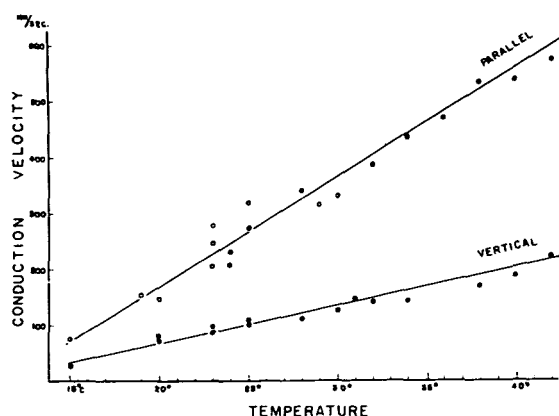


FIG. 4. Relation between conduction velocity and temperature. Open and solid circles denote conduction velocity parallel and vertical to myocardial fibers, respectively. This example was derived from a myocardial strip isolated from the epicardial side of the dog ventricle. Note that parallel conduction velocity showed steeper increase with the rise of temperature.

Effects of Drugs on Directional Difference of Conduction Velocity. Caffeine, allo-p-oxocamphor, strophanthin, Lanatoside C, quinidine, and procaine amide were added to the Tyrode solution, and their effects on the directional difference of the conduction velocity were examined. With each cardiac muscle strip only one drug was tested. Caffeine, 1:8,500 to 1:500 dilution, did not cause any significant change of the conduction velocity either in parallel or in vertical direction. G-strophanthin, $1:13 \times 10^5$ to $1:6.6 \times 10^5$ dilution, Lanatoside C, 1:235,000 to 1:82,000 dilution, and allo-p-oxocamphor (Vitacampher), 1:18,800 to 1:3,760 dilution, decreased conduction velocity, but the effect was usually more marked in the vertical than in the parallel direction. Parallel conduction time often did not show any appreciable prolongation in the afore-mentioned concentration. Procaine amide 1:1,000 to 1:450 dilution, and quinidine, 1:50,000 to 1:5,500 dilution, usually showed the same tendency to delay vertical conduction more than parallel, as is shown in figure 5A. However, in some instances, as is seen in figure 5B, the parallel conduction time was prolonged more markedly than the vertical, and occasionally the former even exceeded the latter. Thus in later stages much more complicated relations

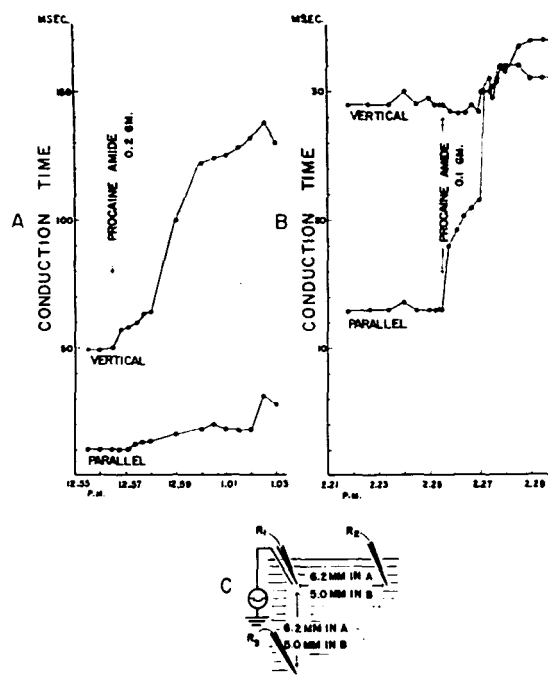


FIG. 5. Effects of procaine amide on directional difference of conduction velocity. From two experiments on external strips. Immediately after 0.2 Gm. procaine amide was added to 90 ml. Tyrode solution, conduction time vertical to myocardial fibers was prolonged more markedly than that parallel to them in figure 5A, whereas the latter was more prolonged than the former in figure 5B, when 0.1 Gm. procaine amide was added to 100 ml. Tyrode solution. In the case of figure 5B, parallel conduction time even exceeded the vertical at certain time points after administration of the agent. In these experiments the first recording microelectrode (R_1 in figure 5C) was inserted close to the stimulating electrode and was only 380 μ from it. The second (R_2) and third (R_3) remote microelectrodes were inserted at points of equal distance from the first electrode in parallel and vertical directions to the myocardial fibers, respectively, the distances being 6.2 mm. in figure 5A and 5.0 mm. in figure 5B.

were observed between both, suggesting the occurrence of intraventricular block. Such tendency by various drugs was the same either with external strips or with internal strips, although the latter showed larger conduction velocity in general.

DISCUSSION

The chief difference between the external (subepicardial) and internal (subendocardial) strip is that the latter generally showed

greater conduction velocity. The internally located muscle often showed conduction rates exceeding 1,000 mm./sec. to 2,219 mm./sec. in the parallel direction; values of this order were almost unknown in the external strips. We believe this to indicate a contribution of Purkinje fibers in the internal strips. Such an assumption is supported by several previous reports on the conduction velocity of Purkinje fibers, i.e., 1,300 to 3,200 mm./sec.³ and 2,000 to 3,000 mm./sec.,¹ as well as by histologic evidence of their scantiness in the external muscle.

Our findings on the external strips, that the range of vertical conduction was narrow while that of parallel conduction was wide, and that the former was equal to the lower limits of the latter, seem to indicate that conduction velocity along the longitudinal axis of the myocardial fiber is great, probably having values close to the upper limits of parallel conduction, viz., 450 to 750 mm./sec. Although the two recording microelectrodes were placed in a direction parallel to the myocardial fibers, it is impossible by our method to be certain that both were always inserted into the same myocardial fiber. When the two electrodes were not in the same myocardial fiber, smaller values must have been obtained for parallel conduction velocity. Slight deviation from the same fiber would have resulted in a considerable drop of conduction velocity, even to the level seen in the vertical measurements, which probably represent the result of the excitation waves making the longest possible detour over the distance between the two recording microelectrodes. The presence of two peaks in the frequency distribution of parallel conduction velocity in figure 3A may be related to this situation. From this figure, and from the afore-mentioned interpretation, the conduction velocity along the longitudinal axis of the myocardial fiber of the dog ventricle is assumed to be 450 to 750 mm./sec. or more. Whether this range actually represents variations in different myocardial fibers, or whether its upper limit is the real conduction velocity, could not be determined from this experiment. These values are consistent with

several reports studied by other less accurate methods; for instance, Swain and Weidner⁵ reported 450 to 650 mm./sec. and Lewis and Rothschild⁶ assumed 300 to 500 mm./sec. to be the conduction velocity of the myocardial fibers.

Since these values of 450 to 750 mm./sec. are about two to ten times larger than those of vertical conduction velocity, 50 to 200 mm./sec., whereas the conduction velocity of Purkinje fibers, some 2,000 mm./sec., is also about three to five times that of the former, it is concluded that the possibility that the conduction velocity of myocardial fibers varies in different directions. Therefore it is postulated from this study that more attention should be given, in the theory of electrocardiography, to the course of the myocardial fibers.

In the internal strips both parallel and vertical conduction velocity ranged widely, although the former generally showed larger values. This is probably because the situation becomes complicated by the presence of Purkinje fibers.

The findings that various drugs, such as digitalis glycosides, quinidine, and procaine amide, increased the differences of conduction velocity in different directions deserve comment in conjunction with the fact that in the effects of drugs on isolated cardiac muscle the surface electrograms were far from the second derivatives of the monophasic curves of the action potentials obtained by microelectrodes intracellularly. This matter is being studied further.

SUMMARY

Directional difference of conduction velocity of myocardial fibers was examined by two or three microelectrodes with isolated cardiac muscle strips from the epicardial side and the endocardial side of the dog ventricle. The chief difference between external (epicardial) and internal (endocardial) strips was that the latter showed larger conduction velocity, including some of more than 1,000 mm./sec. which were never present in the former. This was interpreted as due to contribution of Purkinje fibers in the latter. In

external strips, presumably free of Purkinje fibers, the conduction velocity parallel to myocardial fibers ranged widely from 73 to 709 mm./sec., whereas conduction vertical to the fibers was at this lower limit and ranged narrowly from 80 to 199 mm./sec. Since the two microelectrodes could not always be inserted into the same myocardial fiber, it is concluded that the upper limits of parallel conduction velocity (450 to 750 mm./sec.) are the conduction velocity along the longitudinal axis of the myocardial fiber. This is from two to ten times larger than that of vertical conduction velocity, a difference which cannot be ignored when compared with the rapidity of conduction in Purkinje fibers. Thus, it is postulated that more attention should be given in the theory of electrocardiography to the course traversed by myocardial fibers.

Directional difference of conduction velocity became more marked with a rise of temperature. Mildly acting drugs, such as caffeine, did not alter such directional difference significantly, but some drugs, such as digitalis glycosides, quinidine, and procaine amide, increased it.

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SUMMARY IN INTERLINGUA

Le differentia directional del velocitate de conduction in fibras myocardial esseva examine per duo o per tres microelectrodos in isolate pecias de musculo cardiac prendite ab le latere epicardial e le latere endocardial del ventriculo canin. Le major differentia inter pecias externe (epicardial) e pecias interne (endocardial) esseva que in le secunde de iste due casos le velocitate de conduction esseva plus grande. In certe experimentos, valores de plus que 1000 mm/sec esseva observate in specimens endocardial. Un tal velocitate esseva nunquam incontrate in specimens epicardial. Iste phenomeno esseva interpretate como un effecto del fibras de Purkinje in le specimens endocardial. In specimens epicardial, supponitemente libere

de fibras de Purkinje, le velocitate del conduction parallel al fibras variava extensamente, i.e. inter 73 e 709 mm/sec, durante que le conduction vertical al fibras habeva basse e paucio variabile velocitates, i.e., inter 80 e 199 mm/sec. Viste que il esseva non semper possibile inserer le duo microelectrodos in le mesme fibra del myocardio, le conclusion se impone que le valores le plus alte inter le mesurationes del velocitate del conduction parallel (ab 450 usque a 750 mm/sec) es valores pro un conduction in le axe longitudinal del fibra myocardial. Isto es inter due e dece vices plus grande que le valores del velocitate de conduction vertical. Il se tracta hic de un differentia que es certo non negligibile, e il es postulate que plus attention deberea esser prestate al direction del fibras myocardial como factor de importantia in le theoria electrocardiographic.

Le differentias directional in le velocitate del conduction esseva plus marcate quando le temperatura se elevava. Drogas a action dulce, per exemplo caffeina, non afficeva le differentias directional de maniera significativa, sed certe altere drogas accentuava los. Tal drogas es glycosido de digitalis, quinidina, e procainamido.

REFERENCES

1. TRAUTWEIN, W., GOTTSTEIN, U., AND DUDEL, J.: Der Aktionsstrom der Myokardfaser im Sauerstoffmangel. *Pflüger's Arch. ges. Physiol.* **260**: 40, 1954.
2. SCHAEFER, H., AND TRAUTWEIN, W.: Ueber die elementaren elektrischen Prozesse im Herzmuskel und ihre Rolle fuer eine neue Theories des Elektrokardiogramms. *Pflüger's Arch. ges. Physiol.* **251**: 417, 1949.
3. DRAPER, M. H., AND WEIDMANN, S.: Cardiac resting and action potentials recorded with an intracellular electrode. *J. Physiol.* **115**: 74, 1951.
4. SANO, T., ONO, M., AND SHIMAMOTO, T.: Intrinsic deflections, local excitation and transmembrane action potentials. *Circulation Research* **4**: 444, 1956.
5. SWAIN, H. H., AND WEIDNER, C. L.: A study of substances which alter intraventricular conduction in the isolated dog heart. *J. Pharmacol. & Exper. Therap.* **120**: 137, 1957.
6. LEWIS, T., AND ROTHSCHILD, M. A.: Cited by Swain and Weidner.⁵