AN INVESTIGATION OF THE PROPERTIES OF CARDIAC MUSCLE AND PURKINJE TISSUE

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by

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INTRODUCTION

Although it is nearly 70 years since Waller (1887) first recorded the human electrocardiogram, there is still a divergence of opinion among various workers as to the genesis of the electrocardiogram. It is generally agreed, however, that one of the principal factors involved is the order in which the various parts of the heart are excited. The finer details of the manner in which the excitation wave spreads both in the auricles and in the ventricles are still not clear and therefore various assumptions have had to be made in regard to this for the analysis of the electrocardiogram.

The purpose of this study was to investigate certain aspects of the spread of excitation in cardiac tissues as it relates to the genesis of the electrocardiogram. This entailed an investigation of some fundamental properties of these tissues. In Part I of the thesis, observations on the monophasic action potentials from different species of mammalian hearts, recorded by means of a Ling-Gerard intracellular microelectrode are described. This part is in general a correlative study of the monophasic action potential and the electrocardiogram obtained from the same animal.

Structurally, the heart is complex in that (1) it is a syncytium, (2) there are several muscle bands running in different directions which form the walls of the atria and the ventricles and (3) a specialised conducting tissue is widely distributed in the ventricular walls and sometimes in the atria. An investigation of the spread of

excitation in the heart would have to take into account these structural characteristics of the pathway. Part II describes observations on the structural aspects of the subject.

It is also difficult to really understand the spread of excitation in the heart until some knowledge has been obtained about the rate of conduction in the various cell types making up the excitable tissues of the cardiac musculature. As will be clear from the review in Part III, this property has never been studied adequately. Particular attention has therefore been paid to this aspect of the subject in the present study and this investigation is described in Part III.

Various theories of the genesis of the electrocardiogram have been advanced as our knowledge of the manner of spread of excitation in the heart has increased. The development of ideas regarding the spread of excitation in the mammalian heart has provided a general background to the present studies and it has therefore been briefly reviewed. The literature on the subject is so extensive that to give a complete review is neither possible nor relevant to the present study. Only those papers which in one way or other relate to the observations made in this investigation have been included in the survey. Early work has been reviewed by Gaskell, (1900), Erlanger, (1913) and by Davies & Francis, (1946). The literature on the genesis of the electrocardiogram has been extensively reviewed by Katz, (1928, 1947).

The Spread of Excitation in the Heart: A Brief Historical Survey

Although early physiologists certainly recognised the regular sequence of contractions of the different chambers of the heart, it seems that Harvey (1628) was the first to make a clear statement regarding it. In his book "De Motu Cordis" he wrote (p. 39) "Two sets of movements occur together, one of the auricles, another of the ventricles. These are not simultaneous, but that of the auricles precedes that of the rest of the heart. The movement seems to start in the auricles and to spread to the ventricles". He did not make any statement regarding the mechanism by which this regular sequence of contractions is brought about. He did, however, show that if the heart is cut into small pieces, the separate parts contract and relax (Harvey, 1628, p. 42). It seems that this simple demonstration of the property of inherent rhythmicity and contractility of heart muscle was overlooked by physiologists of that time who held the idea that the heart being an involuntary organ must be under the control of the central nervous system and therefore its automaticity and co-ordination due to its connection with the central nervous system. Thus Willis and later Borelli put forward the view that the automaticity of the heart was due to the 'animal spirits' or 'nerve juice' elaborated in the central nervous system and passed to the heart through the cardiac nerves (Howell, 1936).

In 1757, Haller showed that the contractions of the heart were independent of its connections with the central nervous system. He thought that the blood acted as a

stimulus for the heart muscle to contract and therefore the sequence of contractions of the auricles and the ventricles was due to the blood flowing from the auricles into the ventricles during its circulation. This view was disproved when it was soon apparent that the heart could still continue beating when excised from the body.

Towards the middle of the 19th century, following the teaching of Bichat (1801-3), the view was held that the somatic and automatic functions of the body were controlled by separate parts of the nervous system acting independently of each other. The sympathetic ganglia were thus regarded as independent and isolated centres. It was natural, therefore, that when ganglion cells were described by Remak (1844) in the sino-auricular junction, by Ludwig (1848) in the interauricular septum and by Bidder (1852) in the auriculo-ventricular junction of the frog heart, the seat of automaticity of the heart was referred to these intrinsic cardiac ganglia, and the co-ordination of the auricular and ventricular contractions thought to be brought about through the nerves passing out from these ganglia. For the next 30 or 40 years, this neurogenic theory of automaticity and conduction in the heart was generally accepted by physiologists.

There were some who sought for mechanical explanations to account for the sequence of auricular and ventricular contractions. Reid (1839) attributed this to the mechanical stimulation of the ventricles by the tug of the auricular contractions. Kurschner (1850) suggested that the contractions of the auriculo-ventricular valves brought about the contractions of the ventricles in sequence to that of the

auricles.

It was about this time that the first paper on the electrophysiology of the heart was published. Kölliker & Müller demonstrated in 1856, exactly 100 years ago, that electrical variations accompanied heart contractions. This work was followed by the investigations of Marchand (1877), Engelmann (1878) and Burdon-Sanderson & Page (1878, 1879) who studied the electrical variations and their time relations by leading off from the base and apex of the ventricles of excised hearts of cold-blooded animals. These workers showed that negativity of the base preceded that at the apex and it was therefore assumed that the wave of excitation in the spontaneously beating heart passed from the base to the apex. This demonstration of the order of excitation of the ventricles together with the work of Engelmann (1875) and Gaskell (1883) put the theory of myogenic conduction on a very firm foundation.

When these electrophysiological experiments were extended to the mammalian heart, variable results were obtained. Thus Waller & Reid (1887) found that sometimes the excitation of the base preceded that of the apex, in other cases, the apex preceded the base, while yet in others, the excitation wave seemed to travel from the base to the apex and then back to the base. Waller (1889) on re-investigating the problem on hearts in situ, found that in most of the cases the excitation of the apex preceded that at the base, their previous variable results being attributable to the unstable condition of the dying excised organ. From this order of excitation in the ventricles and from the high rate of conduction he

found in the sheep ventricle (8m/sec), Waller concluded that the spread of excitation in the ventricles was through nervous channels. Although Bayliss & Starling (1891, 1892) found the order of excitation to be from the base to apex, as in cold-blooded hearts, the high rates of conduction they obtained in the dog ventricles (3 m/sec) led them to suppose as Waller did, that conduction in the ventricles was by the intramuscular network of nerves. This tendency to attribute the transmission of excitation in the mammalian heart to nervous agency by investigators of that period (see also McWilliam, 1888) was due to the fact that up till then no muscular connection has been shown to exist across the atrio-ventricular fibrous junction in the mammalian heart. This, however, was soon to be demonstrated.

In 1892 Kent described a number of muscular connections across the atrio-ventricular junction in the hearts of newly born rats. In 1893 he extended these observations to the hearts of monkeys, dogs, cats, guinea-pigs, hedgehogs, rabbits and by using Gaskell's clamp he was "able to verify for the mammal almost all the effects described by Gaskell as obtained in the frog". In the same year, His, Jr. (1893) described the muscular bundle connecting the auricles and ventricles, and which is now called after him.

In 1906 Tawara published a monograph "Das Reizleitungssystem des Saugethierherzens" in which he showed that the bundle of His did not end by joining with the muscle fibres in the interventricular septum as His and others thought, but that it divided into a right and left branch in the region of the membranous septum and these continue down the

right and left sides of the septum under the endocardium to become continuous with the subendocardial network of the peculiar large muscular cells described by Purkinje in 1845. He also described a network of slender muscular fibres in dense fibrous tissue, now known as the atrio-ventricular node, situated in the auricular septum just above the coronary sinus and with which the bundle of His is continuous. From the anatomical distribution of these special structures in the heart, Tawara suggested that the bundle of His and the subendocardial network of Purkinje fibres carried the impulse rapidly to distant parts of the heart, thus enabling all parts to be stimulated simultaneously. This seems to be the first time the Purkinje fibres were described as conducting tissue. Previous workers had variously described them as developmentally arrested cardiac cells, special muscular tissue of the endocardium, pathological heart muscle cells or even a special motor end organ.

A year after the publication of Tawara's book, Keith & Flack (1907) discovered the 'Sino-Auricular Node', a group of slender muscle fibres similar in appearance to the atrio-ventricular node, situated at the upper end of the sulous terminalis. They suggested that it was the region where "the dominating rhythm normally begins".

These anatomical findings profoundly influenced the ideas concerning conduction in the mammalian heart. It appeared that mammalian hearts differed from cold-blooded hearts in that they have a special conducting system. Once structures which could initiate and conduct the impulse across the atrio-ventricular junction to the ventricles had

been demonstrated anatomically, many physiological experiments were performed to test the postulated function of these newly discovered tissues.

Following up the suggestion of Keith & Flack, many workers attempted to determine the function of the sino-auricular node by excising, destroying by heat or formaldehyde, or by warming or cooling the node. Conflicting results were obtained. Those who did observe a change in the rhythm of the heart by destroying the SA node criticised the experiments of those who did not observe any change, that not all of the SA node had been destroyed or excised in their experiments. As the full extent of the SA node was still not known anatomically, this method of extirpation to determine the function of the SA node was unsatisfactory.

It was, however, due to the electrocardiographic studies of Lewis and his colleagues (Lewis, Oppenheimer & Oppenheimer, 1910; Lewis, 1913) that the SA node came to be generally accepted as the pacemaker of the mammalian heart. Using a string galvanometer, and working on exposed dog hearts, they showed that the earliest point of negativity and hence the first point of activity in the auricles was in the region of the head of the SA node. He also showed that the form of the P wave in the electrocardiogram obtained by stimulating the auricles resembled that found in a normal electrocardiogram only when the point stimulated was in the region of the head of the SA node. It was assumed that only then did the excitation wave taking a normal course produce a normal P wave. The results of these two experiments were held to provide conclusive evidence that the

impulse initiating the mammalian heart beat arose from the SA node.

There were still some investigators, notably Erlanger, (1913) who did not subscribe to this view. He pointed out that the interpretation of Lewis' experiments are open to criticism as the electrical activity was recorded only from the surface of the heart and therefore his method would not detect any primary activity within the auricular wall until it reached the surface. Finding that auricular strips from the region of the mouths of the great veins (which do not contain any sino-auricular or atrioventricular nodal tissue) have a higher rhythmicity than those regions containing nodal tissue (Erlanger, 1910; Moorhouse, 1912), he raised the point whether a region having a lower rhythmicity than another region could act as the pacemaker to the heart. Basing on the embryological work of His (1880) who had shown that the sinus venosus of cold blooded heart becomes the extensive 'sinus reuniens' which lies embedded in the right auricular wall in most of its extent in the human heart, he suggested that the pacemaker in the mammalian heart is the sinus reuniens.

Others (Morison, 1912) pointing out that nerve cells and fibres are abundant in the SA node, questioned the validity of assigning the primary role of stimulus formation to the muscle cells in that region. Recently, Glomset & Glomset (1940) studying the SA node in man, dog, pig, ox and horse have claimed that no such structure exists in these animals - the structure of the SA 'node' being caused by the muscle fibres of the superior vena cava entering the atria

obliquely. According to them similar 'nodes' can be found in the left atrium where the pulmonary veins entered it obliquely, and also in some parts of the ventricles. They drew attention to the numerous ganglia and nerve fibres in this region.

The function of the AV bundle was tested soon after it was described. His in 1895 presented a preliminary communication to the Physiological Congress in Bern in which he described experiments showing that sectioning the bundle brought about a dissociation between auricular and ventricular contractions. This was followed by the work of Humblet (1904), Hering (1905) and Erlanger (1905) who all confirmed His' preliminary observations. Some workers however, failed to produce heart block on sectioning the bundle in the rabbit heart (Kronecker & Busch, 1899; Biggs, 1909). These anomalous results were later shown to be due to variations in the branching of the AV bundle in that animal (Cullis & Dixon, 1912; Lloyd, 1930). The argument brought forward by Kronecker & Busch in support of the neurogenic theory, that the AV delay could not be accounted for by the slow conduction in the AV bundle lost much of its force when the AV node was described by Tawara and after Hering (1910) demonstrated that the AV delay was due to the slow conduction in the AV node.

Wilson (1909), Morison (1912), Glomset & Birge (1945) and Field (1951) finding numerous nerve fibres in the AV bundle maintained that it was these nerves that conducted the impulse across the AV junction. It was contended that all experimental procedures on the AV bundle affected these nerves as well. This possibility had previously been recognised by

Cohn & Trendelenburg (1910) and by Erlanger & Blackman (1909-10). The latter workers in order to answer the question performed an experiment in which they cut the AV bundle in dogs and showed that heart block remained permanent, in one case up to a period of 343 days after the operation. On killing the animal after that time and examining the region of the AV bundle histologically, they found that no regeneration of the muscle fibres of the AV bundle had taken place. It was reasoned that if conduction was by the nerves in the AV bundle, normal rhythm should have been restored after some time since nerves are known to be regenerate whereas they had shown that heart muscle does not regenerate (Erlanger & Miller, 1909). From these experiments, they concluded that AV conduction was by the muscular elements in the AV bundle. Robb, Kaylor & Turman (1948) have recently drawn attention to the possibility that the thick scar tissue might have prevented the fine nerves from regenerating and making functional continuity. It might be worth while to repeat Erlanger's experiment of producing chronic heart block and later, using the improved methods of staining for nervous tissue, determining whether the nerves in the AV bundle have in fact regenerated. The experiments of Cullis & Dixon (1912) who failed to produce heart block in the rabbit by applying 5% cocaine to the bundle of His would seem to prove against the view that the nerves in the AV bundle conducted the impulse from the atria to the ventricles. An objection which might possibly be raised against this experiment is that the cocaine was applied to the endocardial surface of the bundle of His and therefore the nerves within the bundle protected by the

thick fibrous tissue might still be unaffected by the cocaine.

Although numerous clinical reports appeared demonstrating fibrosis in the region of the bundle of His, in patients who had shown heart block before death, there were occasional reports of cases where extensive destruction of the bundle produced no heart block. Martin & Klotz (1910) reported a case of a patient who died of extensive sarcoma of the heart with complete destruction and disorganisation of the AV bundle, who had normal rhythm before death. Agduhr (1933) showed that focal areas of degeneration of the AV bundle in mice produced by high doses of cod-liver oil did not cause heart-block and subsequent histological examination showed intact nerve fibres in the vicinity of the AV bundle. This work was supported by Wahlin (1935). They therefore again raised the question whether it might be the nerves which conduct the impulse across the AV junction.

Glomset & Birge (1945) also supporting the neurogenic theory of conduction contend that in man, dog and monkey the AV bundle is the supravalvular part of an ordinary muscle fasciculus - the ridge fasciculus, and not a specialised bundle. The right bundle branch is the infravalvular part of the ridge. According to them there is no left bundle branch and no AV node in these animals. These anatomical findings have not been confirmed by subsequent workers (Kistin, 1949; Baird & Robb, 1950; Wildron & Lev, 1951; Read, Hegre & Russi, 1953). On only one point do they agree with Glomset & Birge - that the cells in the AV bundle in these animals are not very much differentiated from myocardial cells, a fact known long before (Tawara, 1906; Blair & Davies, 1935).

Davies & Francis (1952) feeling that purely morphological studies are not crucial in determining whether conduction is by the nerves or muscle in the AV bundle, correlated the QRS duration to the degree of histological specialisation of the conducting system and to the heart size. They found that the QRS duration is related to the diameter of the muscle cells in the conducting system and therefore concluded that AV conduction was by muscle. Their experiment is to a certain extent unsatisfactory in that the fibre diameters and the electrocardiograms were taken from different animals, and when it is realised that the fibre diameters as well as the QRS duration may vary to a large degree from animal to animal of the same species. To take any average figures as they did is open to objection. Also it has not yet been proved that the duration of the QRS complex represents the time taken for the impulse to spread through the ventricles.

From the above, it would appear that as long as the function of the nerves in the AV bundle is not known, the role of the muscular elements in the AV bundle in AV conduction will continue to be questioned.

Lewis and his colleagues (Lewis, Meakins & White, 1914; Lewis & Rothschild, 1915; Lewis, 1916) in an attempt to harmonize the course of the excitation wave in the heart with the deflections in the electrocardiogram, studied the spread of excitation in several vertebrate hearts and found that it was much more intricate than the simple base-apex direction as hitherto believed. They showed that in mammals the excitation wave followed the anatomical conducting pathway described by Tawara. This scheme of activation has

found general acceptance though from time to time slight modifications have been suggested. Lewis & Rothschild (1914) had shown that the impulse reached different points on the epicardial surface of the ventricles at different times, the time lag being sometimes as much as 30 msec. They found that in the dog heart, the earliest point of arrival of the wave of excitation is on the anterior surface of the right ventricle just over the papillary muscle, and it arrives later over the conus arteriosus and at points near the atrio-ventricular groove in the right and left ventricle. In 1930 Barker, Macleod & Alexander determing the time of arrival of the excitation wave on the exposed human heart found that there was some difference in the sequence of excitation as measured by them and as found by Lewis & Rothschild in the dog heart. Thus they found that the earliest point of arrival on the ventricular surface is near the atrio-ventricular junction on the right ventricle and over the conus arteriosus and that it arrives at an appreciably later time at the points found by Lewis & Rothschild to be excited first. These time differences they suggested were due to some anatomical differences in the distribution of the conducting system in man and in dogs.

In 1931 Cardwell & Abramson and in 1936 Abramson & Margolin described Purkinje fibres in the interventricular septum and in the ventricular myocardium. In 1937 Abramson & Joachim re-investigated the sequence of arrival of the excitation wave at the ventricular epicardial surface. They found that the whole epicardial surface of the ventricles was excited almost simultaneously and they suggested that

the time sequences obtained by Lewis & Rothschild were due to surface cooling of the heart, as they also obtained similar results if the heart surface was allowed to cool in their experiments. Wiggers (1937) using suction electrodes obtained the same results as Abramson & Joachim and like them attributed his results to the rapid conduction of the impulse to the epicardial surface by the myocardial Purkinje fibres. Harris (1941) on the other hand, obtained results to support Lewis. It is of interest to note that he also obtained results reported by Abramson and Joachim when the exposed thoracic contents were covered by a warm moist chamber as those authors had employed. He, however, maintains that the temperature of the heart surface was approximately that of the blood in his experiments and that therefore the results obtained by Abramson & Joachim needed another explanation. A probable explanation for these conflicting results, which none of these investigators seemed to have mentioned is the wide variation in the distribution of the conducting system from animal to animal in the same species. Fig. 2 in the thesis by Draper (1955) depicts the interior of a number of hearts showing great variation in the distribution of the false tendons.

Kaufman, Chernoff & Nahum (1948) determined indirectly the sequence of ventricular excitation in the dog, by an analysis of the instantaneous points of the QRS complex during successive time intervals in the three simultaneously recorded unipolar limb leads. Having found that their results were in accord with the results of direct measurements on the heart by Lewis & Rothschild, they extended their method to

human hearts. They, however, found that the distribution of negative potential exhibits a sequential order quite in contrast to the orthodox concept of the spread of excitation in the heart (Nahum, Mauro, Chernoff & Sikand, 1951; Nahum, 1955).

Another mode of spread - that along the muscle sheets was proposed by Robb & Robb (1936). According to this view, the excitation wave in the ventricular wall is conducted 'axially' along the muscle layers of the ventricles and not at right angles as Lewis had conceived. This view had also been held by Kraus & Nicolai (1910) and had been used by them to interpret the electrocardiagrm. Gotch (1910) had also conceived of a similar method of spread of excitation in the heart. To account for the activity of the base outlasting that at the apex, which would explain the T wave in the electrocardiogram, he suggested that the excitation wave travelled from the base to apex and then from the apex to the base following the path of the embryonic cardiac tube after it had doubled upon itself. Robb (Robb, Kaylor & Turman, 1948) later conceded that the Purkinje fibres are concerned in conducting the impulse in the ventricular walls after their histological studies had shown that the myocardial Purkinje fibres end parallel to the myocardial fibres, which could thus account for their previous observation that the impulse was conducted axially along the direction of the muscle fibres. That the Purkinje fibre system in the heart is chiefly concerned with rapid conduction is still denied by some workers (Pruitt, Essex & Burchell, 1949).

Lewis in his studies had recorded from endocardial and

epicardial surfaces of the heart and therefore spread in the septum and myocardial walls had to be inferred from surface recordings. During the last decade, interest in this aspect has greatly increased and at least five groups of workers are investigating this problem. Thus Prinzmetal and his colleagues (Kennamer, Bernstein, Maxwell, Prinzmetal & Shaw, 1953), Scher and his colleagues (Scher, Young, Malgren & Paton, 1953; Scher, Young, Malgren & Erickson, 1955), and Burchell and colleagues (Burchell, Essex & Pruitt, 1952) in the United States, Durrer and his co-workers in Holland (Durrer, Tweel & Blickman, 1954; Durrer & Tweel, 1954), and Sodi-Pallares and his co-workers in Mexico (Sodi-Pallares, Rodriguez, Chait & Zuckermann, 1951; Sodi-Pallares, Bisteni, Medrando & Cisneros, 1955) have all been studying the spread of activation in the ventricular walls and in the interventricular septum using intromural electrodes. In general their results seem to support the view that activation in interventricular septum and the ventricular myocardial wall takes place rapidly along the myocardial Purkinje fibres described by Abramson & Margolin.

The differences of viewpoint revealed in the above brief survey are due partly to the fact that individual and species differences have not been appreciated and partly because until recently techniques available were inadequate. Previous studies have tended to be restricted to either morphological or physiological matters and as a result sometimes physiological experiments have had to be interpreted in the light of morphological studies which are not really appropriate for the particular problem, for example,

extrapolations from one species to another or even from one part of the heart to another. Conversely, purely morphological studies often fail to give specific information on aspects relevant to physiological studies, for example, the precise relationship of the Purkinje cells to the cardiac cells or the species differences in the distribution of the conducting system. This variation in morphology and in physiological reactions of the cardiac excitable tissues in different species of animals will be more clearly appreciated when the results of the present study are discussed in subsequent sections.

Previously, cells of the conducting system could not be distinguished easily from ventricular myocardial cells except by histological examination. The shape of the monophasic action potential were always regarded with some doubt because of the imperfections of the method employed in producing and recording them. The development of electronic amplifiers and cathode ray oscilloscopes coupled with the Ling-Gerard intracellular microelectrode has removed most of these difficulties.

PART I

THE MONOPHASIC CARDIAC ACTION POTENTIAL

AND THE ELECTROCARDIOGRAM

PART I

The Monophasic Cardiac Action Potential and the Electrocardiagram

INTRODUCTION

Within recent years much work has been done towards the elucidation of the nature of the cardiac action potential, by the use of the Ling-Gerard microelectrode (Draper & Weidmann, 1951; Burgen & Terroux, 1953; Weidmann, 1951, 1952, 1955, 1956). These studies have indicated that the initial rising phase of the action potential, like that in nerve and striated muscle action potential, is due to a specific increase in the permeability of the surface membrane to sodium ions allowing them to enter the fibre at a fast rate (Hodgkin, 1951; Hodgkin & Huxley, 1952a). The peculiar electrical changes which take place during the repolarisation phase has so far defied any solution. Studies by Hodgkin, Huxley & Katz (1949) and by Hodgkin & Huxley (1952a) have indicated that the falling phase of the action potential in nerve is due to a rapid inactivation of the sodium permeability with an increase in the potassium permeability of the surface membrane. From impedance measurements during the cardiac cycle, Weidmann (1951) suggested that the delay in the repolarisation is due to a great retardation of the increase in potassium permeability.

Previous studies have shown that the repolarisation phase of the action potential is different in various tissues of the heart and in the cardiac tissues from different species of animals. Any theory advanced to explain the

repolarisation phase of the cardiac action potential would have to take into account the great variability in the shape of the action potentials. So far, no worker has attempted to study this subject systematically from the comparative aspect. The observations recorded in this part of the thesis is a step in such a direction. No experimental procedures have, however, been performed on these tissues to test any theory of repolarisation of the cardiac action potential.

There is already much evidence to show that the T wave in the electrocardiagram is due to the repolarisation changes occuring in the heart muscle fibres (for review see Katz, 1928 & 1947). The monophasic cardiac action potential can now be recorded faithfully by means of the Ling-Gerard intracellular microelectrode. This has enabled one to test directly whether the repolarisation of the heart muscle fibre does in fact correspond to the T wave.

METHODS

A. GENERAL EXPERIMENTAL PROCEDURE

Goats, dogs, cats, rabbits, guinea-pigs, rats, a golden hamster and mice were used in this study.

The animal was anaesthetised by an intravenous or intraperitoneal injection of sodium pentobarbitone (Veterinary Nembutal, Abbott) the dosage being 0.5 ml/kilogram body weight. The dosage was the same irrespective of whether the injection was by the intravenous or the intraperitoneal route. The electrocardiogram was then recorded, after which an intravenous injection of heparin (Liquemin, Roche; 1 ml/kilogram body weight) given and the heart removed. The injection of heparin was necessary especially in large animals, as it minimised the formation of blood clots in the heart, particularly around the false tendon network. These clots were found to be very tenacious and extremely difficult to remove, so that once they had formed, there was always the risk of damaging the false tendons by undue stretching in trying to remove the clots round them.

The excised heart was placed in warm oxygenated Tyrode solution, ($30^{\circ}C$) in a shallow dish ready for dissection. Samples of auricular, atrial, and ventricular muscle and false tendons were dissected out and put in a reserve bath containing warm Tyrode solution ($30^{\circ}C$) which was continuously oxygenated with a 95% oxygen/5% CO_2 gas mixture. One tissue sample was then selected and placed in the muscle bath ready for experiment.

B. ELECTROCARDIOGRAPHY

The anaesthetised animal, lying on its back was tied

down to the table. Hypodermic needles stuck under the skin were used as recording electrodes.

Leads The three standard limb leads and precordial leads were used. Following convention, the connections were such that negativity at the right arm electrode in Leads I and II and at the left arm electrode in Lead III produced an upward deflection in the tracing.

Two precordial leads were used. In one, the precordial electrode was placed under the skin over the xiphisternum; in the other it was at the lower end of the right border of the sternum (corresponding to the CR_1 lead in man). In both the precordial leads the 'distant' electrode was on the right arm. Negativity at the precordial electrode produced an upward deflection on the tracing.

The earth connections were as follows: in Lead I, the left leg; in Lead II, the left arm; in Lead III and in the precordial leads, the right arm.

Voltages were not standardised as from the tracings only the heart rate and the form of the electrocardiogram were studied.

The amplifier unit was a resistance capacity coupled amplifier of conventional design (Dickinson, 1950), with a time constant of about 1 sec; consequently the shape of the slower changes in the electrocardiogram cannot be recorded faithfully. The tracings were displayed on a cathode ray screen and photographed.

C. RECORDING OF THE MONOPHASIC CARDIAC ACTION POTENTIAL

For the study of the cardiac action potential, the technique described by Draper & Weidmann (1951) employing a

Ling-Gerard capillary intracellular microelectrode was followed, with some modifications.

EQUIPMENT

Electrodes

1. Microelectrodes These were drawn by hand by the method already described in detail by Draper (1955). Standard glass tubing (O.D. 2.5 mm, Monax) was used. To clean the glass tubing they were cut into lengths of about 4 cm. and these short lengths of tubing were boiled in ordinary soap solution for an hour, after which they were washed thoroughly in running tap water for another hour and finally washed in distilled water. They were dried in an oven and were then ready to be pulled down into microelectrodes.

The electrodes were filled by boiling them in 3-Molar potassium chloride solution for half an hour and leaving them under reduced pressure overnight in the refrigerator. This latter procedure assured that all the electrodes filled with the solution.

The resistance of the electrodes was measured using an 'AVO' Electronic Testmeter. Those having a resistance between 15 and 50 M were kept and finally checked under a water immersion lens (N.A. .94 x 42). Only those having a thin wall, a sharp point with the proper kind of taper and the lumen free from dust particles were finally selected. These electrodes were then stored in 3 M-KCl solution and kept in a refrigerator. This was to prevent the growth of fungi at the tip and shaft of the microelectrodes which occurs if they are left at room temperature for any length of time.

Prepared by this method, the yield of good electrodes

is about 10%.

2. Stimulating Electrodes External electrodes were used to stimulate the tissues. These were chlorided silver wires, 200 μ in diameter, passed down a fine twin polythene tube to a fraction of a millimetre of their open tips. Using a micromanipulator, the electrodes were applied to the surface of the tissue with just enough pressure so that they did not shift their position when the tissue contracted. The distance between the cathode and the anode was about 0.5 mm.

The tissue was stimulated by a rectangular pulse of about a millisecond duration and about 4 times the threshold strength obtained through a Carpenter Relay (Type 3G2) which was driven from a square wave stimulator locked to the time base of the cathode ray oscilloscope sweep circuit.

Amplifier Unit

A direct-coupled push-pull amplifier similar in design to that used by Nastuk & Hodgkin (1950) with a cathode follower input stage was used. The time lag of the recording system was about 100 μsecs. The action potentials were displayed on a cathode ray oscilloscope.

Time Tracings

The time 'pips' were obtained from an oscillator unit controlled by a crystal. In some experiments, sine waves from a signal generator were used.

Photography

The action potentials were displayed on a second cathode ray tube and photographed on Ilford or Kodak 35 mm recording film. The records were studied by enlarging using a Leitz Enlarger.

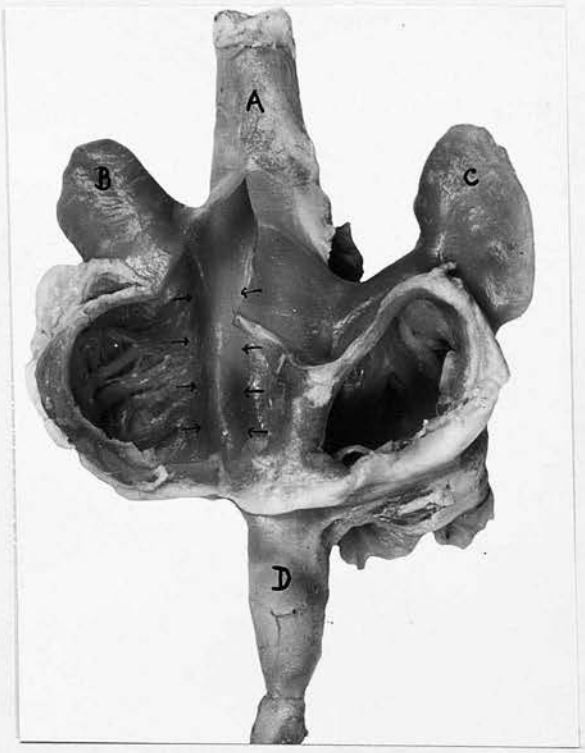

Fig. 1

Goat atria separated from the ventricles at the AV junction. Part of the superior vena cava has been slit open to show the position of the crista terminalis (sulcus terminalis), between the arrows.

A = Superior vena cava

B = Right auricle

C = Left auricle

D = Inferior vena cava

D. TISSUE SAMPLES

General Principles

The general principles which guided the selection and dissection of the tissue samples were as follows:

(1) Thick tissue samples were avoided if possible as oxygenation of the inner cells is not adequate.

(2) Tissue samples were chosen so that as few surfaces as possible were cut.

(3) As little trauma as possible was inflicted on the tissue during dissection. Extreme gentleness is essential.

(4) Stretching of the tissue, a common tendency during its removal, was avoided.

Dissection

1. Sulcus Terminalis This strip of tissue stretching between the entrances of the superior and inferior venacava was taken as a sample of atrial tissue. After many trials, it was discovered that the best way to identify and dissect it out was to expose the endocardial surface of the right atrium by cutting open the right auricle along its lower edge and extending the cut along the junction of the atrium and the AV fibrous tissue, up to a few mm of the point where the inferior venacava enters the right atrium. The free cut edge of the auricle was then lifted up, exposing the endocardial surface. Thus displayed, the sulcus can usually be identified quite easily. It is the strip of muscle which separates the trabeculated auricle from the smooth atria (Fig. 1). If a length of both the superior and inferior venacava is left attached to the heart when it is removed from the animal, identification of the sulcus terminalis is rendered much

easier.

The whole length of the sulcus was excised out and placed in the experimental bath. It was usually found to beat spontaneously. The sulcus terminalis is usually the thickest tissue sample obtained from any heart, consequently it is advisable to start the experiments with this tissue.

2. Auricular Muscle One of the auricular trabeculae running out from the sulcus was usually selected as a sample of auricular tissue. Occassionally an auricular trabecula could be found in which the endocardium enveloped the trabecula all round. It is then easily isolated without inflicting much injury by just lifting up the trabecula at its middle and snipping off its attached ends.

The auricular trabeculae used were mostly from the right auricle. Those in the left auricle, unlike those in the right, have a thick endocardial layer and very few of them are unattached to the underlying musculature. In smaller animals, (guinea-pigs, rats, hamster and mouse), the whole atria was separated off from the ventricles at the AV junction and the whole atria mounted in the bath. Selected spots were then impaled with the microelectrode.

3. Ventricular Muscle In rabbits and cats, the anterior papillary muscle from the right ventricle is long and slender and is easily removed. In goats and dogs, however, this muscle is usually too massive for it to survive long in the bath. Fortunately one can usually find small muscular slips, in these larger animals, arising from the wall of the right ventricle or the inter-ventricular septum near the AV junction and being attached to the atrio-ventricular fibrous ring.

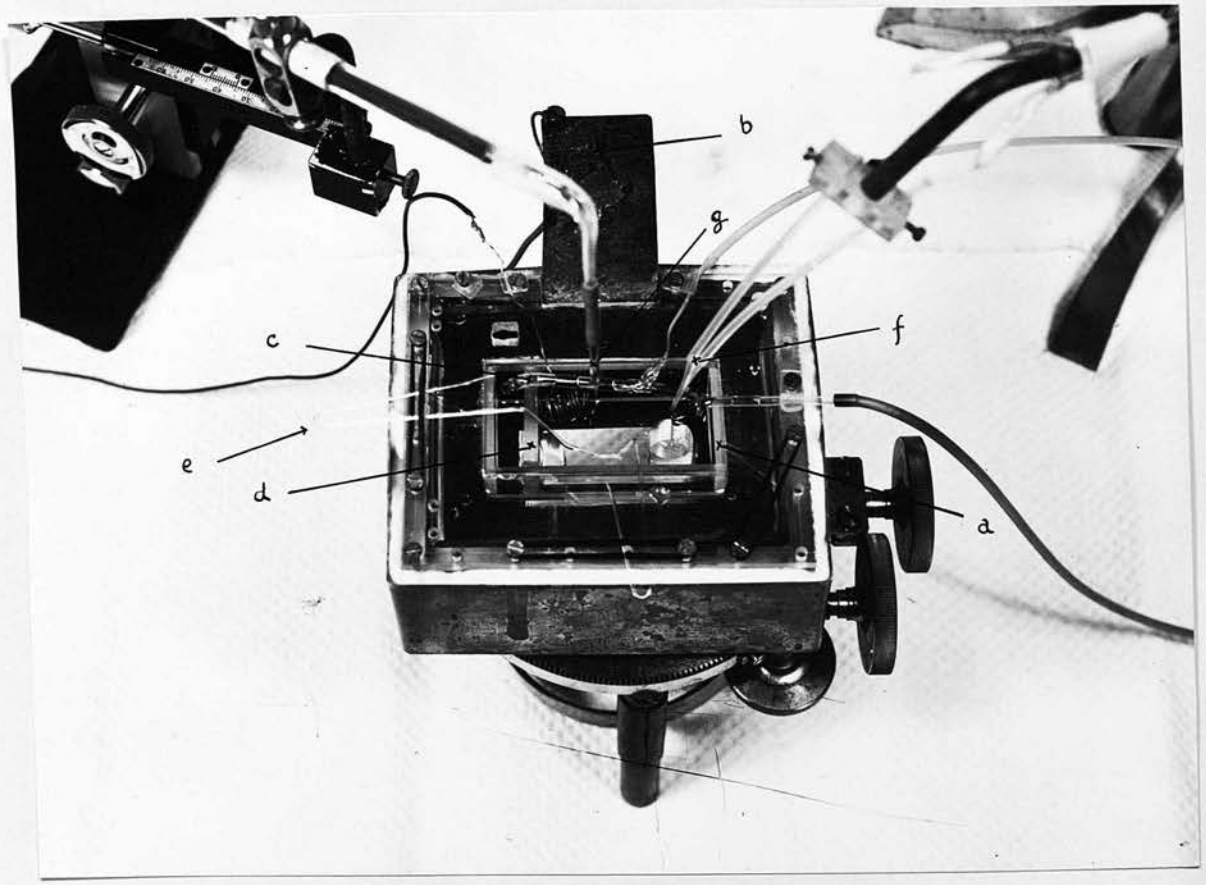

Fig. 2

Experimental Set-up.

a - muscle bath
b - "tongue" of heater copper plate
c - glass "bubbler"
d - glass partition
e - glass hooks
f - stimulating electrodes
g - microelectrode

The small bit of tissue can be seen pinned out by the glass hooks

These have been used. In all these animals the left papillary muscles were too big.

In the smaller animals, the papillary muscles in the right ventricles were very small. The right ventricular wall in these animals was thin so that the whole ventricular wall was dissected out and mounted in the bath, with the endocardial surface facing upwards. Sometimes the left anterior papillary muscles were found to be suitable.

4. False Tendons These were used as samples of Purkinje tissue. The dissection of false tendons for experiment has been described in detail by Draper (1955). His technique has been followed throughout. Essentially, it consists of undercutting the subendocardial portion of a large branch of the false tendon network, using a pair of fine sharp pointed scissors, and tying a piece of nylon thread to its end before detaching it. Then using the piece of thread to steady the false tendon, the subendocardial portions of the remaining false tendons were undercut and the network removed. The important point is to avoid stretching the false tendon at any stage of the dissection.

E. Experimental Set-Up

Fig. 2 shows the experimental set-up.

The muscle bath (Fig. 2a) measuring 6.5 x 3.5 x 1.5 cm. internally was made of glass. This was fitted into a water bath made of Perspex and measuring 11 x 9 x 4.5 cm. externally. The glass muscle bath which was immersed to a depth of about 1 cm. into the water bath, was supported by two cylindrical perspex rods of half inch diamter.

The heating element in the water bath was a thick

copper plate shaped to line the bottom and one side wall of the water bath ending in a 'tongue' projecting horizontally from the upper edge of the side wall (Fig. 2b). The 'tongue' of copper was heated by a small gas flame. Using this method of heating, the temperature of the bath required about 3 hours to stabilise during which time adjustments of the size of the flame had to be made. Once it has stabilised at 99-100°F (37.2-37.8°C), however the temperature of the bath remained remarkably constant, varying between ±1°F. The long time required for the temperature of the water bath to stabilise was not found to be a disadvantage in the present experiments because it usually took about 3 hours for various other preparations, such as making up the solutions, and by the time the tissues were excised from the heart, it was usually about 12 noon, so that if one lit the flames at about 9.00 a.m. the water bath was ready by about noon.

The advantage of this heating system is its simplicity. It also eliminates electrical circuits for immersion heaters near the recording equipment and thus minimises 'pick-up'.

The temperature of the solution was always checked by keeping a small spirit thermometer in the muscle bath, through out the experiment.

The solution in the experimental bath was circulated and at the same time aerated by bubbling a gas mixture of 95% oxygen and 5% CO_2 through a specially shaped glass 'bubbler' (Fig. 2c), whereby the solution was transferred from the larger compartment to the smaller one by means of an air-lift mechanism. A small niche at the middle of the lower edge of the glass partition (Fig. 2d) enabled the solution in the

smaller compartment to flow back into the larger.

That the circulation of the fluid by means of the overhead air-lift mechanism was efficient was shown by the fact that a drop of methylene blue placed into the small compartment was quickly circulated round and the fluid in both compartments was uniformly stained within one minute.

The solution in the experimental bath was changed every half-hour by running into the smaller compartment fresh solution from a heated reservoir placed about 20 cm above the experimental bath, while continuous suction was being applied to the fluid surface in the larger compartment. The rate of inflow of fresh solution was such that the solution in the bath (15 ml.) was completely exchanged in 3 minutes.

The tissue specimens were pinned out by specially shaped glass hooks (Fig. 2e).

F. Solutions

Tyrode solution (Table I) was used throughout the investigation for all tissues. One reason for this was that out of the 4 main groups of workers on the heart using the microelectrode technique, three have used Tyrode solution or some slight modification of it as the bathing fluid with considerable success. Thus Draper & Weidmann (1951), in this country, Trautwein & Zink (1952) in Germany, & Hoffman & Suckling (1953) in the United States have all used Tyrode solution. Burgen & Terroux (1953) in Canada however used Ringer-Locke solution. A comparison of the ionic concentration of the original Tyrode solution (1910) and the Ringer-Locke solution as used by Burgen & Terroux shows that the essential difference is the high concentration of potassium in the Ringer-Locke. Though

this concentration approximates that found in the plasma of most of the animals studied (Table I), there is evidence that at least half the potassium present in the plasma is in the 'bound' state, (Fenn, 1936). It was, therefore, felt that Tyrode solution with its lower concentration of free K ions was the better choice.

Another reason for choosing Tyrode solution was that the majority of the workers (see above) had used this solution in their studies and if the results in the present studies are to be compared with theirs, a comparable solution had to be used.

A third reason for using Tyrode solution for all the tissues is shown in Table I. The concentration of the different ions in Tyrode solution compare favourably with the ionic concentration in the plasma of those animals in which the plasma ionic concentrations are available. Potassium and Calcium ionic concentration in Tyrode solution is lower than in the plasma, but as mentioned previously, there is evidence that only about half the amount of these ions are present as free ions in the plasma. It was therefore felt that the Tyrode solution could be used satisfactorily for all tissues in all the animals studied.

Finally, Draper & Weidmann (1951) in the dog showed that the results obtained with Tyrode were essentially the same as those obtained with plasma.

The Tyrode solution was freshly prepared from stock solutions of molar and millimolar strengths, on the morning of the experiment. BDH 'Analar' reagents and resin distilled water were used to make up the stock solutions.

TABLE I

The millimolar composition of the Tyrode solution used in the present experiments compared with that of plasma of some of the animals. Cat plasma values from Fenn, Cobb, Mannery & Bloor (1938). Rat plasma values from Conway (1945). Other values from Albritton (1952).

	Modified Tyrode soln	Goat plasma	Dog plasma	Cat plasma	Rabbit plasma	Guinea pig (serum)	Rat plasma
Na^+	149.08	-	150	178.5	136	-	157
K^+	2.68	-	4.4	5.32	5.1	6.5	6.7
Ca^{++}	1.70	2.45	2.6	2.79	3.6	4.8	3.4
Mg^{++}	0.49	1.90	0.9	1.08	1.0	4.0	1.7
Cl^-	143.92	85.0	106	128.0	105	-	125.5
HCO_3^-	20.0	-	20.5	22.0	28	-	25.5
Phosphate	0.32	-	1.8	2.29	1.9	-	-
Sulphate	-	-	1.0	-	-	-	-

Values for cat serum from Albritton:

Na^+ = 151 mM; K^+ = 4.3 mM; Mg^{++} = 2.2 mM.

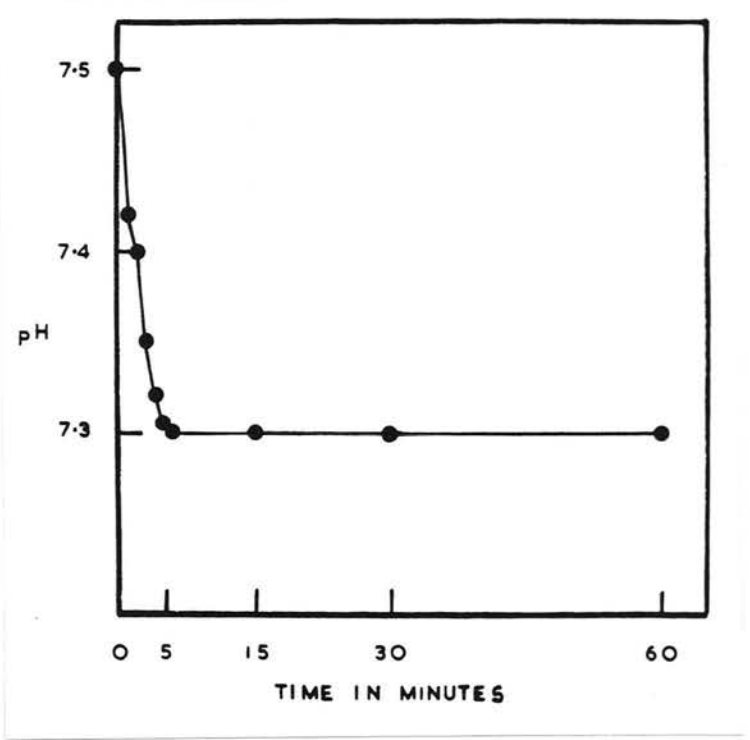

Fig. 3

Graph showing the changes in the pH of the Tyrode solution on bubbling 95%O_2/5%CO_2 gas mixture through it.

These stock solutions were changed every fortnight as there was a tendency for fungi to grow in them especially in the KCl and NaH_2PO_4 solutions.

Effect of passing 95% O_2 5% CO_2 mixture into the solution, on the pH

Changes in the pH of the Tyrode solution in the muscle bath was followed through a period of one hour, by performing a blank experiment in which the glass and calomel electrodes of a Pye direct reading pH meter, were immersed in the solution in the muscle bath while the solution was being aerated by means of the air-lift mechanism.

Fig. 3 shows the results. The solution in the reservoir (about 100 ml.) was aerated by bubbling in the O_2/CO_2 mixture vigorously for about 5 minutes. This usually brought down the pH of the solution in the reservoir to about 7.5. Continuous aeration of the solution in the muscle bath brought down the pH to 7.4 within one minute and to 7.3 within five minutes which level was maintained throughout the one hour period.

Since in actual experimental conditions, the solution in the experimental bath was changed every half hour, there was a wide margin of safety before the pH of the solution fell to a sufficiently low level to affect the results.

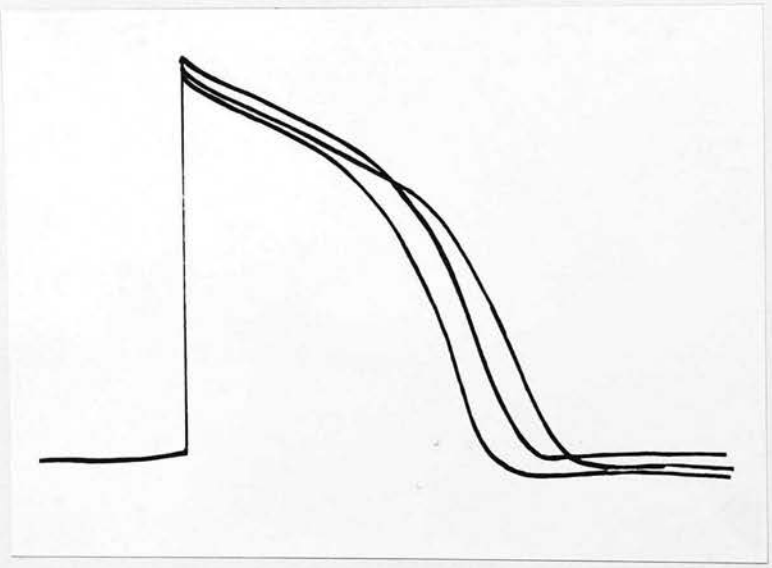

Fig. 4

Three tracings of the goat ventricular action potential from a single impalement, superimposed to show the variation in the duration of the action potential.

RESULTS

MAGNITUDE OF THE CARDIAC RESTING AND ACTION POTENTIAL

General Considerations

According to Draper & Weidmann (1951) the form and magnitude of the action potential recorded from the same Purkinje fibre in a false tendon is remarkably constant and reproducible. The present observations show that this is not so in auricular and ventricular muscle. Although it is fairly easy to penetrate the cell, it is difficult to stay in it for more than a few beats and even then the action potentials are not always consistent in shape and magnitude. Fig. 4 shows the variations in the duration of the action potential recorded during a single impalement of a goat ventricular fibre. Results like this are not uncommon.

Several reasons may be advanced to account for the difference in behaviour from false tendons. Firstly, these tissues, unlike false tendons are usually beating vigorously and thus the chances of the electrode being jerked out is greater. In addtion there is rather less connective tissue over the cardiac muscle cells than around the false tendons. This connective tissue helps to prevent movement of the electrode relative to the excitable cells. Secondly, the cells are much smaller than the Purkinje fibres (See Part II), and this together with the vigorous contraction of the tissue could easily cause damage to the membrane during impalement.

Definition of the term 'Resting' Potential

The atrial and ventricular strips have to be stimulated as they normally do not beat spontaneously unless a bit of sino-auricular nodal tissue or Purkinje tissue is present in

the atrial or ventricular strip respectively. The action potentials from these tissues do now show a slow depolarisation during diastole which is characteristic of spontaneously beating Purkinje fibre action potentials (Fig. 7). The definition of the resting potential in these tissues is thus rendered much easier than in Purkinje cells. It has been arbitrarily defined as the highest negative potential reached during the cardiac cycle (Draper, 1955). In these cells this is the same as the potential level just before the upstroke of the action potential.

Measurement of the Resting Potential

The procedure adopted in recording the action potential was as follows: one beam of a double-beam cathode ray tube was used to record the zero potential line while the other beam was used to record the action potential. With the electrode outside the cell, the two beams were first aligned so that both of them coincided. The fixed beam thus represented the zero potential base line. The cell was then impaled and the action potential together with the base line was photographed. The electrode was then withdrawn and any shift in the base line checked.

The resting potential was measured by projecting the action potential on to a calibration grid of 10 mV steps, using a photographic enlarger, and measuring the potential difference between the base line and the point of highest negativity in the action potential.

Recent work by Adrian (1956) has shown that there is a potential at the tip of some of the microelectrodes during impaling, which would tend to shift the zero potential base

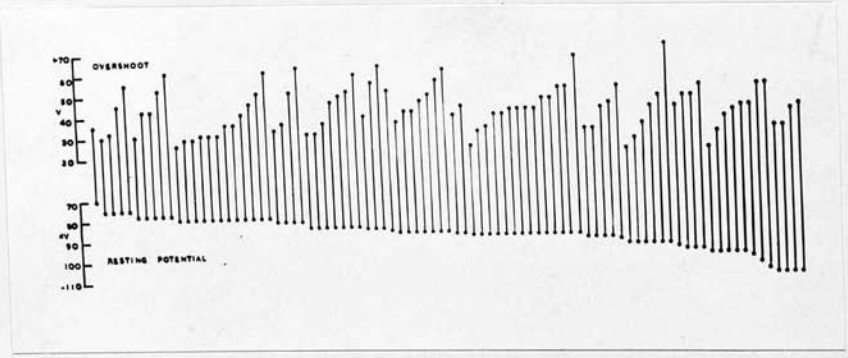

Fig. 5

Graph showing the magnitude of the resting potential, and overshoot of all the action potentials recorded from the atria of 4 goat hearts.

line by as much as 15 to 20 mV. This has been called the 'tip potential'. The actual cause of this tip potential is still not clear but it is presumed to be due to some protein from the myoplasm sticking at the tip of the electrode, acting as a "rectifier". It is also presumed that the protein blocking the tip is left behind because no change in the zero base line can be detected when the electrode is withdrawn in the present experiments. This shift in the base line during the course of impaling caused by the tip potential therefore makes the resting potential values which have been measured, to some extent uncertain. The only value one can have confidence in is, therefore, the total action potential magnitude.

As an example of typical results, the measurement of the magnitude of the resting and action potentials in the goat atria, obtained in the present experiments will now be considered in detail in the light of the above work. Fig. 5 summarises the results. All the action potentials obtained from the atria of 4 goat hearts have been plotted to show the magnitude of the resting potential and the overshoot of each of the action potentials. It can be seen that a number of the action potentials have high resting potentials and a number have high overshoots. More detailed examination reveals that some of those with high resting potentials have low overshoots i.e. below the normal range of values found by other workers for cardiac tissues (summarised by Weidmann, 1956). Conversely, some of the action potentials have low resting potentials and high overshoots. Such results can be explained by a shift in the zero level while inside the cell due to the influence of a tip potential. However such an

explanation will not suffice for a high resting potential with a high overshoot.

Fig. 5 also shows a number of action potentials having

(1) high resting potentials with high overshoots;

(2) high resting potentials with normal overshoots;

(3) normal resting potentials with high overshoots.

All these three types have values for the total action potential above the normal and therefore the high resting potentials and the overshoots recorded in these action potentials cannot be accounted for by a shift in base line due to the tip potential. As other sources would tend to lower rather than raise the magnitude of the resting and action potentials, these high values would have to be regarded as the real magnitudes in these particular fibres. Bennett, Ware, Dunn & McIntyre (1953) also found high resting potentials in the mouse skeletal muscle measured in vivo, but as they did not record the total action potential, the amount of overshoot is not known and therefore the possibility that the tip potential might have been responsible for some of their high values cannot be ruled out.

The cause of the high resting potential and overshoot found in these fibres is still not clear. Bennett et al (1953) suggested that the resting potential is a labile voltage depending on metabolism, and that high potentials are recorded when abnormal metabolic activity produce a transient hyperpolarisation of the membrane.

Can the intracellular potassium concentration in the myocardium account for the high resting potentials? On the basis of the equation $E_K = 61.5 \log_{10} \frac{K_i}{K_o}$ (37°C)

with K_o = 2.7 mM (Tyrode) and E_K = 110 mV (the highest value recorded), the intracellular potassium works out to about 166 mM. The only recent figures available for intracellular K for mammalian cardiac tissue is by Robertson & Dunihue (1954) for the cat cardiac muscle (151 mM/kg water). Considering the uncertainties involved in the measurement of the intracellular K and also the variations in different species, it is not impossible for the calculated potassium concentration to be found intracellularly. It is also possible that some cardiac fibres may have slightly more intracellular K than others. It has to be remembered that the present method of measuring the resting potential in single cells is far in advance of the methods for estimating the intracellular concentration of potassium in these cells, which at best is a mean for all the cells in the population, and any correlation between the mean of the resting potential and the mean of the intracellular K concentration would have to be an approximation. The estimate for the intracellular sodium concentration for the cat heart given by Robertson & Dunihue (1954) is 6.5 mM/kg fibre water. The equilibrium potential for sodium according to this equation is 84 mV. The highest value of the overshoot of 70 mV recorded in the present experiments is thus not unreasonable.

Magnitudes of the cardiac resting and action potentials

High values for resting potentials and overshoots, similar to that found in the goat atria have also been observed in the goat and cat ventricle.

Despite the uncertainty involved in the measurement of the resting potential, discussed in the previous sections,

TABLE II

Magnitudes of the resting and action potential and the overshoot in the atria and ventricles of different animals. The figures in brackets under the animal column indicate the numbers of animals from which the values were obtained. The order of the figures under the resting potential, overshoot and action potential columns are (1) The mean value (2) the standard deviation (in brackets) (3) the range of the values.

ATRIUM

Animal	No. of impale-ments	Resting Potential mV	Overshoot mV	Action Potential mV
Goat (4)	87	89, (9) 70-110	41, (11) 20-70	130, (14) 105-165
Dog	-	-	-	-
Cat (4)	29	85, (8) 73-100	34, (7) 22-45	119, (9) 98-133
Rabbit	-	-	-	-
Guinea-pig (2)	13	83, (4) 75-90	32, (10) 20-50	125, (15) 95-130
Rat (3)	14	89, (8) 80-100	26, (6) 16-35	115, (8) 104-131
Golden Hamster(1)	7	73, (7) 58-78	11, (8) 0-22	83, (14) 64-100
Mouse (3)	13	80, (13) 62-110	22, (12) 0-40	103, (5) 75-125

VENTRICLE

Animal	No. of impale-ments	Resting Potential mV	Overshoot mV	Action Potential mV
Goat (4)	44	98, (8) 80-115	41, (15) 20-75	138, (15) 115-180
Dog (2)	10	96, (8) 85-110	41, (12) 27-60	137, (12) 117-155
Cat (6)	60	92, (10) 75-110	37, (11) 15-64	129, (10) 108-145
Rabbit (1)	7	89, (8) 80-102	46, (9) 40-65	135, (13) 120-157
Guinea-pig (2)	37	87, (8) 70-100	43, (8) 28-60	129, (10) 110-145
Rat (1)	12	92, (6) 80-95	29, (7) 18-40	120, (8) 110-138
Golden Hamster(1)	14	73, (11) 55-90	17, (12) 0-35	90, (21) 60-115
Mouse (1)	7	61, (4) 60-70	15, (5) 5-20	77, (2) 75-80

the means between groups of observations on different tissues are in reasonable agreement. These results are summarised in Table II. In addition, the mean values for the resting potential, overshoot and the action potential obtained for most of the animals fall within the range of values found in other cardiac tissues by previous workers. Slightly lower values have been obtained from the smaller animals (mouse and hamster). This may have been due to the cells being damaged more easily due to their smaller size or might have been due to the deteriorating condition of the tissue itself. Too much reliance cannot be placed on these values as the number of impalements were too few and were obtained from a single animal in each case.

A number of action potentials recorded from these smaller animals, showed no membrane reversal phase. This finding can however be explained by the work of Weidmann (1955) who showed that the amount of overshoot depends on the resting potential. By 'clamping' the potential at about 60 mV in calf Purkinje fibres, he showed that the crest of the action potential just failed to reach the zero potential line. In the present observations, all the action potentials which did not show a membrane reversal or did show only very low ones, had resting potentials of 50-75 mV. This would, therefore, support the idea that in heart muscle, as in Purkinje fibre (Weidmann, 1955) and nerve (Hodgkin & Huxley, 1952b), the amount of overshoot depends on the potential difference which had been acting on the membrane previous to depolarisation.

RATE OF RISE

The rates of rise of the action potentials were not

studied systematically but a few estimates were made from some of the records obtained for conduction velocity measurements. As the sweep speeds were not always fast enough, the measurements are subject to some error in addition to the lag in the recording system previously referred to.

Atrial and ventricular action potentials appear to have the same rate of rise. The goat and cat atrial and ventricular action potentials from which most of these measurements have been made, rise with an average rate of 175 V/sec (range 79-360 V/sec). In smaller animals the average rate of rise was found to be 30 V/sec (16-62 V/sec). This slower rate of rise may also be accounted for by the low resting potentials recorded from these cells (Weidmann, 1955).

The rates of rise found in both atrial and ventricular action potentials is thus much slower than the rate of rise of the Purkinje fibre action potential (610 V/sec in the dog; Draper & Weidmann, 1951).

CONFIGURATION OF THE ACTION POTENTIALS

Duration of the action potential

It is difficult to decide how to describe the duration in such a complicated and variable action potential as exists in the cardiac tissues of different animals. If the total duration of the action potential is given, as is usually done for nerve and striated muscle action potentials, it will not describe adequately the time course of the action potential. In addition, the action of certain drugs on the action potential indicates that the duration of the other parts of the action potential may be altered without its total duration being appreciably affected. Fig. 6 shows as an example, the

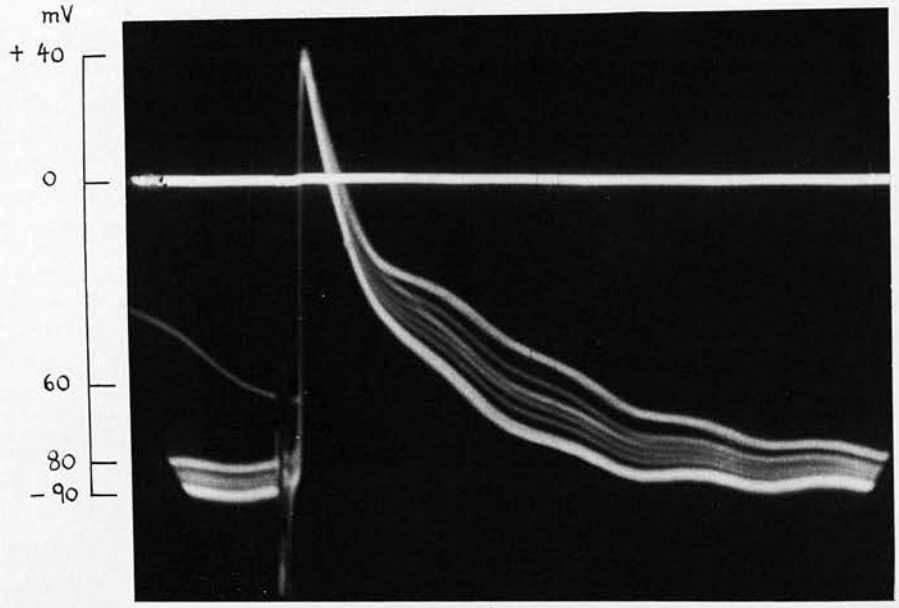

Fig. 6

Action of acetylcholine on the mouse auricle showing the shortening of the action potential and the hyperpolarisation.

action of acetylcholine on the mouse auricular action potential. The electrode was left in situ as the acetylcholine was added to the bath (final concentration 1.5×10^{-8}), and the successive action potentials exposed on the same frame. The action potential is seen to shorten definitely in its falling phase between the 30 and 60 mV range, by as much as 50%, whereas the total duration is not very much changed. In fact it is extremely difficult to decide where the action potentials actually end.

As the action potential is complex, and as there is evidence to suggest that the different phases of the action potential have different underlying mechanisms, it is more informative to describe the duration of the cardiac action potential at several levels.

In the present experiment the following have been chosen:

(1) Duration of the membrane reversal phase ('A' in fig. 7A).

(2) Time to repolarise to the 60 mV level ('B' in fig. 7A).

(3) Time for the action potential to be 95% complete ('C' in fig. 7A).

The duration of the phase of membrane reversal is of great interest because the mechanisms whereby the membrane can remain reversibly polarised for as long as 150 msecs. in some tissues has so far not been explained in terms of ionic shifts.

The time taken for the action potential to repolarise to 60 mV has been chosen because it has been shown by Weidmann (1955) in the Purkinje fibre and by Draper (1955) in atrial and

TABLE III

Temporal Characteristics of the Action Potentials shown in Fig. 7

A. Duration of the membrane reversal phase (in msec)

	Goat	Dog	Cat	Rabbit	Guinea-pig	Rat	Hamster	Mouse
ATRIUM	95	46	46	13	23	12	9	3
VENTRICLE	137	93	115	73	124	4	3	5

B. Time to repolarise to 60 mV (in msec)

	Goat	Dog	Cat	Rabbit	Guinea-pig	Rat	Hamster	Mouse
ATRIUM	207	136	105	80	60	23	34	24
VENTRICLE	217	155	165	129	168	13	14	35

C. Duration of the 95% complete action potential (in msec)

	Goat	Dog	Cat	Rabbit	Guinea-pig	Rat	Hamster	Mouse
ATRIUM	245	205	145	126	80	50	43	49
VENTRICLE	227	180	190	146	177	24	22	100

ventricular muscle that the absolute refractory period ends when the membrane has repolarised to about 60 mV. Measurement of the duration of the action potential at this level would therefore be useful as it would give some indication of the duration of the absolute refractory period as well.

By definition, the membrane reversal and the 60 mV repolarisation level would have to be measured from the zero potential base line. As discussed previously, there is some evidence that the base line may sometimes shift when the electrode is in the cell, due to the presence of a tip potential. This possibility of the base line shifting therefore introduces some uncertainty in durations measured at these two levels.

As mentioned before, the only measurement that would not be affected by the presence of a tip potential is that of the total action potential. Measurements of the duration of the total action potential would thus be free from the criticism levelled against the measurements of the above two phases. As the action potential usually tails off very slowly, making it difficult to decide when it ends, the duration for the action potential to be 95% complete has been chosen arbitrarily. Table III gives the durations of the action potentials shown in Fig. 7 measured at these three levels. The durations given for the atrium and ventricles of the different animals are typical for those tissues in the particular species.

Comparison between the configuration of the atrial and ventricular action potentials

In Fig. 7 are shown the atrial and ventricular action

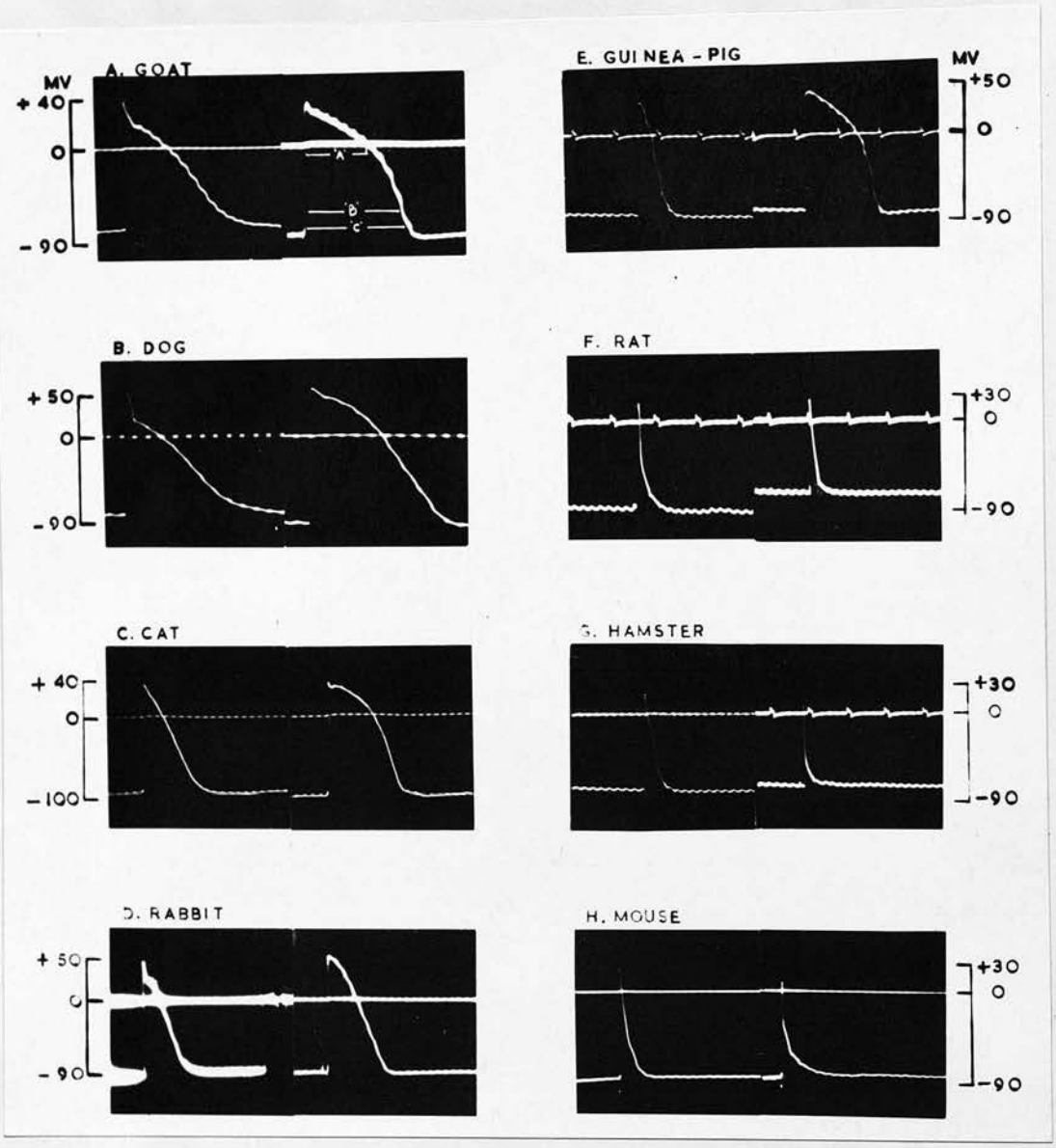

Fig. 7

Action potential configurations of the atria and ventricles of different mammals. In each pair of records the atrial action potential is on the left and the ventricular action potential on the right. All records are at approximately the same sweep speed. 'A', 'B', & 'C' on the goat ventricular action potential are the levels at which the duration of membrane reversal, the time to repolarize to 60 mV and the time for the action potential to be 95% complete respectively were measured. The action potentials from the dog and cat hearts have been retouched. Time in B, C & G (atrial action potential) in 20 msec. Time in E, F & G (ventricular action potential) in 100 msec.

potentials from the different animals used in this study. If there is any change in the configuration of the action potential with time (see below), the action potential recorded nearer to the time of death of the animal has been shown. All the records with the exception of the ones from the dog are very nearly the same sweep speed. The dog atrial and ventricular action potentials are on a slightly faster time base. All the records have been obtained from the tissues which have been stimulated at about the same rate (60/min).

In larger animals (goat, dog, cat, rabbit and guinea-pig), there is a consistent difference between the atrial and ventricular action potentials. In general, the atrial action potential is much shorter and the repolarisation phase falls much more rapidly than the ventricular action potential. In the latter, there is a "plateau" in its repolarisation phase thus making its duration much longer than the atrial action potential. The repolarisation phase of the atrial action potentials of some of the animals (goat, dog and rabbit) show a slight indication of a plateau, but it is not maintained.

In the smaller animals (rat, hamster and mouse), the atrial and ventricular action potentials resemble each other both in shape and duration. The ventricular action potentials in these smaller animals normally show no plateau phase (but see p. 43 and Fig. 9c). Occasionally, the duration of the ventricular action potential is found to be shorter than the atrial action potential (Fig. 7, rat; hamster).

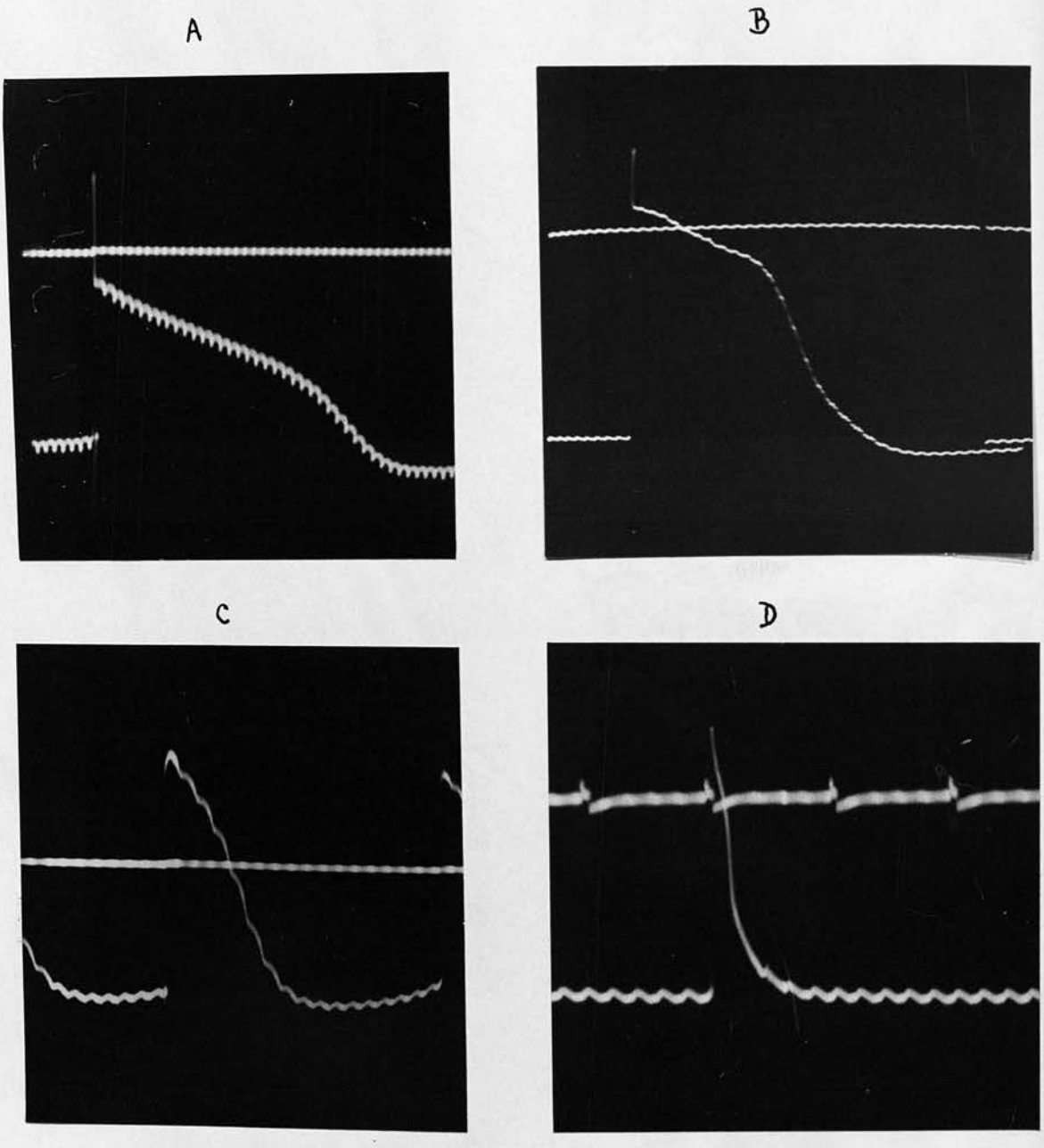

Fig. 8

Configuration of the action potentials from 'Purkinje fibres' in the goat (A), dog (B), cat (C), and rat (D) false tendons. At different sweep speeds. 100 msec time markings in (D). The zero potential line in (C) shifted about 20 mV negative.

Comparison between the configuration of the 'Purkinje fibre' action potentials in the goat, dog, cat and rat

The configuration of the goat and dog Purkinje fibre action potentials is well known. It has an initial spike followed by a long plateau phase which may be a little above or below the zero potential line, and then a rapid repolarisation phase. Though various workers have stated that they have been able to record from the false tendons of cats and rabbits, no records have been published. In the present experiments, it has been possible to obtain records of a few action potentials from the false tendons of cats and of a rat, in addition to those from goats and dogs (Fig. 8). Both the action potentials from the cat and the rat false tendons do not show a spike like those from the goat and dog false tendons. There is a slight suspicion that the shape of the cat 'Purkinje fibre' action potential is due to slight anoxia as it resembles Purkinje fibre action potentials of dogs which have been subjected to oxygen lack (Trautwein, Gottstein & Dudel, 1954). All the 'Purkinje fibre' action potentials obtained from cat false tendons so far, have been of this configuration.

The rat 'Purkinje fibre' action potential resembles its ventricular action potential, both in shape and duration (c.f. Fig. 7). In this case there is no reason to believe that the tissue was suffering from anoxia.

All the false tendons from which records were obtained have been examined histologically (see Part II).

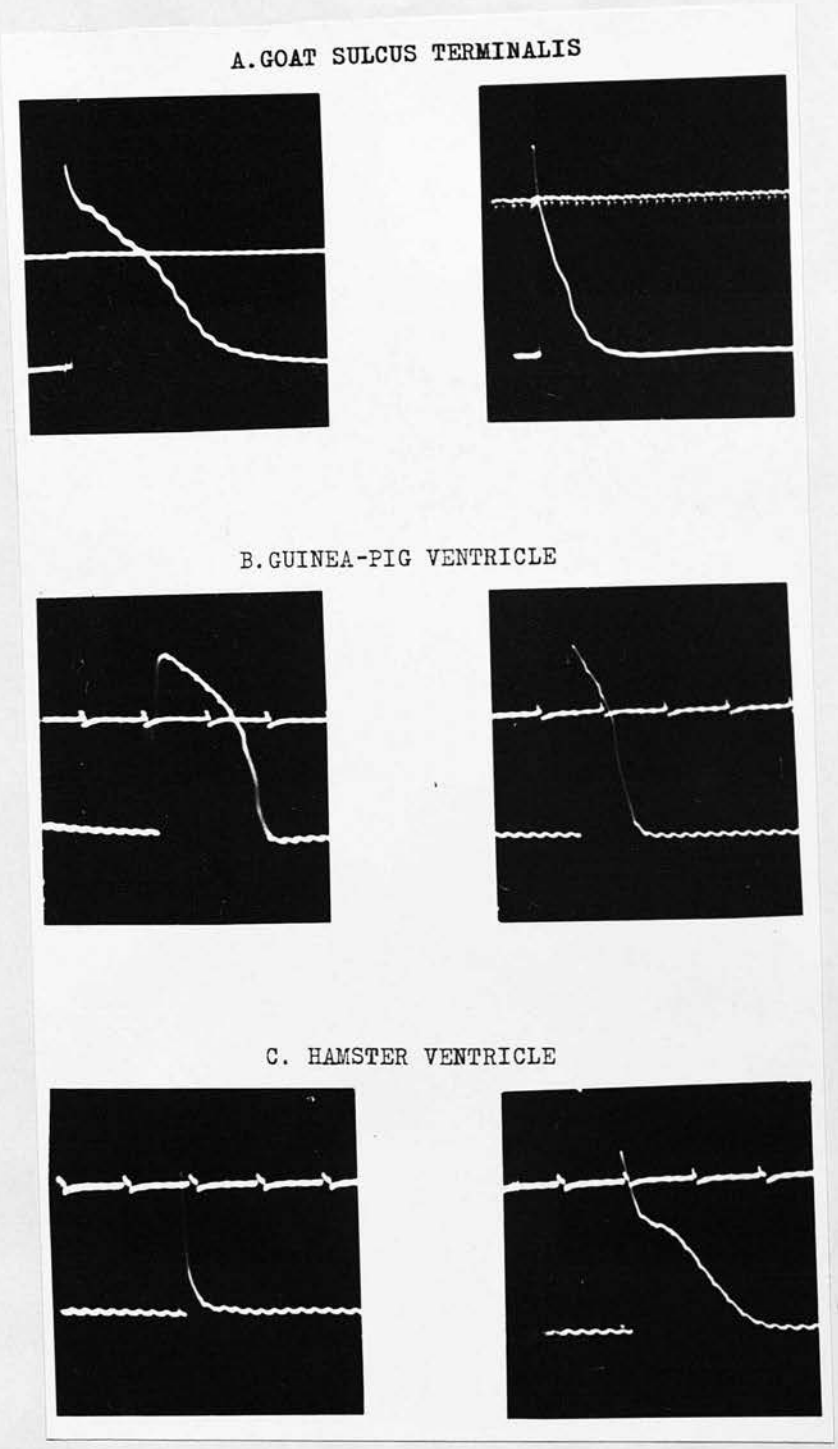

Fig. 9

Change in the duration and configuration of the action potential with time.

A. Goat sulcus action potential at the beginning of the experiment (left) and 12 hours later (right).

B. Guinea-pig ventricular action potential at the beginning of the experiment (left) and 75 minutes later (right)

C. Hamster ventricular action potential at the beginning of the experiment (left) and 6 hours later (right).

100 msec time markings in B and C

Comparison between the configuration of the action potentials in different animals

Large animals have a longer action potential both in the atria and in the ventricles. The ventricular action potential begins to lose its "plateau" phase as the animal gets smaller. The action potentials in these smaller animals repolarises rapidly and all of them have reached the 60 mV level within 50 msec, (Table III). The course of the recovery phase after that is more variable. It may continue the rapid decline and complete 95% of the total action potential within 60 msec. On the other hand, the recovery phase may slow down considerably after it has repolarised to about 60 mV. Both these variations have been observed in the action potentials of both atrium and ventricles from the three smaller animals (rat, hamster and mouse).

Change in the action potential configuration with time

It has been observed on several occasions that during the course of an experiment the shape of the action potential recorded from the same tissue and from about the same spot, greatly differed. This was observed most frequently in the goat sulcus, once in the guinea-pig ventricle and once in the hamster ventricle (Fig. 9). As most of the changes observed were towards shortening of the action potential (goat sulcus and guinea-pig ventricle), the possibility that the tissue had become anoxic could not be ruled out, as anoxia is known to shorten the action potential in these tissues (Trautwein, Gottstein & Dudel, 1954).

In the hamster ventricle, however, after a period of about 5 hours in the bath, the action potential became longer, a

TABLE IV

Data of the body weight, heart weight and heart rate of the animals from which the records shown in Fig. 7 were obtained. Body weight in kilograms; heart weight in grams; heart rate per minute. $\frac{\text{Heart rate}}{\text{Body weight}}$ in arbitrary units.

	Body weight	Heart weight	Heart rate	$\frac{\text{Heart rate}}{\text{Body weight}}$	$\frac{\text{Heart rate}}{(\text{Body weight})^{0.1}}$
Goat	27	119	150	6	108
Dog	8.2	67	144	18	117
Cat	3.1	14.5	165	53	147
Rabbit	4.0	9.5	270	68	235
Guinea-pig	.55	1.9	260	473	277
Rat	.3	1.0	300	1,000	337
Hamster	.16	0.5	467	2,500	563
Mouse	.037	0.2	450	10,000	625

plateau phase being now added on to the original action potential. With the present state of our knowledge it is difficult to explain this observation unless it is assumed that the reaction of the hamster ventricular tissue to anoxia is different from the other cardiac muscular tissues.

Correlation between the metabolic rate of the animal and the action potential configuration

Clark (1927) has shown that

$$\text{Pulse rate} \propto \frac{\text{metabolic rate}}{\text{heart output per unit weight}} \quad \ldots\ldots(1)$$ p. 93

$$\text{Heart output per unit weight} \propto \frac{1}{(\text{Body weight})^{0.1}} \quad \ldots\ldots(2)$$ p. 93

Hence $$\frac{\text{Heart rate}}{(\text{Body weight})^{0.1}} \propto \text{metabolic rate} \quad \ldots\ldots(3)$$

In Table IV are given the body weight, heart weight and heart rates of the animals from which the records shown in Fig. 5 were obtained. $\frac{\text{Heart rate}}{(\text{Body weight})^{0.1}}$ values are calculated and given in arbitrary units. This is proportional to the metabolic rate (Equation 3). The table shows, as expected that as the animals get smaller and the metabolic rate rises the heart rate increases. This correlates with the gradual shortening of the action potential as the animal gets smaller.

It is well known that increasing the heart rate shortens the action potential (for recent evidence see Hoffman & Suckling, 1954). It was therefore though that the mere rapidity of the heart rates in the smaller animals could account for the shortened action potential found in the hearts of these animals. However, two observations were against this view: (1) the action potential still remained short even when the tissue was stimulated at a slow rate (ca. 60/min). (2)

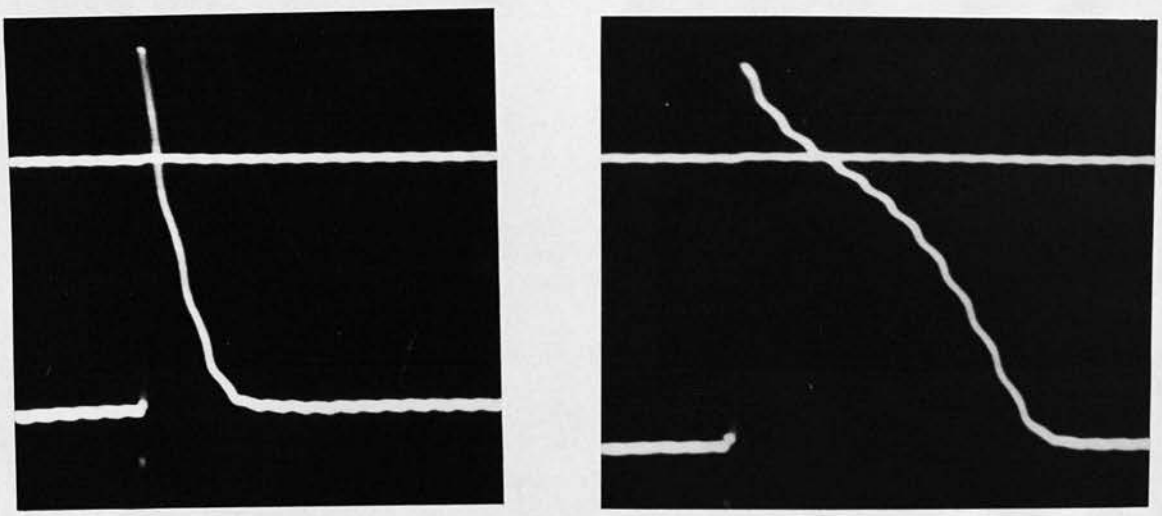

Fig. 10

Guinea-pig ventricular action potential
at 100°F (left) and at 82°F (right)
Same impalement.

If the rate of stimulation of the tissues of larger animals was made to approach the rate found in smaller animals the changes in configuration and duration never approached the values found in the small animals (Hoffman & Suckling, 1954).

Two other observations support the view that the difference in the shape of the action potentials could be due in part to a difference in the rate of metabolic activity of the tissue. (1) Cooling the heart tissue in small mammals makes their action potential not unlike that found in larger animals. Fig. 10 shows the change in configuration of a guinea-pig ventricular action potential on lowering the temperature of the bathing solution. (2) Action potentials from the hearts of fishes, frogs, and tortoises, which have a low metabolic rate show a pronounced plateau phase. Fig. 11 shows the action potentials from a frog ventricle (A) and from a tortoise auricle (B). Brooks, Hoffman, Suckling & Orias (1955) show in their monograph a photograph of the action potential from the ventricle of a gold fish, which resembles the action potentials shown in Fig. 11.

According to the above view, it may be that the atrial action potential is shorter than the ventricular because of a difference in the metabolic rate of the tissues of the atria and ventricles. No evidence however is yet available regarding the relative oxygen consumption of the atrial and ventricular tissues of the same heart, to test this possibility.

Concerning the evolution of the configuration of the cardiac action potential

It can be argued that the atrial type of action

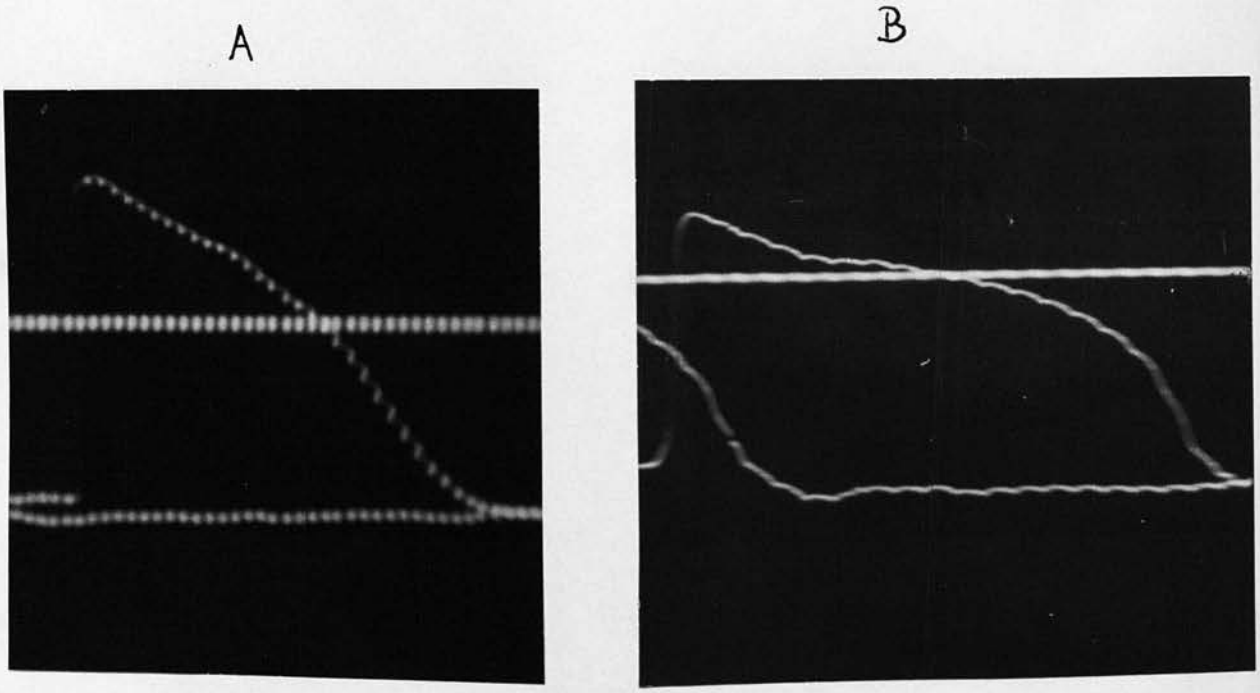

Fig. 11

Configuration of the tortoise auricular (A) and frog ventricular (B) action potentials At room temperature (18°C)

potential is the basic action potential in cardiac tissues and that extra mechanisms are added to it to produce the 'plateau' phase in the ventricular and Purkinje action potentials. This stems from the observations that the plateau phase is extremely sensitive to anoxia and to certain drugs which therefore suggested that these agents knock off a specially developed mechanism, and thus the action potential reverts to its 'primitive' type.

Phylogenetic and embryological considerations, however, suggest that the action potential with the plateau may be the basic type. In all the lower vertebrates hearts so far studied (piscian, amphibian and reptilian), the long action potential with the plateau has been found. Embryological evidence also seems to show that the action potential gets shorter as the heart develops. Measurements of duration have been made on published records of the monophasic action potential from the chick embryonic heart (Fingl, Woodbury & Hecht, 1952), of the Q-T interval in the electrocardiograms and electrograms from chick embryo hearts (Bonsdorff, 1950) and from the hen (Sturkie, 1954). Taking the Q-T interval as indicating the duration of the monophasic action potential (see next section and Hoffman & Suckling, 1954), it appears that the action potentials get shorter as the heart develops in spite of the progressive slowing of the heart rate. However, it is realised that it is not quite fair to compare the duration from the records obtained from different authors who worked under different experimental conditions. It would be worth following the changes in the configuration of the action potential through the various stages of the

development of the heart, under standard experimental conditions.

On this view that the ventricular type of action potential is the more basic action potential, it might be asked why the atrial action potential is more 'developed' than the ventricular. It is possible that the short type of action potential with possibly a simpler mechanism of repolarisation is an adaptation of the tissues enabling it to respond to rapid rates of stimulation, not unlike that in nerve and striated muscle. It is also possible that this might be the reason why the action potentials from both the atria and ventricles from small mammals have short action potentials.

With regard to the Purkinje fibre action potential, from the differential action of oxygen lack on the plateau phase in the Purkinje fibre and the ventricular action potential (prolonging it in Purkinje action potential and shortening it in the ventricular action potential), it would appear that the same mechanism may not be operating in the two tissues to produce the plateau. Moreover, the configurations are not the same, the pronounced spike being absent in the ventricular action potentials. It is possible that the Purkinje fibre action potential also developed from the basic ventricular type of action potential, but along different lines from that of the atrial action potential. It has been shown that Purkinje tissue has a much lower oxygen consumption than ventricular muscle (Yamazaki, 1930; Murray, 1954).

THE ELECTROCARDIOGRAM AND THE MONOPHASIC ATION POTENTIAL

In Fig. 12 are shown the electrocardiograms from

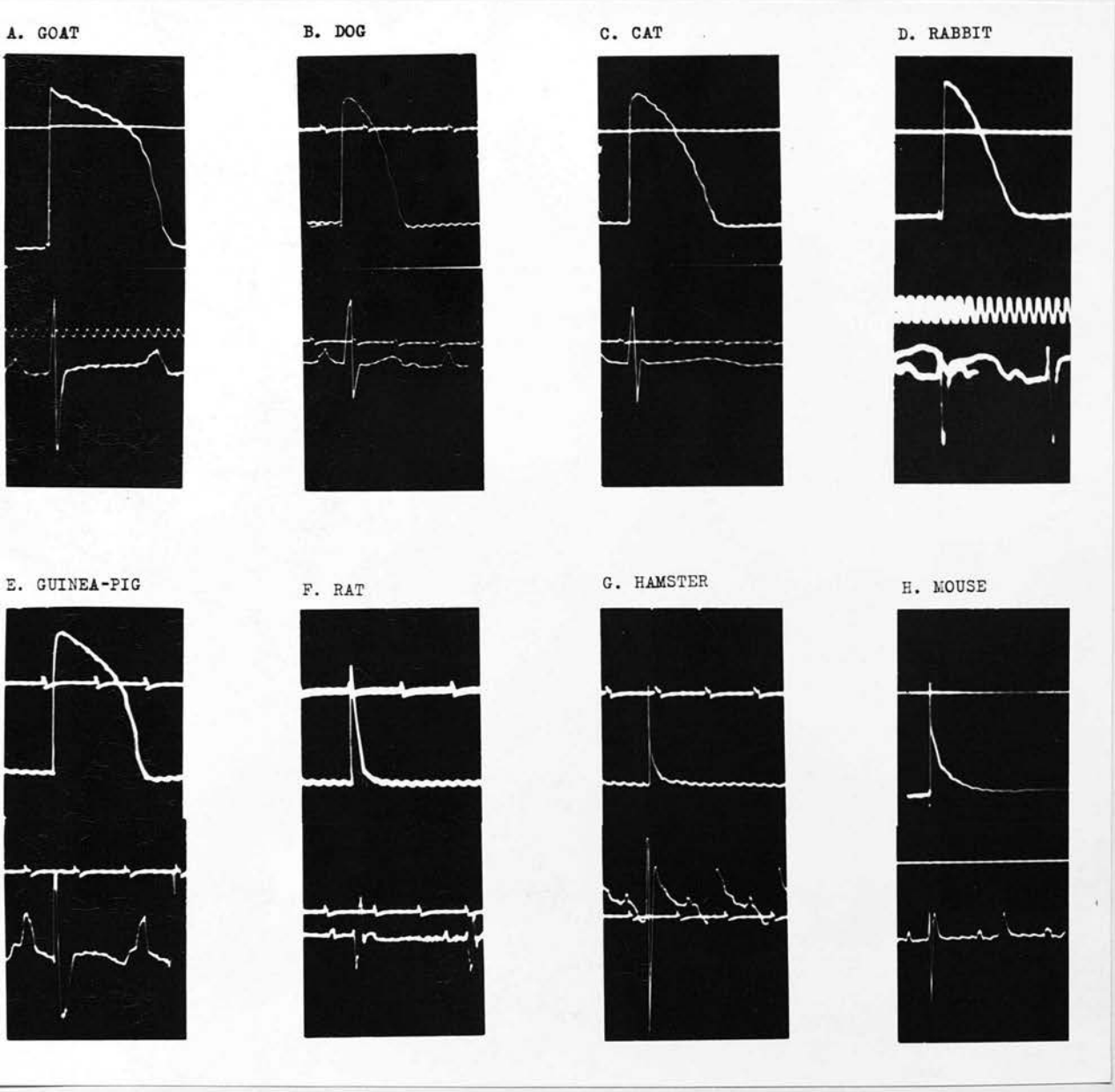

Fig. 12

Correlation between the duration of the ventricular action potentials and the Q-T interval. The monophasic action potential and the corresponding e.c.g. are at the same sweep speed, and they have been aligned so that the upstroke of the action potential and the 'R' wave of the e.c.g. are on the same co-ordinate. Lead CR_1 in goat, rat and mouse. Lead II in rabbit. Precordial (xiphisternum) lead in dog, cat, guinea-pig and hamster. The upstrokes of the monophasic action potentials and the QRS complexes have been retouched. 20 msec time markings in A & D. 100 msec time markings in B, E, F & G.

different mammals, together with the ventricular action potentials recorded from the same animals. The electrocardiogram and its corresponding action potential are recorded on the same sweep speed and the two records are aligned so that the upstroke of the R in the electrocardiogram coincides with the rising phase of the action potential.

The electrocardiographic data for the records shown in Fig. 12 are given in Table V. The heart rate was calculated from the R-R interval. The P-R interval was measured from the beginning of the P wave to the beginning of the R or the Q if that is present. The Q-T interval was measured from the beginning of the Q or the R to the end of the T wave. In most of the smaller animals the form of the electrocardiogram is complicated especially in its S-T segment. This introduces a considerable error in the measurement of the Q-T interval as the T wave is incorporated in the QRS complex making it difficult to decide where it ends.

In Table V, the duration of the 95% complete action potential is also given. The Q-T interval in the electrocardiogram is usually regarded as the duration of the electrical activity in the ventricles, and the T wave thought to be brought about by the repolarisation of the cardiac muscle fibres (Katz, 1947). This has been tested by comparing the Q-T interval with the duration of the ventricular action potential recorded from the same heart. Considering that the Q-T interval represents the temporal dispersion of the depolarisation and repolarisation processes in the whole heart, the ~~whole~~ correspondence between its duration and that of the 95% complete monophasic action potential from the ventricle

TABLE V

Electrocardiographic data for the records shown in Fig. 12. Duration of the 95% complete ventricular action potential is also given for comparison with the duration of the Q-T interval. The durations are given in msec.

	Age	Weight (kilo-grams)	Heart weight (grams)	Heart rate /min.	P-R inter-val	QRS Dura-tion	Q-T inter-val	95% Vent. action potential
Goat	7 months	26	119	230	98	16	258	240
Dog	2 years	6.5	65	200	63	25	143	135
Cat	5 years	4.0	30	145	75	22	250	190
Rabbit	adult	4.5	9.5	270	80	16	140	145
Guinea-pig	adult	.550	1.9	260	72	25	160	177
Rat	adult	.300	1.0	300	53	23	50	24
Hamster	adult	.160	0.5	467	48	17	81	60
Mouse	adult	.037	0.2	400	48	16	40	100

ventricle is remarkable. The ventricular action potential in the mouse appears to be very much longer than the corresponding Q-T interval. This is due to the peculiar slowing down of the final phase of repolarisation in the action potential. However, it is possible that the electrical forces during this slow phase would cancel out and no potential change would show up in the electrocardiogram.

Fig. 12 shows that as the animal gets smaller and the ventricular monophasic action potential gets shorter, the T wave progressively moves in closer to the QRS complex. In the hamster and the mouse, the action potential is so short that the T wave follows immediately after the S, or may sometimes be incorporated in the latter part of the QRS complex. Lombard (1952) studied the electrocardiograms of a number of small mammals and finding no T wave in some of the records suggested that the repolarisation phase in small mammalian hearts is a long process which results in cancellation of the electrical forces thus producing an isopotential T wave. The direct recording of the action potential using an intracellular microelectrode in this study shows that the repolarisation is, on the contrary very rapid in these small mammals. The above correlations indicate that the complicated QRS wave is due to the T wave being incorporated in it.

One other observation may be mentioned here although it is more relevant to the findings in the two other parts of the thesis. Reference to Table V shows that the P-R interval is shortened by a factor of two only between that in the largest animal studied (goat) and the smallest (mouse). The heart sizes between these two animals are however greatly

different. The approximate relation between the heart weight and the size of the left ventricular cavity has been worked out by Clark (1927):

$\sqrt[3]{\text{heart weight}} \propto$ length of L.V. cavity (p. 84).

Calculations of the approximate length of the left ventricular cavity in the goat and mouse from the heart weights given in Table V gives estimates of 0.6 cm. for the mouse and 5.0 cm for the goat. These dimensions are comparable to those measured on actual specimens. The length of the left ventricular cavity therefore shortens by a factor about 8 times. Since the P-R interval shortens by a factor of only 2, it suggests that conduction from the auricles to the ventricles in the smaller animals is relatively slower. This finding is relevant to the observations on the histological structure of the conducting tissue in the small animals, to be presented in Part II.

PART II

STRUCTURAL ASPECTS OF THE SPREAD OF EXCITATION IN THE HEART

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INTRODUCTION

The special features of the structure of the mammalian heart have to be taken into consideration in the study of the spread of excitation in it. Firstly, cardiac muscle is a syncytium. Consequently the excitation wave is capable of travelling in all directions. This, together with the different directions in which the muscular sheets composing the walls run, introduces complications in the measurement of the velocity of spread of excitation.

Secondly, there is a specialised muscular bundle connecting the auricles and the ventricles and also a specialised conducting tissue distributed widely in the latter. There is already evidence that the excitation wave is conducted rapidly throughout the ventricles by the conducting tissue. The finer anatomical distribution of this system have, however still not been worked out. Even in a study of the spread of excitation in portions of excised cardiac tissue which have been selected as far as possible macroscopically for regularity in structural arrangement and purity in cell type, the above two complicating factors have to be taken into consideration. The observations described in this part of the thesis stem from the histological examination carried out as a routine to determine the structural arrangement of the muscle fibres and to determine the type of excitable cell present in the tissues used for experiments.

MATERIAL

Excised tissues of the sulcus terminalis, auricular trabeculae, papillary muscles, false tendons and the atrio-ventricular bundle have been studied. Most of these tissues had been used for experiments described in Parts I and III and were studied histologically for correlation with the experimental findings.

METHODS

The tissues were fixed in 10% solution of formalin in Tyrode, immediately after the experiment. They were then blocked in paraffin and serially sectioned at 6 μ.

The following stains were used:

1) Modified van Gieson (Haemalum, Aurantia & van Gieson) (Marshall, 1946).
2) Gomori stain (Chrome-alum haematoxylin; Phloxin) (Gomori, 1941).
3) Masson's Trichrome stain (Heidanhain's haematoxylin; Mallory II, & Aniline Blue). (Cowdry, 1943, p. 117).
4) Lead Tetra-acetate Schiff method for glycogen. (Pearse, 1953, p. 438).

Van Gieson and Masson stains were found to be good general stains. Gomori stain which was initially used in this study to investigate the ganglion cells in the atrium, was found to delineate the boundaries of the cardiac fibres well. Consequently, this stain was used when fibre diameters were to be measured.

RESULTS

The nature of the cells in which the excitation wave travels

There is already much evidence to show that the excitation wave in the heart travels through muscular channels (see Historical Survey, p.10). The muscular elements are, however, far from uniform in structure. According to the orthodox conception, spread of excitation in the auricles is through ordinary muscular fibres. Conduction across the atrio-ventricular fibrous junction is by means of a special muscular bundle - the atrio-ventricular bundle - arising from the AV node situated in the interauricular septum. Spread in the ventricles is through the AV bundle, its two branches and along the subendocardial network of Purkinje fibres. From the subendocardium, the impulse travels into the muscle, radially in an endocardium-epicardial direction.

The cells constituting this pathway will now be considered as observed in the strips of excised tissues which had been used for experiments.

Auricular and ventricular myocardium

No histological differentiation can be made between these two tissues in spite of the differences in the configuration of their action potentials (p. 40).

Their histological character are well known and need no detailed description. The following points need mentioning for comparison with the Purkinje cells:

1) The fibres are generally smaller than the typical Purkinje fibres found in the goat and dog (see p. 60, fibre diameters).

2) The nuclei are usually single, centrally situated, and

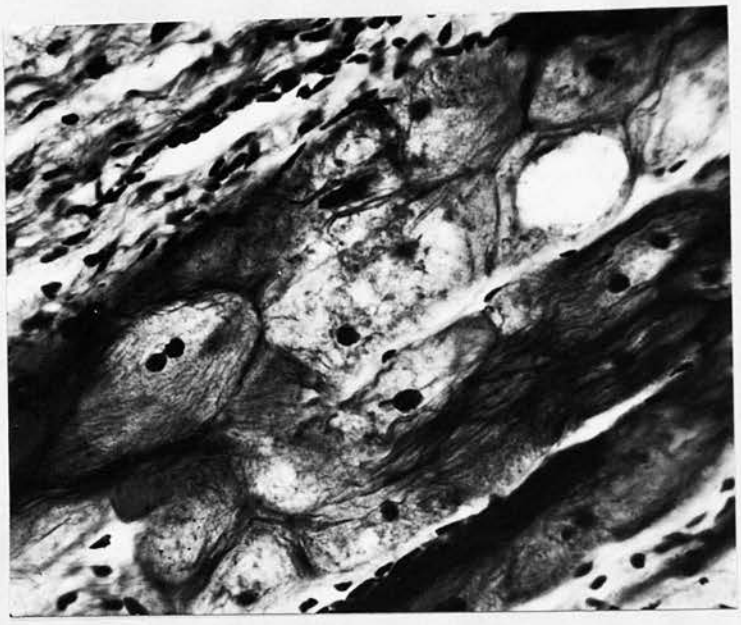


Fig. 13

Typical Purkinje fibres from a goat false tendon. (x 400). Characteristic features are the round nuclei, (seen paired in one cell), the central clear spaces and the loose myofibrils. (Gomori.)

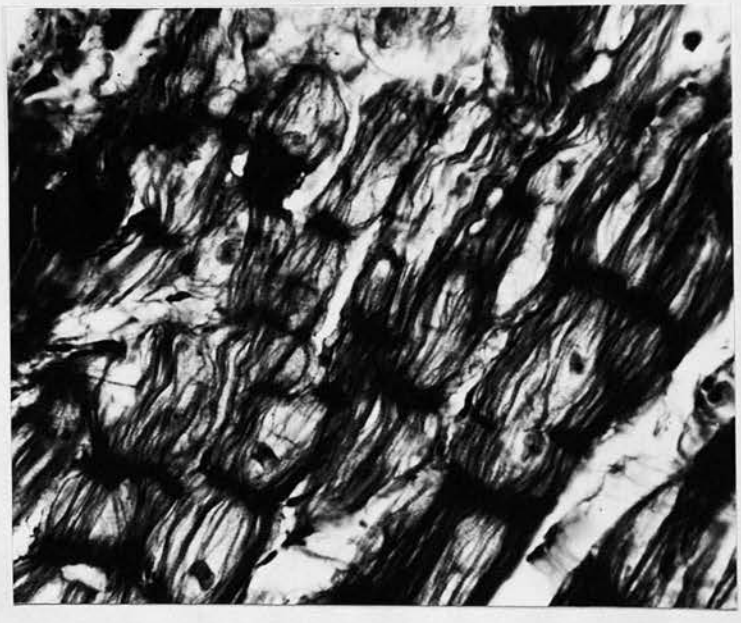


Fig. 14

Typical Purkinje fibre from a dog false tendon (x 400). The round nuclei, central clear space and loose fibrils can be clearly seen. (Gomori)

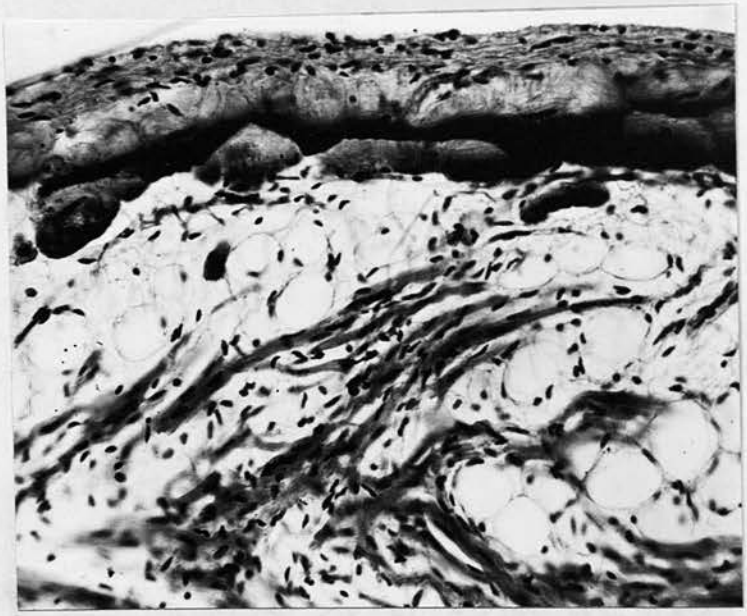


Fig. 15

Longitudinal section of a goat false tendon (x 260) showing both typical Purkinje fibres and ordinary cardiac muscle. The dark areas in the Purkinje cells are areas which showed a positive Schiff reaction. The section has been treated by the lead-tetra-acetate-Schiff method. The result of the measurement of conduction velocity in this false tendon is shown in Fig. 33.

are flattened and elongated though they may sometimes tend to be slightly oval.

3) There is no clear space around the nuclei.

4) The myofibrils are compact and fill the whole fibre.

Purkinje cells

The Purkinje cells studied in this investigation have mainly been from the false tendons.

The histology and distribution of false tendons have already been described in detail by Draper (1955). The excitable cells in these structures in the goat and dog are usually 'typical' Purkinje cells, (Figs. 13 & 14). The characteristic features of typical Purkinje fibres have been described by so many workers and they are so well known that a detailed description is not necessary (vide Todd, 1928).

These 'typical' Purkinje cells have the following characteristics:

1) The fibre diameters are definitely larger than the auricular and ventricular myocardial fibres.

2) The nuclei are round, in groups of two or three, surrounded by a clear space.

3) The myofibrils are loosely packed.

4) They contain a large store of glycogen.

The fact that false tendons may sometimes contain ordinary cardiac muscle, a mixture of typical Purkinje cells and ordinary cardiac muscle (Fig. 15) or an intermediate or 'transitional' type of Purkinje cell, is not so well known. This has made it necessary for all the false tendons experimented on to be checked histologically.

The transitional cells have been described by Todd

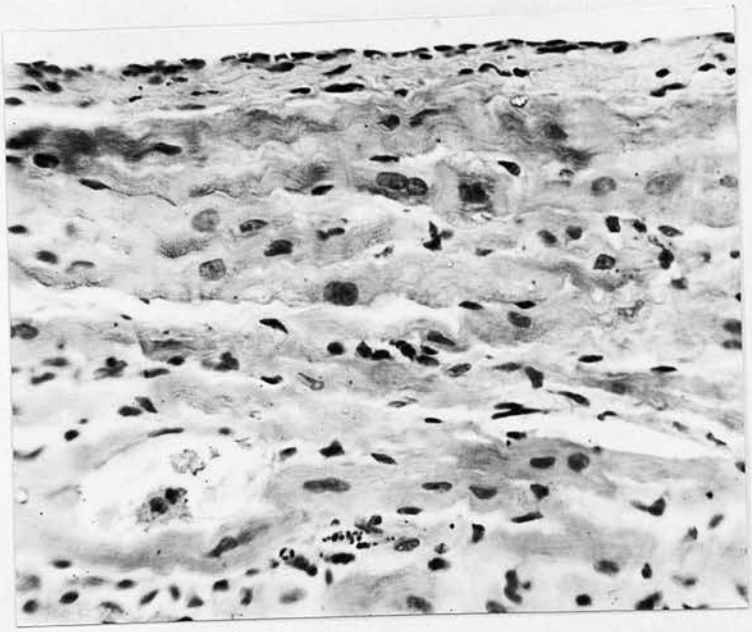


Fig. 16

Section of a dog false tendon (x 400) showing "transitional" cells. Round nuclei, some of them paired, and loosely packed myofibrils can be seen in these cells.
(Modified van Gieson.)

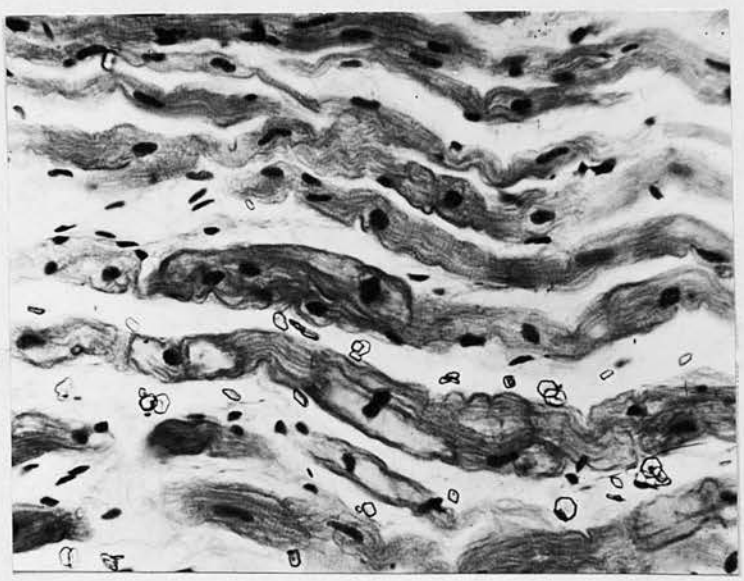


Fig. 17

Longitudinal section of a cat false tendon (x 400) showing the cat "Purkinje fibres". In some of the cells round paired nuclei, and central clear spaces can be seen.
(Modified van Gieson.)

(1928), Truex & Copenhaver (1947) and by Draper (1955). According to these authors, these cells are in every way intermediate in their characters between the typical Purkinje cell and the typical cardiac muscle. Thus their size is in general much larger than the ordinary cardiac muscle but smaller than the typical Purkinje fibre. Their nuclei may be round or oval, having no obvious vacuole around them, and there are more myofibrils in their sarcoplasm than the Purkinje fibres.

Fig. 16 is a section of a false tendon from a dog. A large number of the cells have the characteristics of transitional cells described above. This false tendon was one used for the measurement of conduction velocity and the results in relation to the histological structure will be considered in Part III.

Purkinje fibres in the cat, rabbit and rat false tendons

Purkinje cells of the type described above as 'typical' are found only in the goat and dog out of the series of animals studied. In the cat and rabbit false tendons so far studied, cells resembling the transitional cells have been observed. In the rat false tendon, however, the cells resemble closely the ventricular muscle fibres. The typical Purkinje fibres found in the goat and dog have never been seen in these smaller animals.

Figs. 17, 18 & 19 show the cell structures in the cat, rabbit and rat false tendons. Comparison with the Purkinje fibres found in the goat and dog (Figs. 13 & 14 which are at the same magnification), and with the ordinary cardiac muscle shows that they resemble the latter much more than they

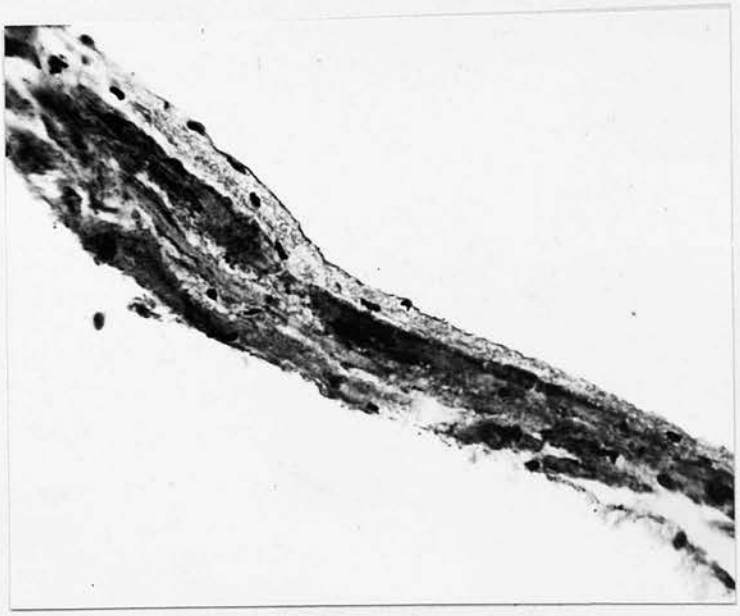


Fig. 18

Longitudinal section of a rabbit false tendon (x 400)
treated by the lead-tetra-acetate-Schiff method.
Structural details of the cells cannot be made out by the
presence of glycogen in these cells can be seen from the dark areas.

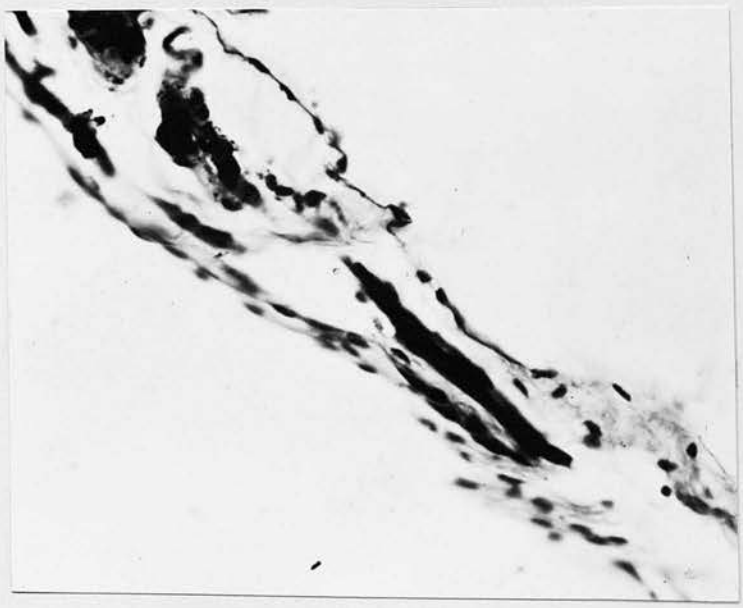


Fig. 19

Longitudinal section of a rat false tendon (x 400)
treated by the lead-tetra-acetate-Schiff method.
The cells are filled with glycogen.

resemble the typical Purkinje cells.

The records of action potentials obtained from the false tendon of a cat shown on p. 42 (Part I) was obtained from the false tendon shown in Fig. 17. Though the differentiation from ordinary cardiac muscle is not as great as in the goat and dog Purkinje tissue, the vacuolated appearance with the round nuclei suggest that the Purkinje tissue in the cat have these characters. Supporting evidence is the finding that these cells contain much more glycogen than the surrounding ventricular myocardial fibres. In the rat false tendon, shown in Fig. 19, the cells are not so well defined. But it can be made out that they are about the same size as the ventricular fibres. In contrast to the myocardial cells, however, these cells are loaded with glycogen, thus suggesting they do belong to the conducting system.

Fig. 8D shows a typical action potential obtained from a rat false tendon. The conditions of the experiment were such that this record was almost certainly obtained from a normal tissue. Unfortunately false tendons from other small animals rarely survived long enough to be confident that the records obtained were from normal tissues. Fig. 8C shows a typical record obtained from a cat false tendon. Such action potentials have only been obtained for a short time and the general configuration is such that it would seem most likely to be a record from a dying cell.

Purkinje cells in the atria

From time to time various investigators have claimed that Purkinje fibres are present in the mammalian atria, (Van der Strict & Todd, 1919; Holmes, 1921; Todd, 1928; and

Fig. 20

Longitudinal section of a goat sulcus terminalis, showing a group of "Purkinje cells" (at the upper left corner). x 400. (Gomori)

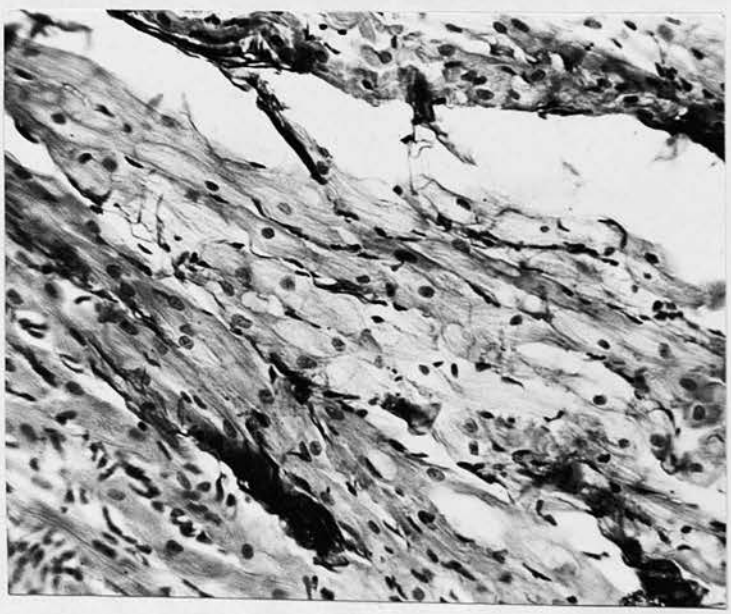


Fig. 21

Longitudinal section of a cat sulcus terminalis showing a strand of "transitional" and a few Purkinje fibre-life cells. Ordinary auricular muscle fibres at the lower left corner. x 400. (van Gieson)

Stotler & McMahon, 1947). Recently Copenhaver & Truex (1952), while denying that there are Purkinje cells in the atria of the sheep, found in one out of the twenty-three adult human atria studied, 'atrial elements which simulated Purkinje fibres which are found in the conducting system distal to the fibrous ring'. In 50% of the human atria studied, they found atrial fibres resembling transitional cells.

In the present study, typical Purkinje cells have been observed under the epicardium in one sulcus terminalis of the goat (Fig. 20). The distribution of these cells could not be studied as they appeared in the first section and rapidly disappeared. Probably most of the cells of that group were left behind with the rest of the atria when the sulcus was excised. It is situated about the middle of the sulcus. Staining with Lead-tetra-acetate-Schiff showed that these cells were filled with glycogen, just as in the typical Purkinje fibres.

In a cat sulcus terminalis, cells resembling transitional cells have also been observed (Fig. 21). Such findings, once again stress the need for a histological check of the tissues after experimenting on it. When action potentials from the atrium are being studied, the possibility that atrial Purkinje fibre action potentials may be recorded will have to be kept in mind. Their histological resemblance to ventricular conducting tissue, however, is no guarantee that their action potentials would be similar. Note, for example the difference in the atrial and ventricular action potential configurations. There is no means of knowing yet, what the shape of the atrial Purkinje action potential would look like.

In all probability, it would not differ much from the typical ventricular Purkinje fibre action potential.

That Purkinje fibre-like cells may be present in the atrium has also to be kept in mind when measuring the conduction velocity in these tissues, as a fast velocity found in atrial tissues may have been due to the presence of these fibres, assuming that they have similar properties to the ventricular Purkinje fibres (see Part III).

Atrio-Ventricular Bundle

It has been shown that the AV bundle can be dissected out in man (Walls, 1945; Kistin, 1949) and in a number of mammals by Holmes (1921). The possibility of isolating and dissecting out the AV bundle without inflicting much damage to it was looked into with a view to excising it and experimenting on it as was done with other bits of cardiac tissue in the present experiments.

An attempt was first made to dissect out the bundle in the hearts of goats and dogs according to the method described by Walls (1945) and Kistin (1949), which involved picking at the dense fibrous tissue piecemeal till the bundle was exposed. It was found that although the bundle could ultimately be isolated, it inflicted such trauma to the tissue that it was not worth experimenting on it for purposes of the present study.

In cat hearts, in was found that the fibrous tissue at the AV junction was not as dense as that in the goat and dog hearts, and therefore the AV bundle could be easily dissected out in about 25% of the hearts. As this is likely to be a useful preparation, the method of isolation will be described

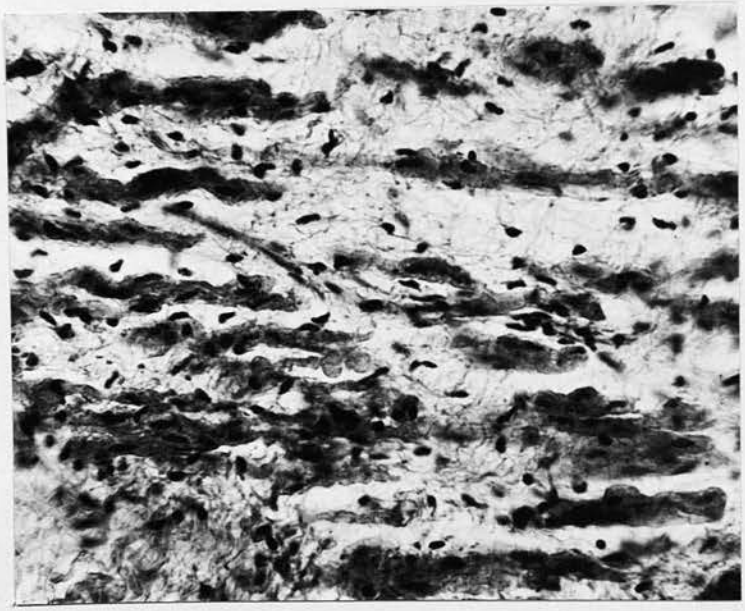


Fig. 22

Longitudinal section of an isolated A.V. bundle of the cat (x 400). The small cells are embedded in the thick fibrous tissue. (Lead-tetra-acetate-Schiff.)

in detail. After opening both the right and left ventricles, the interventricular septum was inspected from both the right and left ventricular surfaces. On the right side, the medial cusp of the tricuspid valve was cut away close to its upper attachment. The medial cusp of the aortic valves was likewise removed. The atria was then separated from the ventricles at the AV junction. This can be safely done without damage to the bundle, by inserting one blade of a small sharp pointed pair of scissors into the upper region of the membranous interventricular septum and cutting away the atria, thus exposing the upper surface of the interventricular septum with the cartilaginous AV fibrous tissue covering it. A portion of the right atrium in front of the coronary sinus is left behind. In about 25% of the hearts, inspection of the interventricular septum on both sides reveals, usually on the left side, a whitish strand through the endocardium running just below the region where the valves are attached to the AV ring. This strand is the AV bundle. The endocardium over it is very loosely attached and can be removed easily by incising the endocardium above and below the bundle and peeling it off gently. Following the bundle distally, it can usually be seen to divide into its right and left branches. Proximally it is more difficult to follow as it passes into the right auricular muscle. A length of about 5-6 mm of the AV bundle can be obtained by this method. With practice it takes about only 5 minutes to isolate and excise it.

The AV bundles thus excised have been found to contract on stimulation. No spontaneous contractions have been

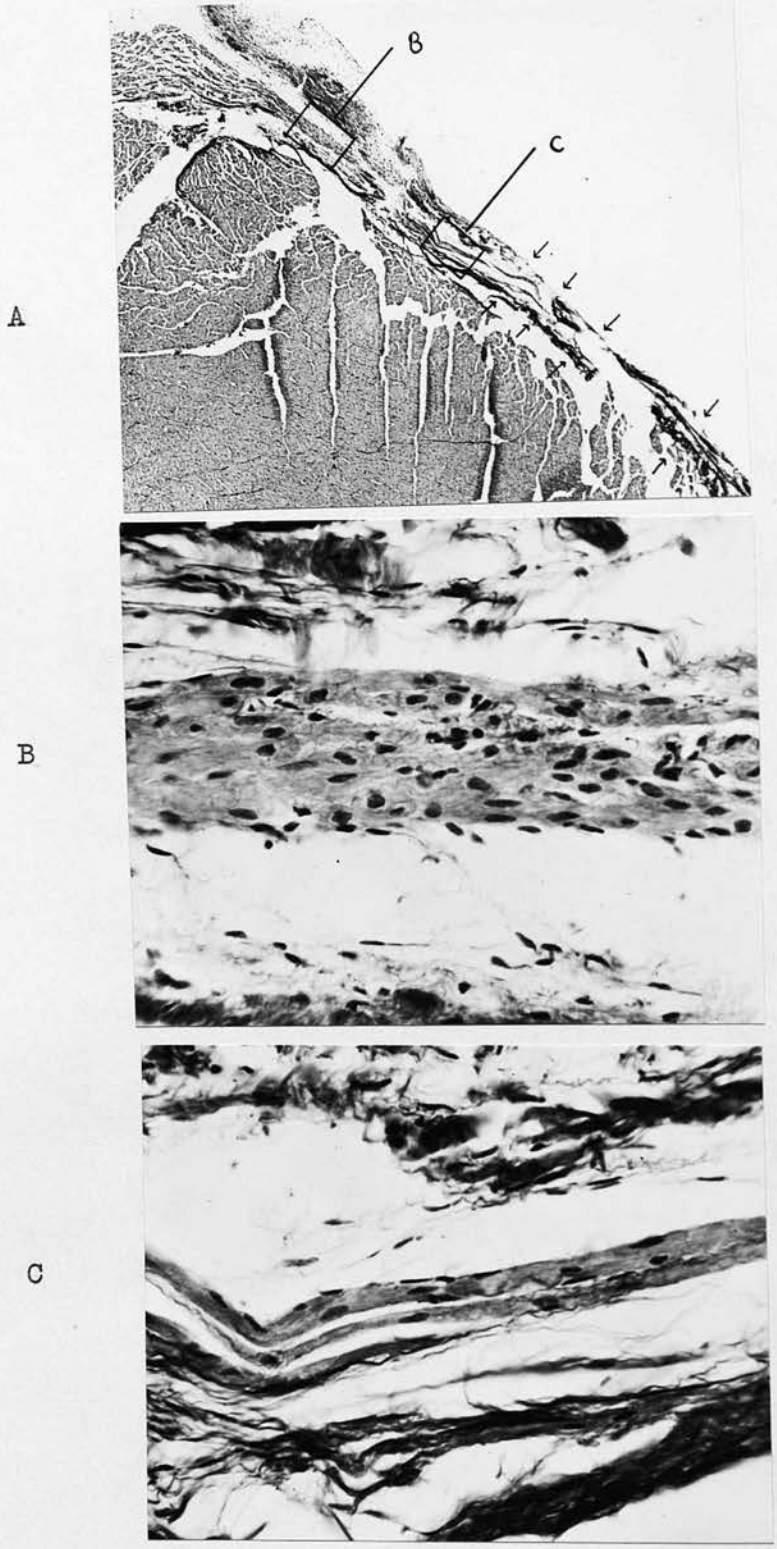


Fig. 23

A. Longitudinal section of a cat interventricular septum, (x 35) showing the AV Bundle and its continuation with the right bundle branch (between arrows).
B. Higher magnification of the AV bundle shown in upper box (x 400).
C. Higher magnification of the upper part of the right bundle branch shown in lower box (x 400).

observed. Fig. 22 shows the histological structure of such a bundle. The small cells embedded in thick fibrous tissue can be seen. The two factors have so far prevented any impalement of the cells of the bundle with microelectrodes. The cells are seen to resemble closely ordinary ventricular myocardial cells. The problem therefore arises whether the bundle dissected out is merely an ordinary muscular fasciculus from the upper part of the interventricular septum as suggested by Glomset and Birge (1945). Against this possibility is the finding that the cells in the isolated bundles always contain more glycogen than the neighbouring ventricular myocardial cells and are always surrounded by a fibrous tissue sheath. Fig. 23 shows the AV bundle with the right branch isolated according to the above method, but kept in continuity with the interventricular septum at its extremities.

Fibre diameters

To obtain some quantitative idea of the cell type and cell size, fibre diameters of the atrial, auricular, ventricular and Purkinje tissues were measured. Many workers have previously measured the diameters of ventricular myocardial cells and Purkinje fibres (for review see Ashley, 1945). Measurements of the atrial or auricular fibre diameters have not been found in the literature.

In a syncytium, it is difficult to define the limits of a cell. An obvious structure which could be regarded as a transverse cells boundary in the cardiac fibres is the intercalated disc, and various authors have suggested this. In this connection, the observations of Van Breeman (1953) and

of Bourne (1953) are of interest. Van Breeman, studying ventricular myocardium of dogs under the electron-microscope, claimed that the intercalted discs are 2 laminated layers continuous with the sarcolemma, and that the myofibrils are not continuous through the intercalated discs. Bourne found a localisation of dephosphorylating enzymes (succinic dehydrogenase and non-specific alkaline phosphatase) in the intercalated discs and concluded that since these enzymes are usually found in cellular sites where the production of free energy is necessary, the intercalated discs may also be regions where release of energy takes place. These observations therefore indicate that the intercalated discs are not artifacts as some authors have believed. Whether they are actually transverse cell boundaries is still not settled.

The lateral boundaries are more easily defined, especially in auricular, atrial and ventricular muscle fibres. In the Purkinje tissue, on the other hand, it can sometimes be extremely difficult to define the lateral boundaries of the cells.

Because of these uncertainties in defining the actual limiting boundaries of a cardiac cell, the measurement of 'fibre diameter' must of necessity be to a certain extent arbitrary. Previous investigators (Blair & Davies, 1935; Ashley, 1945; Davies & Francis, 1952) have taken the 'fibre diameter' as the measurement across the fibre at the position of a nucleus. It is not well known, however, what the relation is between the number of nuclei per unit amount of myoplasm, or whether the nuclei have any plan of distribution. The branching of the fibres also do not appear to have any

TABLE VI

Diameters of goat cardiac fibres

Auricular, atrial and Purkinje tissue fibre diameters of an 8 months old goat. Ventricular fibre diameters from another goat of the same age, and same heart weight.

Tissue	No. of fibres	Mean (μ)	S.D.	Range (μ)
Auricular	85	14	2.2	10-21
Atrial	80	12	1.5	9-17
Ventricular	58	9	1.7	6-14
Purkinje	25	38	12.0	19-72

TABLE VII

Diameters of cat cardiac fibres

Auricular, ventricular and Purkinje tissue cells from same animals, atrial and AV cells from 2 other animals.

Tissue	No. of fibres	Mean (μ)	S.D.	Range (μ)
Auricular	51	10	1.9	6-15
Atrial	70	11	2.7	8-19
Ventricular	37	10	2.1	7-14
Purkinje	74	13	2.31	8-17
AV cells	9	8	1.1	7-11

regularity. For want of a better method, the same procedure as used by the above authors has been followed in the present study.

The fibre diameters were measured from longitudinal sections by means of an eye-piece micrometer, and using 10X eye piece and an oil immersion objective (N.A. 1.30; 100X). The fibre diameters are given to the nearest μ.

In Table VI are given the diameters of the auricular, atrial and Purkinje fibres from an 8-month old goat and of the ventricular fibres from another goat of the same age, having the same heart weight. Comparison of the auricular, atrial and ventricular fibre sizes with that of the Purkinje tissue shows that they are definitely smaller than the Purkinje cells. There is not as much difference in size between the fibres in the auricular, atrial and ventricular tissues, though the ventricular fibres appear to be somewhat small. In contrast, the diameters from a cat heart (Table VII) shows that the fibres from all the tissues are very much the same. In Table VIII the mean fibre diameters of the auricular, atrial, ventricular and Purkinje cells from the hearts of various mammals are compared. The complete data are given in Tables i-iv (see Appendix I). These tables show that the atrial, auricular and ventricular fibres from all the animals studied have about the same mean fibre sizes, those of the mouse being consistently in the lower ranges. The Purkinje cells in the goat and dog are much larger than the corresponding cells in the cat, rabbit and the rat, the size of the cells in the latter animals being nearly the same as those in the ordinary cardiac muscular tissues.

TABLE VIII

Mean fibre diameters of auricular, atrial, ventricular and Purkinje tissue of various animals.

Animal	Auricular	Atrial	Ventricular	Purkinje tissue
Goat	14μ	12μ	9μ	38μ
Dog	10μ	12μ	11μ	22μ
Cat	10μ	11μ	10μ	13μ (AV cells 8μ)
Rabbit	11μ	12μ	11μ	10μ
Guinea-pig	-	-	13μ	-
Rat	8μ	-	9μ	-
Hamster	9μ	-	10μ	-
Mouse	8μ	-	6μ	-

The cells of the atrio-ventricular bundle in the cat have been found to be much smaller than the ventricular fibres. This is in agreement with the findings of previous workers. The fibre sizes of these cardiac tissues found in this study are comparable to the results obtained by Blair & Davies (1935) and by Davies & Francis (1952) who used the same methods of fixing and sectioning as in the present experiments. Ashley (1945) on the other hand measured the diameters using frozen sections, and thus obtained slightly higher values obtained by the above workers and in the present experiments.

These histological findings will be taken into account when the results of the conduction velocity measurements are considered in Part III.

Fibre direction

The direction of the fibres in the tissue samples has to be taken into account when measurements of the conduction velocity are being made. This will be discussed in relation to the conduction velocity measurments.

The main value of the histological study has been to define the cell type present in the tissues under consideration. The structure of the muscle is such that the measurement of "fibre diameter" is a somewhat unsatisfactory procedure. Nevertheless, it is interesting to observe that in large animals such as the goat and the dog, the conducting tissue is very definitely a much bigger cell-type than the muscular tissue. In the small hearts, this big difference is not apparent. Finally, it is interesting that in all the hearts examined the diameter of the constituent muscular cell is approximately the same.

PART III

THE CONDUCTION VELOCITY IN MAMMALIAN CARDIAC TISSUES

PART III

Historical Survey of the Measurement of Conduction Velocity in Mammalian Cardiac Tissues

Basic to the study of spread of excitation in the heart is the measurement of conduction velocity in the different cardiac excitable tissues. A survey of the literature will show that very few attempts have been made to measure accurately this fundamental property in these tissues.

The first workers to measure the rate at which the contraction wave passes over the ventricles of the mammalian hearts (guinea-pigs, rabbits, cars, dogs and a sheep) were Waller & Reid (1887). They used the graphic method, placing 2 levers, one on the apex and the other on the base of the ventricles and measuring the time difference between the 2 contractions. These measurements were carried out on excised spontaneously beating dying hearts 1-13 minutes after removal from the animal. Because of this rather crude method of measuring the conduction rate, it is no wonder that they obtained such a wide variation in their results. Moreover, they did not differentiate between the contraction wave and the wave of excitation.

Bayliss & Starling in 1891 while studying the electrical changes in the exposed heart of the dog, measured the propagation rate of the excitatory process in the ventricles, using a capillary electrometer. One electrode was placed on the apex while the other was on the base, and from the time interval between the beginning and the culmination of the initial wave, the propagation rate was calculated. This method had already been used by Burdon-Sanderson & Page (1879) to

measure the propagation rate in the frog ventricle. Bayliss and Starling obtained a conduction rate of about 3 m/sec in the dog ventricles, but they themselves stated that since the form and direction of the wave is very sensitive to slight changes in the temperature of the different parts of the heart surface, their value for the conduction rate in the ventricles was not a very accurate one.

Waller in 1889, ~~using the Sanderson-Page method~~ estimated the rate of transmission in the human ventricle from the electrocardiogram to be about 5 m/sec. This, he admits is a very rough approximation.

In 1902, Schlüter, in spite of the electrical recording instruments available then, used the frog muscle-nerve preparation to determine the rate of conduction in dog and cat hearts, perfused by the ~~Hagendroff's~~ Langendorff's method. His method resembled the classical method used by Helmholtz to determine the conduction velocity in nerves. He found the rate of conduction in the ventricles of both the animals to vary from 2-4 m/sec.

Gotch (1910) measured the propagation rate of excitation in the rabbit ventricle using much the same method as Bayliss & Starling (1891) and found rates varying from 1.5 to 2 m/sec.

Hering (1910) estimated the conduction rate in the AV bundle of the dog. In a perfused heart, he sectioned the AV bundle and by stimulating first the distal cut end of the bundle and then the ventricular muscle wall, recorded the ventricular contractions on a kymograph. From the time interval between the contractions he calculated the conduction rate in the AV bundle. He does not give actual values but states

that the conduction rate in the AV bundle is the same as in ventricular muscle. It is of interest to note that this is the only paper in the literature where an attempt has been made to measure directly the conduction rate in the AV bundle.

In 1912, Erlanger, studying the properties of Purkinje tissue, measured the conduction rate in calf false tendons, this being the first time the conduction velocity was measured in Purkinje tissue since the suggestion by Tawara (1906) that it conducts more rapidly than ordinary ventricular muscle. In a perfused calf heart cut open to lay bare the false tendons, he calculated the conduction velocity in them, from the ventricular contraction times brought about by stimulating the cut false tendon at two points a known distance apart. He found the rate to be 0.7 m/sec, but he states that the actual rate would be much faster because his measurements were done on lax false tendons.

Because of the various technical imperfections, none of the values obtained by the above mentioned investigators can be accepted. The first comprehensive work on the measurement of conduction velocity in the heart was done by Lewis and his colleagues. Using an Einthoven galvanometer with two strings, they measured the conduction velocity in the atria, ventricles and Purkinje tissue of dogs. From these results they also estimated the propagation rate in the AV node and AV bundle. They used dogs for all their experiments and carried out the measurements on normally beating exposed hearts, using unipolar or bipolar recording electrodes.

For measurement in the auricles, Lewis, Meakins & White (1914) placed 2 pairs of electrodes, 7 mm apart along an

auricular trabecula on the epicardial surface of the heart and measured the time interval between the two 'intrinsic' deflections. They measured the conduction rates along the taenia terminalis and along the different muscle bands of the auricles and obtained values ranging from 0.596 to 1.899 m/sec with an average of 0.859 m/sec in the right auricle and 1.252 m/sec in the interauricular band.

For measurement of the conduction rate in the ventricular muscle, Lewis & Rothschild (1915) placed 2 electrodes at a measured distance apart on the ventricle along a muscle band, and the stimulating electrode in line with the two recording electrodes. From the time difference between the deflections at the two electrodes, the conduction rate was calculated. Using this method, they found conduction rates of 1000 to 1500 mm/sec in the right ventricular wall, whereas in the left ventricular surface, the conduction rates were 300 to 500 mm/sec. They considered that the actual velocity in ventricular muscle is slow, i.e. 300 to 500 mm/sec and the faster velocity found in the right ventricle was because of the thin wall and therefore allowing the impulse to enter the endocardium to be carried rapidly by the subendocardial Purkinje fibres and out again through the muscle to the distal recording electrode.

Measurement of the conduction rate in the subendocardial Purkinje fibre network was carried out in an indirect way by the same authors. Two electrodes were placed opposite each other on the endocardial and epicardial surfaces of the right ventricle and the ventricle was stimulated at some distance on the epicardial surface. Thus arranged, there are two

alternative pathways to the epicardial recording electrode: (1) along the superficial muscle fibres, and (2) through the thickness of the muscle at the point of stimulation to the subendocardial Purkinje fibre network, along that network to the endocardial recording electrode and then out again through muscle to the epicardial electrode. The distance of the stimulating electrode was so adjusted that the impulses travelling via both the routes reached the epicardial recording electrode simultaneously. By measuring the thickness of the muscle and the distance between the stimulating and the recording electrodes, the relative rates of conduction in muscle and Purkinje tissue were calculated. They calculated the conduction rate in the subendocardial Purkinje tissue to be 1000mm to 1500 mm/sec. However, since this velocity was in a network, they estimated that the actual velocity in "straight Purkinje fibres, i.e. the chief divisions of the bundle should be 50-100 per cent faster". They thus estimated the rate in Purkinje tissue to be 3.0 to 5.0 m/sec.

De Boer (1925) criticised the above procedure of Lewis and Rothschild and he maintained that the increased velocity found in the Purkinje tissue can be explained by a difference in temperature between the epicardial surface which was undoubtedly cooled during the experiment, and the warm endocardial surface.

The above procedure for measuring the conduction velocity in Purkinje tissue is unsatisfactory in other respects. It was assumed that the impulse spread from the stimulating electrodes through the muscle in a perpendicular fashion from the epicardium to the endocardium. At the time of their

experiments myocardial Purkinje fibres (Abramson & Marjolin, 1936) had not yet been demonstrated. The presence of these Purkinje fibre elements, in addition to the different directions in which the muscular bundles run in the wall of the ventricle, would certainly complicate the interpretation and calculations in the above experiment.

Conduction velocity in the AV bundle was not measured but it was assumed to be the same as in Purkinje tissue, i.e. 3 to 5 m/sec. An estimate of the conduction rate of 0.2 m/sec in the AV node was also made by Lewis (1925), his calculations being based on:-

(1) the rate of transmission from the SA node to the septal muscle overlying the AV node;

(2) the approximate length of the AV node;

(3) the time of appearance of the R wave in the electrocardiogram being taken as the time of transmission along the AV bundle (conduction velocity 4m/sec.) to the apex of the right ventricle.

After the studies of Lewis, all subsequent investigators, with the exception of Lapicque & Veil (1927) used electrical methods to measure the conduction rate in the heart. Lapique & Veil measured the conduction velocity in dog auricles and false tendons in connection with their studies on the chronaxie of these tissues. Using the method of Erlanger (1912) they found values of 1.5 m/sec for the auricles and 0.5 m/sec in the false tendons.

Table IXgives a summary of the results of the conduction velocity measurements by workers who used electrical recording methods. None of these workers have measured it as

thoroughly as Lewis and his colleagues have. In fact, many of them do not even mention the method by which they measure the conduction velocity. They presumably used the '2-point' method of Lewis, i.e. placing two recording electrodes a known distance apart, the conduction velocity being calculated from the transmission time between these two points.

As can be seen from the table, there is very little agreement between the workers as to the conduction velocity even in the same tissues from the same species. For example, the conduction velocity measured in the atria in dogs have ranged from 0.49 to 4 m/sec. Likewise, the conduction velocity in the dog ventricle has ranged from 0.15 to 2.4 m/sec. To make matters worse, species differences have not been fully realised and the values obtained from one species have been freely used to interpret data obtained from experiments on a different species. For example, the conduction rates quoted in textbooks of Physiology (Lovatt Evans, 1952, Best & Taylor, 1955), and Cardiology (Katz, 1941) as occuring in the human heart, are the values obtained by Lewis in the dog heart. Actually, measurements of the conduction rate in the human heart have been very few. So far, only three attempts have been come across in the literature. As mentioned previously, Waller (1889) estimated the conduction rate in the human ventricles from the axial electrocardiogram. Recently, Kossman, Berger, Brumlik & Briller (1947) directly measured the conduction velocity in the exposed human heart, using a string galvanometer and Lewis' '2-point' method. They found rates of 1.8 to 2.7 m/sec in the atria. Prinzmetal, Corday, Brill, Oblath & Kruger (1952) measured the

rate of spread of excitation in the human atria from the electrocardiograms of two simultaneously recorded oesophageal leads, separated by a fixed known distance. They obtained a value of 0.5 m/sec. This measurement, although carried out on intact subjects, is in a way less satisfactory than that of Kossman et al as it is not certain whether the spread is being measured in one auricle only and because the direction of the fibres could not be taken into account. In spite of the importance of this property in human cardiac physiology, these have been the only attempts to measure it in the excitable tissues of the human heart.

Some recent workers have used direct-writing electrocardiographic machines to measure the conduction rates in the heart. This is unsatisfactory as the paper speed in these machines cannot be made fast enough and in the measurements of conduction times which are of the order of milliseconds, considerable error will arise especially when the velocity is being measured in short distances.

From this survey, it is clear that no satisfactory studies of this interesting and important aspect of cardiac physiology have been made. One of the major difficulties has been the problem of how one can measure the velocity of spread of excitation in such a complex tissue. One of the chief objects of the work described in this thesis was to develop a technique to measure accurately the conduction velocity in the different cardiac excitable tissues.

TABLE IX

Summary of the conduction velocities in mammalian cardiac tissues obtained by investigators who used electrical recording methods

Species	Tissue	Prepa-ration	Method	Conduction Velocity m/sec.	Authors
Man	ventricle	in situ	axial e.c.g.; Sanderson-Page method. Cap. electrometer.	5.0	Waller, 1889
Man	atria	in situ exposed	direct; '2-point method. String Galvo.	1.8-2.7	Kossmann, Berger, Brumlik & Briller 1947
Man	atria	in situ	oesophageal leads, e.c.g; Direct-writing machine	0.5	Prinzmetal, Corday, Brill, Oblath & Kruger, 1952, p. 172
Dog	auricles	in situ exposed	direct; '2-point method; double string galvo	0.596 - 1.899 av. 1.0	Lewis, Meakins & White, 1914
Dog	auricles	in situ exposed	direct; '2-point method; oscilloscope	0.5-1.2 av. 0.8	Brendel, Raule & Trautwein, 1950.
Dog	auricles	excised	not mentioned; microelectrode oscilloscope	1.0	Hoffman & Suckling, 1953
Dog	auricles	in situ exposed	direct; '2-point method; Sanborn Polyviso direct writing machine	0.487-1.3 4.0 along taenia	Peuch, Esdavissat, Sodi-Pallares & Cisneros, 1954
Dog	ventricle	in situ	direct; Sanderson-Page method	3.0	Bayliss & Starling, 1891
Dog	ventricle	in situ	direct; '2-point' method; double string galvo.	0.3-0.5	Lewis & Rothschild, 1915
Dog	ventricle	in situ	"	2.4	Robb & Robb, 1936
Dog	ventricle	in situ	direct; oscilloscope	0.88	Schaefer & Trautwein, 1949.
Dog	ventricle	in situ	direct; '2-point' method; oscilloscope	0.5-1.2	Brendal, Raule & Trautwein, 1950
Dog	ventricle	in situ	direct; oscilloscope	0.15-0.5	Scher, Young, Malmgren & Paton 1953

TABLE IX (Cont.)

Species	Tissue	Preparation	Method	Conduction Velocity m/sec.	Authors
Dog	ventricle	in situ	transmural; direct writing ecg.	0.3-0.4	Rodriguez & Sodi-Pallares, , 1952.
Dog	ventricle	excised	not mentioned; microelectrode	1.2	Hoffman & Suckling 1954.
Dog	ventricle	in situ	transmural oscilloscope	0.5	Durrer, Tweel & Blickman, 1954.
Dog	Purkinje tissue	in situ	indirect; string galvo	3.0-5.0	Lewis & Rothschild, 1915.
Dog	False tendon	?	? string galvo	2.0-3.5	Maeno, 1930.
Dog	False tendon	excised	'2-point' microelectrode; oscilloscope	1.3-3.2 av. 2.0	Draper & Weidmann, 1951.
Dog	Purkinje tissue	in situ	direct writing e.c.g.	4.0	Rodriguez & Sodi-Pallares, , 1952.
Dog	False tendon	excised	not mentioned microelectrode oscilloscope	2-3	Trautwein, Gottstein & Federschmidt, 1953.
Dog	Purkinje tissue	in situ	not mentioned oscilloscope	1.0	Scher, Young, Malmgren & Erickson, 1955.
Dog	AV node and its branches	in situ	direct multipolar intramural; oscilloscope	1.4	Scher, Young & Becker, 1955.
Dog	AV bundle	-	indirect	4.0	Lewis, 1925.
Dog	AV node	-	calculated	0.2	Lewis, 1925.
Calf	False tendon	excised	not mentioned oscilloscope	4.0	Curtis & Travis, 1951.
Kid	False tendon	excised	'2-point' method; microelectrode; oscilloscope	2.4	Draper & Weidmann, 1951.
Cat	auricle	excised	not mentioned; microelectrode oscilloscope	0.3-0.5	Burgen & Terroux, 1953.
Cat	ventricle	excised	not mentioned microelectrode oscilloscope	0.96	Trautwein, Gottstein & Federschmitt, 1953.
Rabbit	ventricle	in situ	direct; Sanderson-Page method Cap. electrometer	1.5-2	Gotch, 1910.

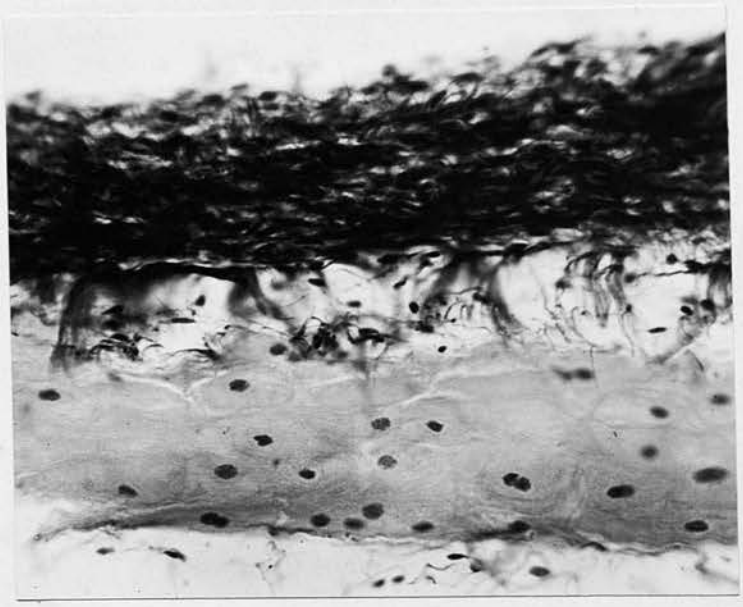


Fig. 24

Typical Purkinje fibres in a goat false tendon showing regular arrangement of the fibres. x 400. (Modified van Gieson.)

METHOD

TISSUE SAMPLES

The muscular architecture of the heart is so complex that if one used the whole organ for measurements of conduction velocity in the different cardiac tissues, there is bound to be a great deal of uncertainty in the actual pathway taken by the excitation wave. Moreover, Purkinje tissue is so widely distributed in ventricular myocardium that it would be difficult to determine the part contributed by the separate components in the total conduction time.

It was therefore decided to use isolated strips of cardiac tissue. Ideally, for the purpose of measuring conduction velocity, these strips should have a regular structure, namely a parallel arrangement of the fibres and with no mixture of other excitable tissues.

Conduction velocities have been measured in the auricular, atrial, ventricular and Purkinje tissues of goats, dogs, cats and rabbits.

1. Purkinje tissue Purkinje fibres are conveniently isolated in the false tendons and these have, therefore, been used to measure the conduction velocity in Purkinje tissue. In slender false tendons, the arrangement of the cells are simple and consistent - usually consisting of just a few strands of Purkinje fibres running parallel along the length of the false tendon, (Fig. 24). Unfortunately, however, the type of excitable element in these false tendons is not as consistent as its structural arrangement. As mentioned in Part II, the most common excitable cells present are the Purkinje fibres. Sometimes, however, they may consist

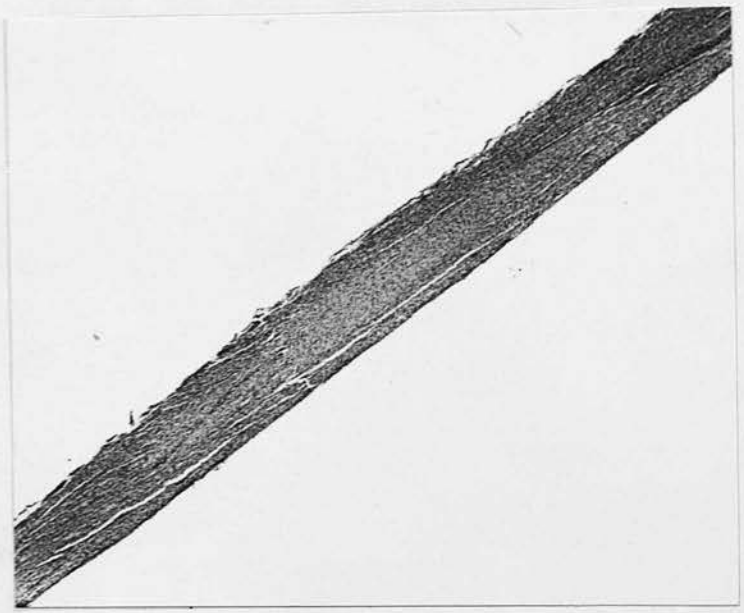


Fig. 25

Longitudinal section of a cat papillary muscle to show the regular and parallel arrangement of the fibres.
x 25. (Modified van Gieson.)

entirely of ordinary cardiac muscle. In some there may be a mixture of typical Purkinje fibres and cardiac muscle; occasionally they may contain an intermediate type of Purkinje cell. Therefore, before accepting a conduction velocity measured in a false tendon as relating to Purkinje tissue, it is necessary to demonstrate that the only excitable cells present are Purkinje cells. All the three variations in the cell constituents of the false tendons have been met with in the present study.

The procedure for dissection of the false tendons has already been described (p. 27).

2. Ventricular muscle The muscular arrangement of the papillary muscle is regular, the fibres being more or less parallel (Fig. 25). They therefore offered the best situation in which to measure the conduction velocity in ventricular myocardial tissue.

The right anterior papillary muscles were selected in cats and rabbits because they were long and slender. In goats and dogs, since this muscle was too big, small ventricular slips were used.

No sample of ventricular muscle can be assumed to consist solely of muscular tissue. Even macroscopic examination can show the strands of conducting tissue ramifying over the base of the papillary muscles. Thus in fact it has been quite difficult to find samples of "pure" ventricular muscle with a suitable arrangement of fibres.

3. Auricular muscle Auricular trabeculae were dissected out as described on p. 26. One often finds the histological structure of these trabeculae to be very irregular in spite

of its apparent regularity as seen from macroscopic inspection. They do not therefore present an ideal situation in which to measure the conduction velocity. Occasionally one can obtain an auricular trabecula with a regular structure for a short stretch. Measurements have been made on four such trabeculae.

4. Sulcus Terminalis (Crista terminalis) This strip of muscle has been taken as a sample of atrial tissue. It is of special interest to measure the conduction velocity in the sulcus terminalis because the SA node is situated at its upper end and it has been claimed that the excitation wave originating in the SA node reaches the AV node via this path in a normally beating heart, it being the shortest distance between the SA and the AV nodes.

Muscular arrangement of the fibres in the sulcus is very irregular, but the direction of the surface fibres can usually be discerned through the endocardium. This has been noted during the experiment and later checked with the histological structure.

The procedure for dissecting out the sulcus has been described already (p. 25).

THE CONDITION OF THE TISSUE

One important point which has to be settled when using isolated strips of cardiac tissue in an artificial bathing solution is whether they are in as good condition as in whole hearts. This is all the more important as on it will depend whether the conduction velocity results obtained on isolated strips are comparable to the velocity actually occuring in intact hearts.

The condition of the isolated cardiac muscular tissue may be assessed from:

(1) its ability to respond to stimulation by contraction;

(2) the nature of the electrical response.

Studies of the cardiac action potential using intracellular microelectrodes by Trautwein, Gottstein & Dudel (1954) and by Draper (1955, p. 73) suggest that the shape of the action potential and the magnitude of the resting potential and the overshoot may be used as an indicator of the condition of the tissues. Trautwein et al. have shown that the first change which occurs when the tissue is subjected to oxygen lack is a shortening of the action potential due to the loss of its plateau phase. Later on, the magnitude of the resting potential and the over shoot decreases, together with a slowing of the initial rate of rise and a decrease in conduction velocity.

The conditions for assessing the state of the Purkinje tissue is slightly different from cardiac muscle.

(1) The mechanical beat cannot be taken as an absolute prequisite for the good condition of the tissue, as some false tendons, especially in goats and calf do not normally show contractions (Draper, 1954).

(2) The behaviour of Purkinje cells subjected to oxygen lack is different from that of cardiac muscle in that it is far less sensitive and the first change is a loss of the spike followed by a lengthening of the plateau. The resting potential magnitude does not alter significantly for some time (Trautwein, Gottstein & Dudel, 1954; Draper, 1955, p. 150).

The magnitudes of the resting and action potentials obtained by Trautwein & Zink (1952) from isolated strips of mammalian cardiac muscle compare favourably with the results obtained by Hoffman & Suckling (1952) who measure the resting and action potentials on intact hearts in situ. The results of Draper & Weidmann (1951) and of Trautwein & Zink (1952) on isolated false tendons also gave comparable results as far as the magnitudes of resting and action potentials are concerned. This, therefore, indicates that the excised strips of cardiac tissue experimented in an artificial media behave similarly to whole hearts experimented in situ, as judged by these criteria.

Taken separately, the mechanical and electrical properties of the excised strip of cardiac tissue may not indicate the true condition of the tissue, but taking both into consideration, one can make a reasonable assessment of the state of the tissue.

METHOD OF MEASURING CONDUCTION VELOCITY

Essentially the most convincing results of previous workers have been obtained by one or other of the following methods. The first of these is the 'Sanderson-Page' method described on p. 63. It consists of placing a pair of electrodes in line with the point of stimulation, and the time taken to travel between the two electrodes is calculated from the beginning to the culmination of the negative phase of the action potential. This method is not very reliable as the duration of the intial wave is sensitive to change in temperature as Bayliss & Starling (1891) have pointed out. Moreover, Wilson, Rosenbaum & Johnston (1947) have pointed out that

there is uncertainty in relating the arrival of the impulse immediately under the electrode and a point on the action potential wave.

The other method used by Lewis and most other workers (Table IX), may be called the '2-point' method. The two recording electrodes (either unipolar or bipolar) are placed on the tissue along the line of stimulation, a known distance apart and the conduction velocity calculated from the time taken by the impulse to travel between these 2 electrodes. An objection which can be raised against this method is that because of the complicated nature of the structure of the heart, there is always the possibility that the 2 recording electrodes being placed on 2 sets of fibres running in different directions thus giving a value which is not the true conduction velocity for that tissue.

The method which has been used in this study may be called the 'multiple-point' method. In principle, it resembles the method commonly used to determine the conduction velocity in nerve (e.g. Sanders & Whitteridge, 1946). It consists in recording the action potentials at varying distances from the stimulating cathode and plotting on a graph the conduction distances on the ordinate, against the corresponding conduction time on the abscissa. The conduction velocity is then obtained from the slope of the regression line fitted to these points.

Certain modifications have had to be developed to suit the particular conditions which arose out of the fact that usually only very short strips of tissue were available. Firstly, the method of measuring accurately conduction

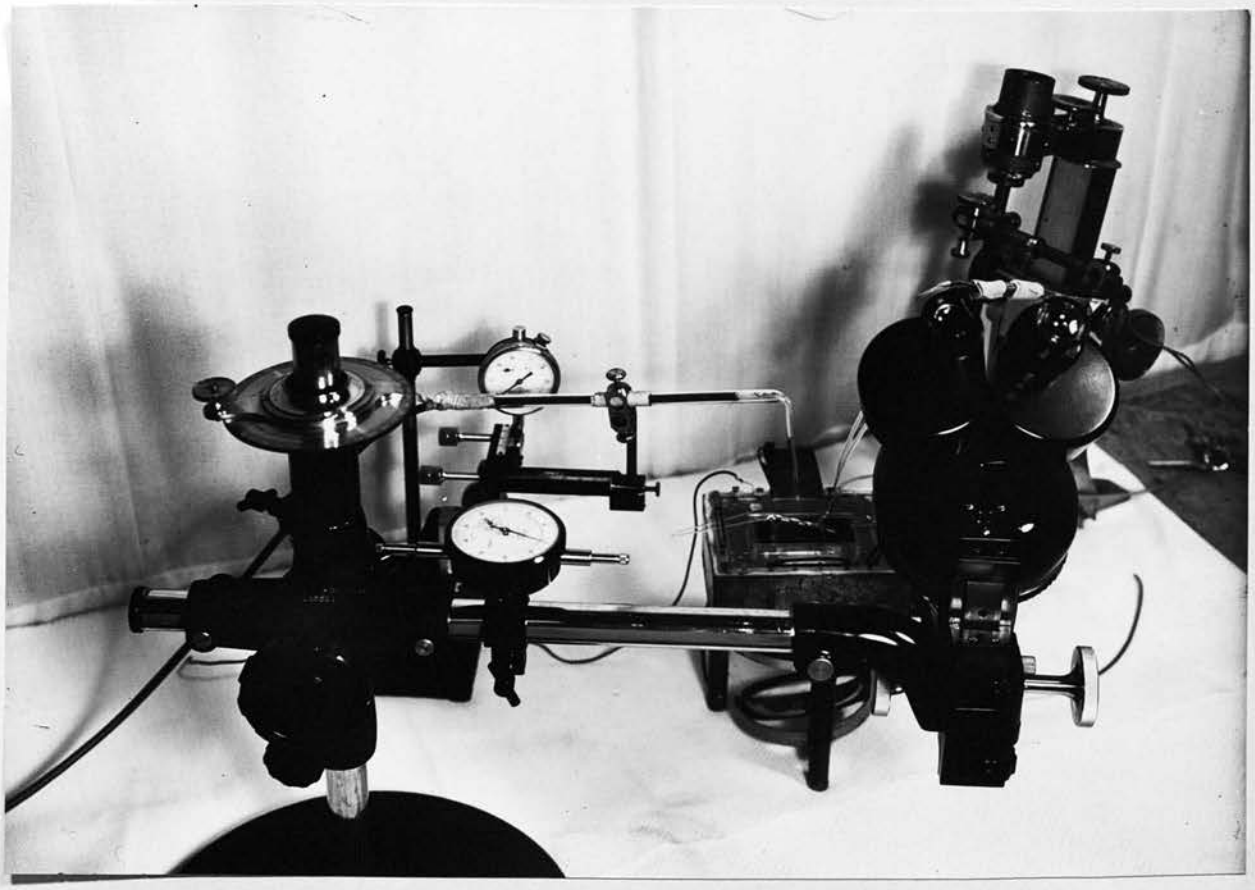


Fig. 26

Set-up for measuring distances
on the tissue.
See text for description.

distances was of great importance. In the very early experiments, a relatively crude method was used to measure conduction distances. A pointed needle was fixed on the horizontal bar of the binocular microscope. Displacements of the needle as the microscope traversed the tissue were measured with an ordinary steel ruler. The two required points on the tissue were 'fixed' alternately by means of cross hairs in the eyepiece of the microscope. In later experiments, a dial-gauge was substituted for the ruler (see below).

Secondly, to obtain many recording points on this short strip of tissue, very small electrodes had to be used. Ling-Gerard internal microelectrodes were found to be ideal for this purpose. In some cases, where longer stretches of tissue were obtained, external electrodes were used.

MEASUREMENT OF CONDUCTION DISTANCE

The general experimental set-up has already been described (p. 27). Fig. 26 shows in some detail the set-up used for measuring distance on the tissue. A dial-gauge is attached to the horizontal bar of the microscope with its plunger resting against a stop fixed firmly to the vertical stand. Any slight shift of the microscope along its traverse will thus be registered on the dial gauge. The actual procedure in measuring the distance on the tissue was as follows: the tissue was first orientated so that the projected line of measurement on the tissue was in line with the traverse of the horizontal bar of the microscope. The stimulating electrodes were then placed at one end of the tissue, the cathode being towards the recording electrode. The position of the middle of the cathode was 'fixed' under the

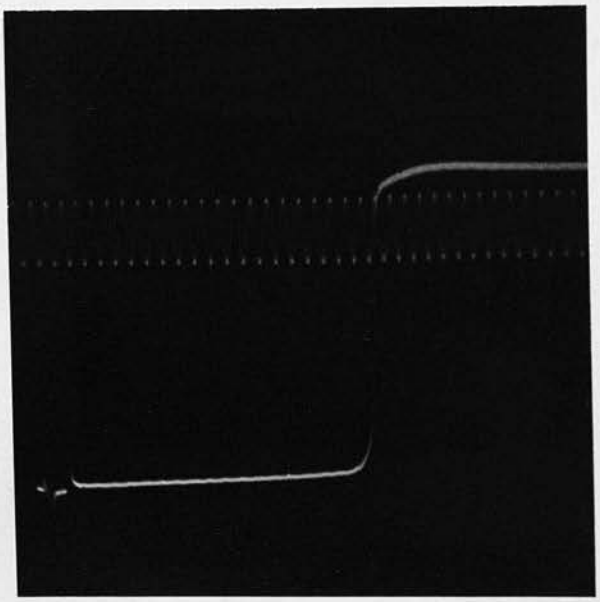


Fig. 27

Intracellular record of a cat ventricular action potential on a fast sweep. Time in msec.

microscope using cross hairs placed in one eyepiece and the reading on the dial gauge taken. The microscope was then shifted along the length of the tissue and the position of the tip of the recording electrode was 'fixed'. The dial gauge was again read, the difference between the two readings thus giving the conduction distance. The dial gauge readings were taken to the nearest 0.01 mm.

RECORDING ELECTRODES

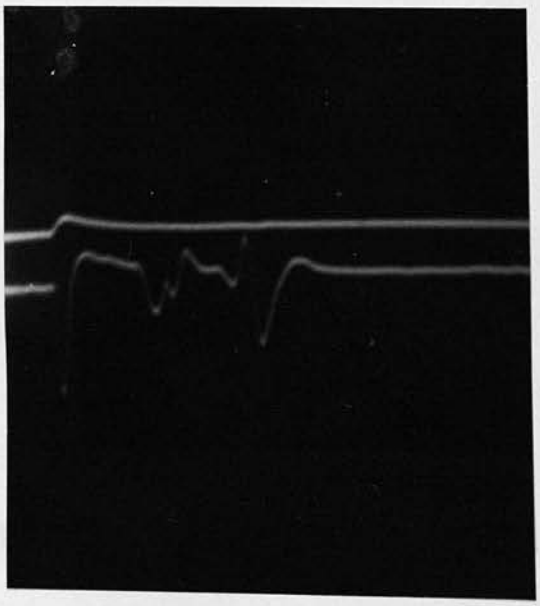
For recording the action potentials, internal electrodes were used almost entirely. In certain situations external electrodes were more convenient. The internal capillary microelectrode has many advantages over the external electrode in the measurement of conduction velocity in the heart.

Firstly, it enables one to measure the resting and action potential and to study the shape of the action potential of the cells and thus assess the condition of the tissues.

Secondly, it gives an indication of the cell type in the tissue being investigated. As shown in Part I, the shape of the action potential in the atrial, ventricular and Purkinje tissue is characteristic in each tissue.

Thirdly, the shape of the action potential recorded is simple. Fig. 27 shows an intracellular record of an action potential on a fast sweep. The zero potential line passes through the middle of the 1000 cycle sine waves. The resting potential can therefore be measured on the same record as the one on which the conduction times are being measured. The consistent shape and the abrupt upstroke of the action potential makes the measurement of the conduction time relatively easy. In contrast, records obtained by external electrodes

A



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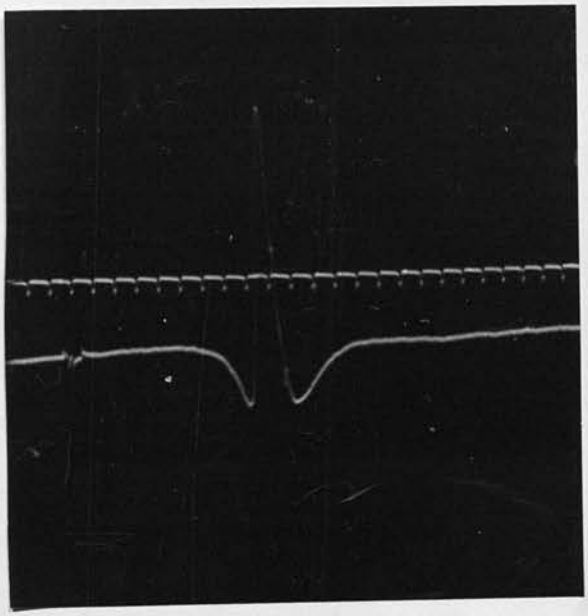


Fig. 28

A. Externally recorded action potential from dog false tendon (bipolar recording).

B. Externally recorded action potential from a goat papillary muscle (unipolar recording).

are complex. Fig. 28A shows the complex action potential from a false tendon recorded by bipolar external electrodes. In Fig. 28B is shown the action potential from a papillary muscle recorded externally by the unipolar method. The action potential in this case is relatively simpler. But unfortunately such good and simple records of action potentials are not easy to obtain even with the unipolar method of recording. The form of the action potential also usually changes as the electrode is shifted from point to point, presumably due to the variability in the underlying muscular architecture, and this makes it difficult to decide which point to select on the action potential wave to measure the conduction time.

Finally, microelectrodes are much smaller than the external electrodes commonly used with resistance-capacity coupled amplifiers, and thus many records can still be obtained from small pieces of tissue.

External electrodes, however, have their use in certain situations. In some false tendons, it is extremely difficult to impale the cells especially along the projected line of measurement because of the thick connective tissue sheath. In such situations, external electrodes can be used. One other possible advantage which records obtained with the external electrodes have is that they give the velocity of the fastest fibres in the cell population being studied.

That the conduction velocity results obtained by measurements of externally recorded action potentials under suitable conditions, do not differ from those obtained using microelectrodes is shown in Fig. 29. The regression line drawn

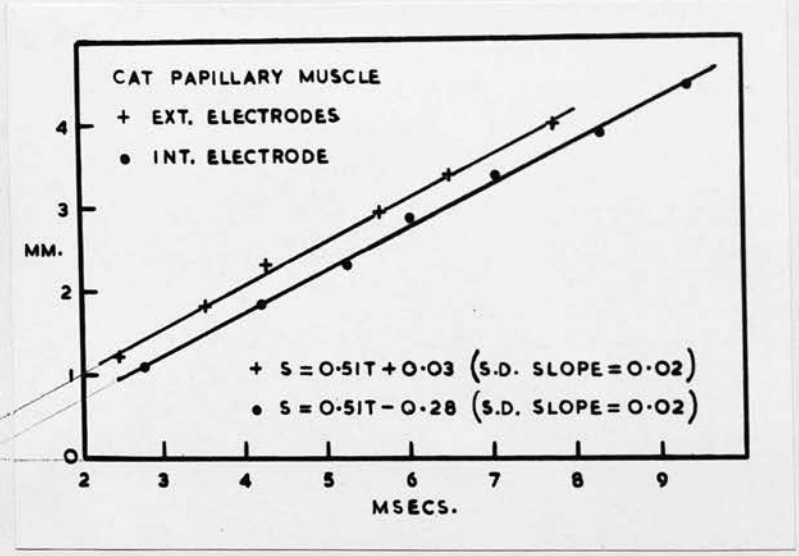


Fig. 29

Graph of the conduction distance against time in a cat papillary muscle, recorded by external as well as internal electrodes. Conduction velocity in both instances = 0.51 m/sec. (S.D. ± .02)

through the crosses gives the result obtained in a cat papillary muscle using external electrodes and the line through the dots that obtained by using a microelectrode. It is seen that both lines have the same slope of 0.51 with a S.D. of 0.02. The line through the crosses is shifted towards the left because in measuring the conduction time in the external electrode records, the point selected on the upstroke of the action potential does not necessarily correspond to the phase of excitation under the electrodes measured in the internal electrode records.

RECORDING EQUIPMENT

This has been described in Part I, p. 23.

MEASUREMENT OF RECORDS

The action potentials displayed on the cathode ray screen were photographed on 35 mm film (reduction 4:1). The photographic records were enlarged about 7 times and the conduction times were measured on the enlarged tracings.

The conduction times were measured on intracellular records from the beginning of the stimulus artifact to either of the following two points on the upstroke of the action potential: (1) the point at the foot of the action potential where it rises rapidly and the tracing suddenly goes faint; (2) a point midway between the base and the crest of the action potential. Either of these points was taken consistently in a series of records for the same measurement.

In externally recorded action potentials, the conduction time was measured from the stimulus artifact to an arbitrary point, selected consistently, on the first upstroke of the action potential.

The conduction times were measured to the nearest 0.05 msec.

TIME

Time measurements were obtained from an "Airmec" signal generator type 702, or from a crystal controlled oscillator.

CONSIDERATIONS ON THE ACCURACY OF THE METHOD

In general the tissues themselves are so variable in structure from part to part in the same heart or even from the same part in different hearts that it is difficult to know, even if the spread of excitation is measured with a high degree of accuracy, how relevant the result is to the structure under examination.

A factor which will influence all results is the temperature at which the experiments were performed. The Q_{10} for conduction velocity in these tissues lies between 1.5 and 2 (see p.100) and so for every 1 °C variation, the result may change by as much as 5%. However in these experiments, a thermometer (reading in degrees Farenheit) was kept permanently in the bath and the temperature was noted after the action potential was recorded for each point. Although the temperature of the solution might have been increasing or decreasing regularly during the course of recording from a series of points, the variation in temperature between each recording and from the beginning to the end of a series of recordings was never more than $\pm$ 1 °F (0.56 °C). The greatest variation to be expected from this factor would be 3%. However in most experiments the temperature variation was less than 0.5 °F.

Another factor which needs to be discussed in connection with the relevance of the results to the heart in vivo is the constitution of the bathing solution. Electrolytes in the

solution, especially the sodium ions are known to affect the conduction rate in nerves (Katz, 1947; Hodgkin & Katz, 1949). The millimolar concentration of sodium in the Tyrode solution used in the present experiments and the concentration of sodium estimated in the dog, rabbit and rat plasma (Table I) do not differ much. It is unlikely, therefore, that this factor is of importance in comparing the conduction rates found in the present study to those occurring naturally in the body in these animals.

The sodium concentration in cat plasma obtained by Fenn, Cobb, Mannery & Bloor (1938) (Table I) appear to be appreciably higher than the sodium concentration in Tyrode solution. The value given by Albritton (1952) however is much lower and is within the range found in other animals and that present in Tyrode solution. Since the magnitudes of the total action potential in the cat and in the other animals are comparable, it would be expected that the internal sodium concentration in cat heart fibres would be higher than in other animals if the value for sodium concentration in the plasma obtained by Fenn et al (1938) is found to be actually present in the cat blood.

In the present state of knowledge concerning the relation between electrolyte concentration and happenings at excitable membranes it seems unlikely that the results obtained in Tyrode solution will differ very much from those that would be obtained if the serum of the animal in question were used. The only experiment actually comparing serum and Tyrode solution was done by Draper & Weidmann (1951) in the dog heart. Here it was shown that the magnitude of the

resting and action potentials obtained in Tyrode solution are comparable to those obtained in plasma. Whatever may be the influence of a variation in effective serum concentration of ions the fact that all the present observations have been made under the same conditions should enable comparisons to be made between the properties of different parts of the same heart and between comparable parts of the heart in different animals.

Finally the pH of the solution has been claimed to affect the conduction rate in the heart (Andrus & Carter, 1924; Drury & Andrus, 1924; Wiggers & Banus, 1926). The pH of the solution in the bath varied between 7.3 and 7.4, never falling below 7.3 (Fig. 3). It is not likely that the conduction velocity measurements were affected by this factor.

Distances were measured with a dial guage to the nearest 0.01 mm. The average length of tissue examined was 10 mm.

Conduction times were measured on the enlarged tracings (x 7) to the nearest 0.5 mm. The magnification was such that 1 msec on the enlarged tracings corresponded to about 10mm.

A regression line of distance upon time was calculated from the observations. The slope of this line indicates the velocity. The standard deviation of the slope was calculated as described by Mather (1949) and this gives an indication of the reliability of the results. The various statistical factors for those results calculated have been set out in Appendix II.

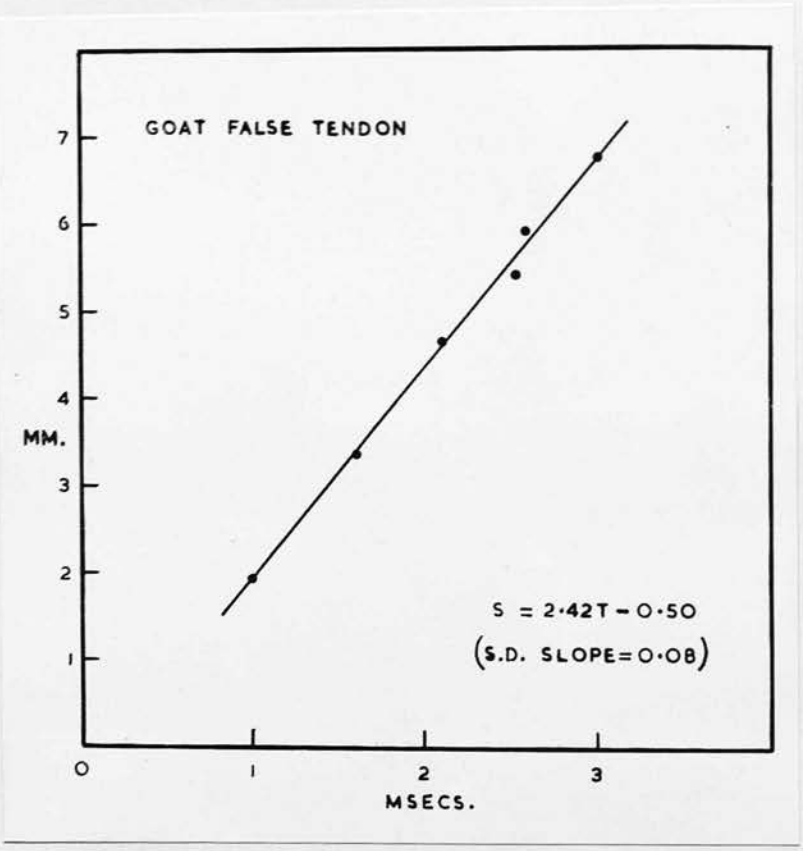


Fig. 30

Graph of the conduction distance against time in a goat false tendon.
Conduction velocity = 2.42 m/sec.

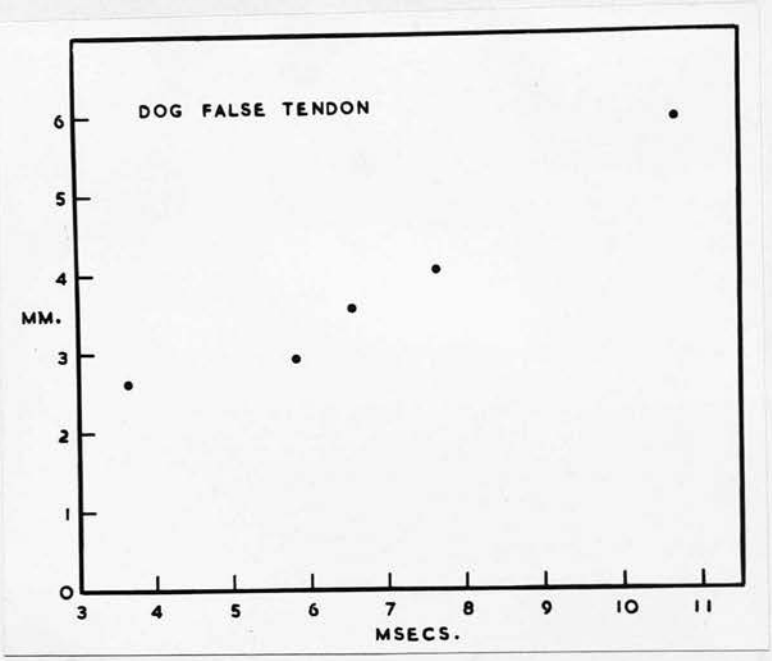


Fig. 31

Graph of conduction distance against time in a dog false tendon. A line drawn by eye to fit all the points give a slope of ca. 0.5. A line drawn through the latter 4 points only, give a better fit, and a slope of ca. 0.6.

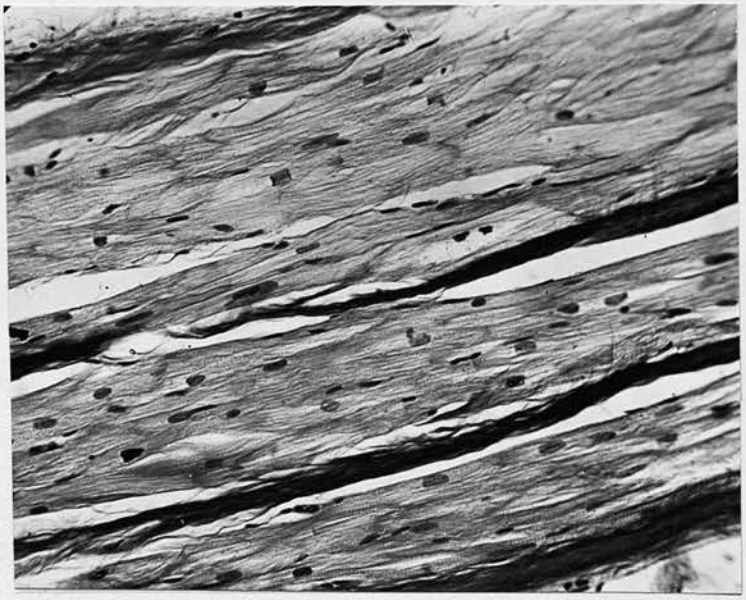


Fig. 32

Longitudinal section of the dog false tendon from which the result shown in Fig. 31 was obtained. The predominantly muscular element in the false tendon can be seen. Some typical Purkinje fibres were seen in the section just beyond the part shown in the upper right corner of the photograph. (x 260). Modified van Gieson.

RESULTS

The conduction velocity in false tendons

Conduction velocity has been measured on goat and dog false tendons. The few attempts which have been made on cat false tendons have so far been unsuccessful because those tissues did not survive for a sufficient time.

Fig. 30 shows the result of an experiment on a goat false tendon. The velocity as indicated by the slope of the regression line is 2.42 m/sec, the S.D. of the slope of the line being 0.08. In Fig. 24 is shown the histological structure of this false tendon. The typical Purkinje fibres arranged in a regular fashion are clearly seen. There were no cardiac muscle cells in this false tendon. It is therefore safe to relate the velocity of 2.42 m/sec obtained in this false tendon to the typical Purkinje fibres present in it.

In two experiments on dog false tendons, conduction velocities of 2.57 and 2.40 m/sec were obtained, the S.D. being 0.09 and 0.05 respectively. The regression lines in both these experiments gave as good a fit as the one shown in Fig. 30. The excitable cells in both these false tendons were entirely Purkinje cells. The section of the false tendon showing typical Purkinje fibres in the dog (Fig. 14) is from the false tendon on which the velocity of 2.40 m/sec was measured. The velocities of 2.57 and 2.40 m/sec can therefore be related to the typical Purkinje cells in these particular false tendons.

In two other false tendons, one from a goat and the other from a dog heart, the points did not fall on a straight

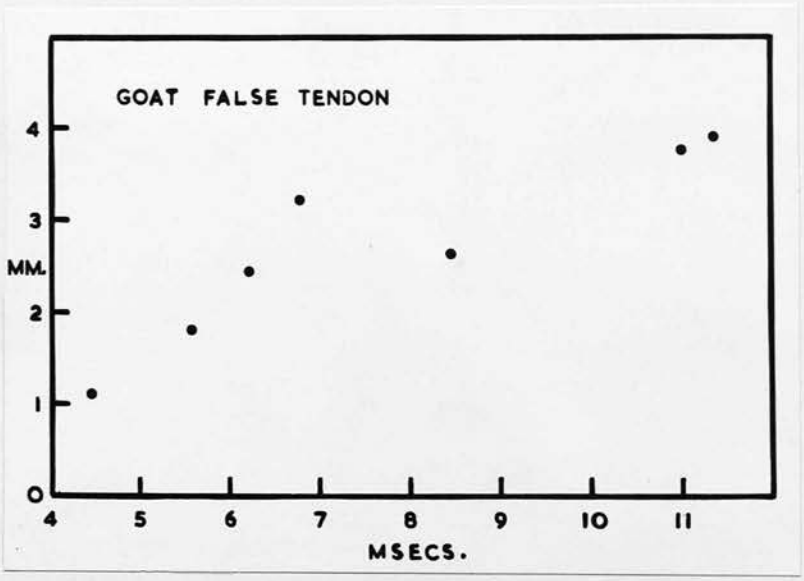


Fig. 33

Graph of conduction distance against time in a goat false tendon. A line drawn by eye through the first 4 points give a slope of 0.7. A line drawn through the 1st, 2nd, 5th 6th & 7th points give a slope of 0.4.

line. Fig. 31 shows the result obtained in the dog false tendon. Inspection of the graph shows that a line can be drawn through the latter 4 points giving a reasonably good fit. The slope of the line is ca. 0.5. A similar line can be drawn through the first four points though it does not fit as well as the line drawn through the latter 4 points. The slope of this line is ca. 0.3.

A similar scatter of the points is seen in the graph of the result obtained from the goat false tendon (Fig. 33). Here also it is possible to fit two lines to the points, one through the first four points (counting from the left) and the other through the 1st, 2nd, 5th, 6th and 7th points. The first line gives a slope of ca. 0.7 and the second a slope of ca. 0.4. No matter how the lines are fitted, it is seen that the velocity in both these false tendons do not approach the values obtained in the false tendons previously described. Histological examination showed that in both these false tendons, the predominant cell type was ordinary cardiac muscle with relatively few strands of Purkinje fibres present (Fig. 32 and 15).

When these experiments were performed, the complexity of the problem was still not fully realised and so the action potentials were recorded only on a fast sweep for each point. The records therefore did not indicate the type of cell impaled at a particular point. From the available data, it could only be surmised that the cause of the scatter of the points in the graphs obtained from experiments on these two false tendons which had a mixed cell population, was due to the records at one or several points being from different

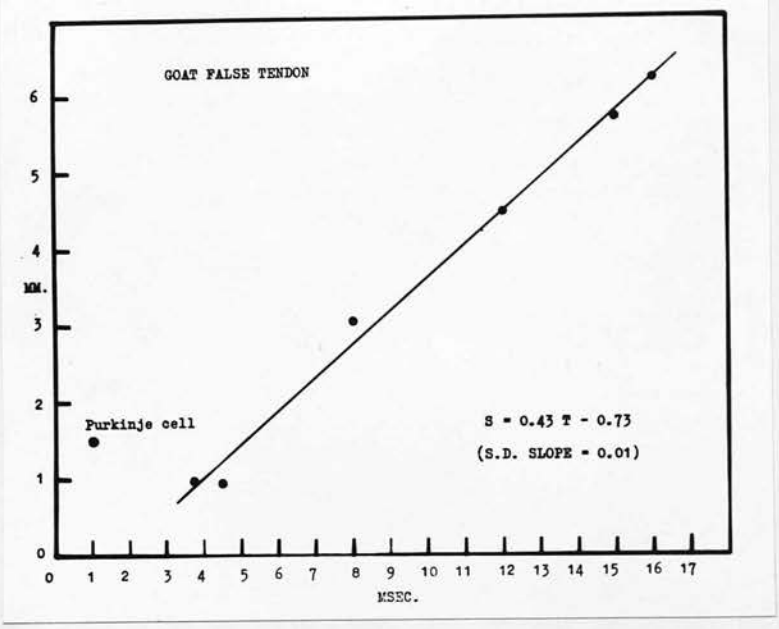


Fig. 34

Graph of conduction distance against time in a goat false tendon. The regression line is drawn through the points from which ventricular action potentials were obtained. At the point on the extreme left, a Purkinje fibre action potential was recorded. Conduction velocity = 0.43 m/sec.

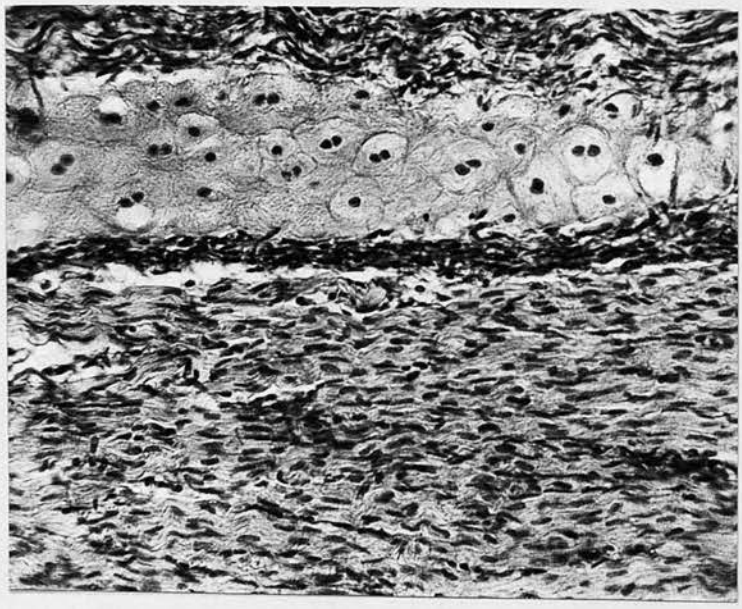


Fig. 35

Showing the presence of typical Purkinje fibres and cardiac muscle in the goat false tendon from which the result shown in Fig. 34 was obtained. (Longitudinal section; x 260; Modified van Gieson.)

cell types.

In one of the latest experiments on a goat false tendon, however, at each point impaled, the action potential was recorded first on a slow sweep to obtain the shape of the complete action potential, and then on a faster sweep to obtain a record suitable for measuring conduction time. In this way the type of cell impaled at each point was determined. Fig. 34 shows the results obtained on this false tendon. Coming in from the furthest distance, ventricular action potentials were recorded at each point, until the 5th point when a typical Purkinje fibre action potential was recorded. Ventricular action potentials were again recorded from the 6th and 7th positions. Histological examination of the false tendon revealed the presence of myocardial cells with some Purkinje fibres (Fig. 35).

It is seen from the graph that except for the point recorded from a Purkinje cell, a regression line can be drawn through all the other points giving a good fit (S.D. 0.01). The velocity of 0.43 m/sec found in these ventricular fibres is also comparable to the velocity obtained in the papillary muscles from goats (see below). If one had not recorded the complete action potentials on a slow sweep in this experiment, one would have been in the same quandaryas in the previous two experiments (Fig. 31 & 33). From the result obtained in this experiment, the results of the other two can be explained more intelligently. In Fig. 31, the first point which seemed to be displaced upwards could have been from a record of an impaled Purkinje fibre. Similarly, in Fig. 33, the 4th and possibly the 3rd points could have been

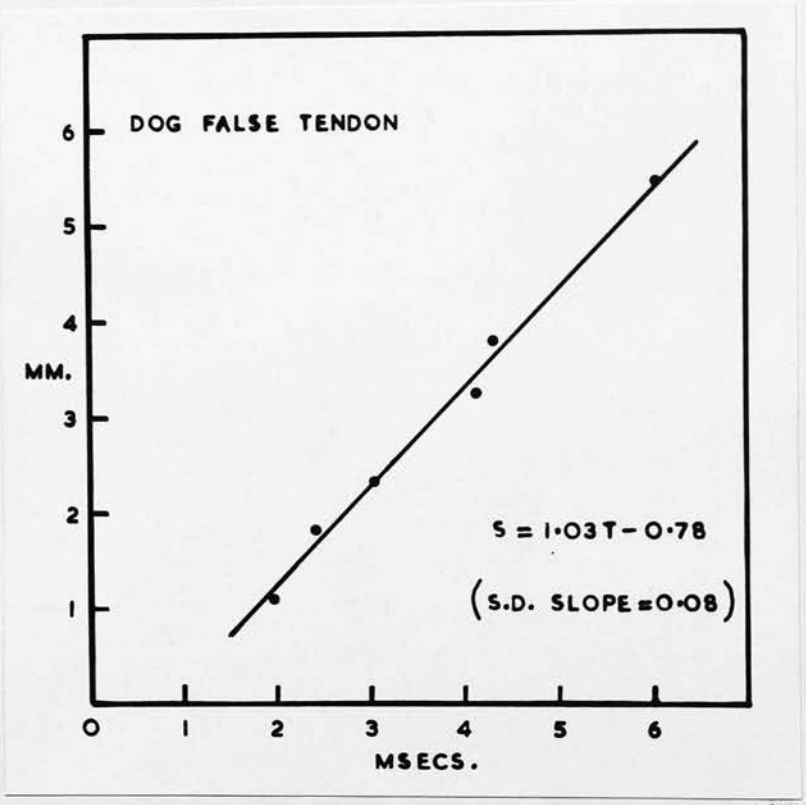


Fig. 36

Graph of conduction distance
against time in a dog false tendon.
Conduction velocity = 1.03 m/sec.

from Purkinje fibres. Neglecting these points in the respective graphs, lines fitted to the remaining points (drawn by eye) gave slopes of 0.5 in the dog false tendon and 0.4 in the goat false tendon. These values are comparable to the values found for the velocity in ventricular muscle in these animals.

On one other false tendon from a puppy, a conduction velocity of 1.03 m/sec was obtained (Fig. 36). The constituent cells in this false tendon were of a myocardial character in general and in regions resembled transitional cells. The section of the false tendon showing transitional cells (Fig. 16) is from this particular false tendon. Measurement of the diameter of the cells in this false tendon gave a mean value of 15 μ (S.D. 2.6) (Range = 10-20 μ). The diameter of these cells is therefore generally much larger than the typical ventricular fibres though much smaller than the Purkinje fibres (Appexdix I, Table i).

From the results obtained from these false tendons, it seems clear that the typical Purkinje fibres in goats and dogs conduct much faster than ordinary cardiac muscle. It would also appear that intermediate Purkinje cell types have an intermediate velocity.

Conduction velocity in ventricular muscle

Consistent results have been obtained from cat papillary muscles. Fig. 37 shows the result from one. Action potentials from 9 different distances were recorded from this papillary muscle and it can be seen that the regression line fits the points very well (S.D. 0.01)., the slope of the line being 0.66. Fig. 38 shows the result from another cat

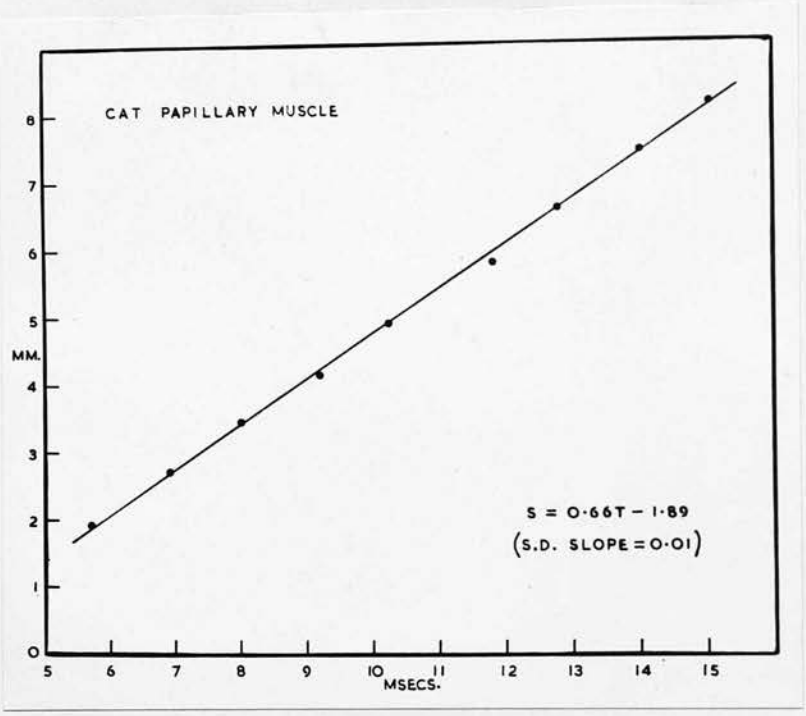


Fig. 37

Graph of conduction distance against time in a cat papillary muscle.
Conduction velocity = 0.66 m/sec.

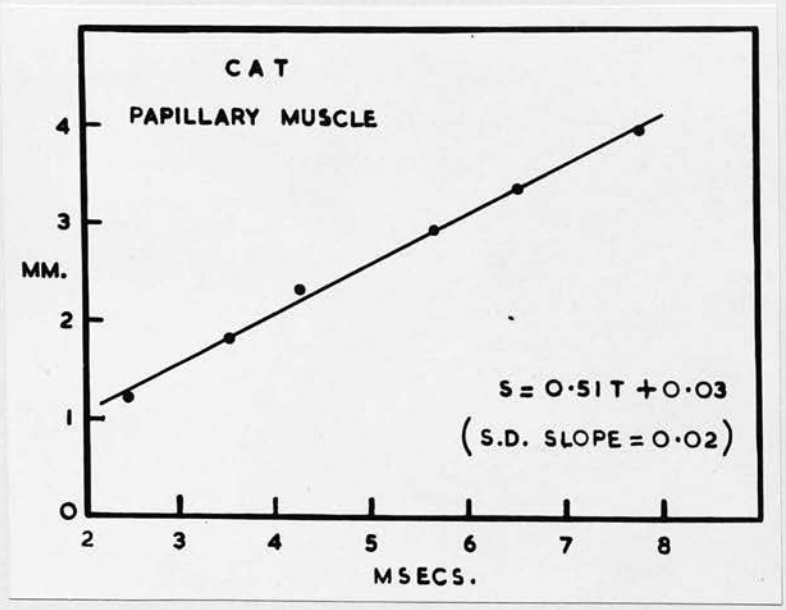


Fig. 38

Graph of conduction distance against time in a cat papillary muscle.
Conduction velocity = 0.51 m/sec.

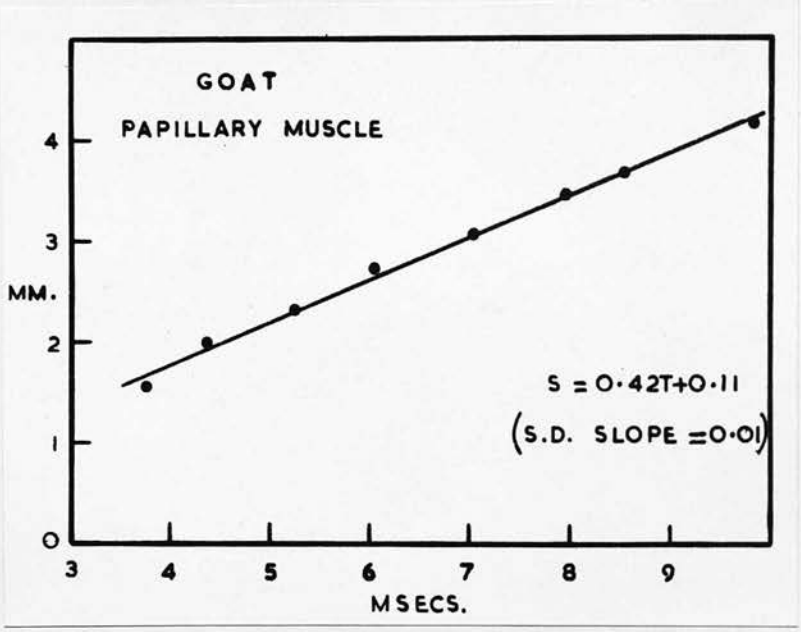


Fig. 39

Graph of conduction distance against time in a goat papillary muscle.
Conduction velocity = 0.42 m/sec.

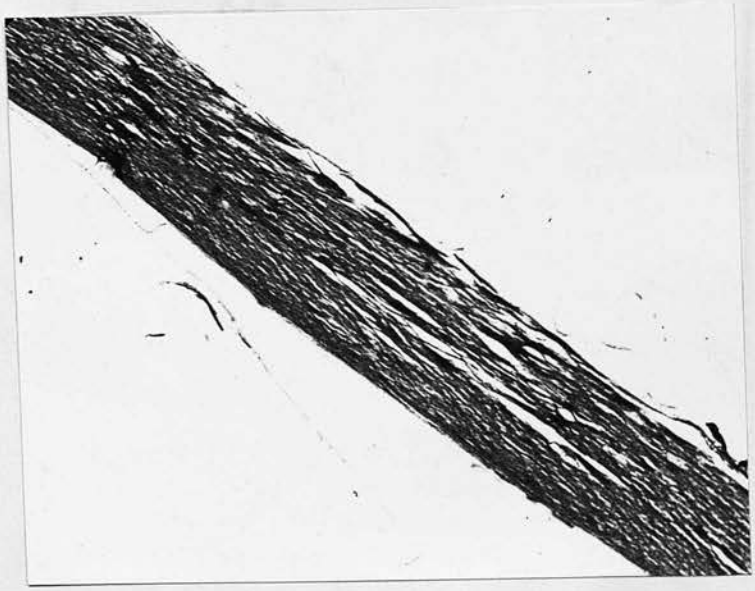


Fig. 40

Longitudinal section of the goat papillary muscle from which the result shown in Fig. 39 was obtained, showing the parallel arrangement of the muscular fibres.
x 11. (Modified van Gieson.)

papillary muscle. The good fit of the regression line can also be seen in this graph, (slope 0.51; S.D. 0.02). Three other cat papillary muscles have given similar results (Table X).

In Fig. 25 the parallel arrangement of the muscular fibres in a cat papillary muscle is shown. All the cat papillary muscles which have been used have had such a regular structure. No Purkinje fibres or transitional cells have been found in these muscle samples. The consistent result obtained from cat papillary muscles is therefore believed to be due to the regularity of its structure and the purity in its muscular cell constitution.

Results have not been as consistent in the papillary muscles from other animals. In the goat, out of 4 experiments, 3 have given good results. Fig. 39 shows the result from one of these papillary muscles. The conduction velocity in the muscle as indicated by the slope is 0.42 m/sec (S. D. 0.01). Fig. 40 shows the longitudinal section of the papillary muscle slip on which the measurement was made. The fibres are seen to be somewhat distorted because this muscle was used to study the effect of stretch on conduction velocity and it was stretched to about 60% of its original length (p. 100). However, one can still see the regular and parallel arrangement of the fibres. No Purkinje cells were found in this muscle slip.

In two other papillary muscle slips obtained from separate goat hearts, the velocities were 0.44 m/sec (S.D. 0.05) and 0.38 m/sec (S.D. 0.03). No Purkinje fibres were found in either of these tissue samples.

In the fourth goat papillary muscle, the graph of the conduction distance against time resembled that shown in Fig. 33 for the goat false tendon, except that in this case, the 2nd and 3rd points appeared to be displaced upwards, instead of the 3rd and 4th in Fig. 33. A line drawn through the first 3 points gave a steeper slope (ca. 1.2) than a line drawn through the remaining 5 points (slope ca. 0.27). Purkinje cells were found to be scattered over the surface at the base of the muscle. It is therefore thought that the displacement of the points could have been due to the impalement of Purkinje cells at those points. The shape of the action potentials recorded from the various points were not recorded and therefore this possibility could not be checked as was done in the experiment shown in Fig. 34. Some of the surface muscular fibres were also found to be running transversely and this might be the reason why a velocity lower than those in the other goat papillary muscles was obtained.

Conduction velocity was measured in 3 papillary muscles from the dog. In one, the action potentials were recorded at only 4 distances. The regression line through the 4 points gave a slope of 0.55 (S.D. 0.08). No Purkinje tissue was present in this muscle.

In one other papillary muscle, 6 points were recorded. The plot of the points showed that the 4th point, counting from the left, was displaced upwards. A regression line drawn through the remaining five points gave a good fit (S.D. 0.03). The slope of the line was 0.7. As Purkinje fibres were found in this muscle also, it is thought that

the action potential at the displaced point may have been recorded from a Purkinje cell.

In the third dog papillary muscle, 5 points were recorded. In the plot of these points, the 5th point was displaced downwards, a line drawn through the first 4 points giving a slope of ca. 1.5. As Purkinje fibres were found to be ramifying at the base of this muscle sample, it is possible that the first 4 points were recorded from Purkinje cells and the 5th point from an ordinary muscle cell.

Conduction velocity was also measured in the papillary muscles from 5 rabbits. In four of these, regression lines could be fitted to all the points recorded in each case, but they showed a wider scatter than those found in the cat. In no instance has a line been obtained with the closeness of fit found in the muscles from the cat and goat (Fig. 37, 38 & 39). No reason can be given for this finding except that the muscle fibre arrangements in the rabbit papillary muscles do not appear to be as regular as that found in the cat and goat muscles.

In the 5th rabbit papillary muscle, out of the 5 points obtained, the 2nd point was displaced upwards. A line drawn by eye through the remaining 4 points gave a slope of 0.67. As mentioned in Part II, typical Purkinje fibres are not seen in the hearts of cats and rabbits. Identification of the cells of the conducting tissue in the papillary muscles of rabbits is thus not easy. In this particular papillary muscle, cells resembling transitional cells (Fig. 21) were seen and it is thus possible that the action potential recorded at the displaced point may have been from one of them.

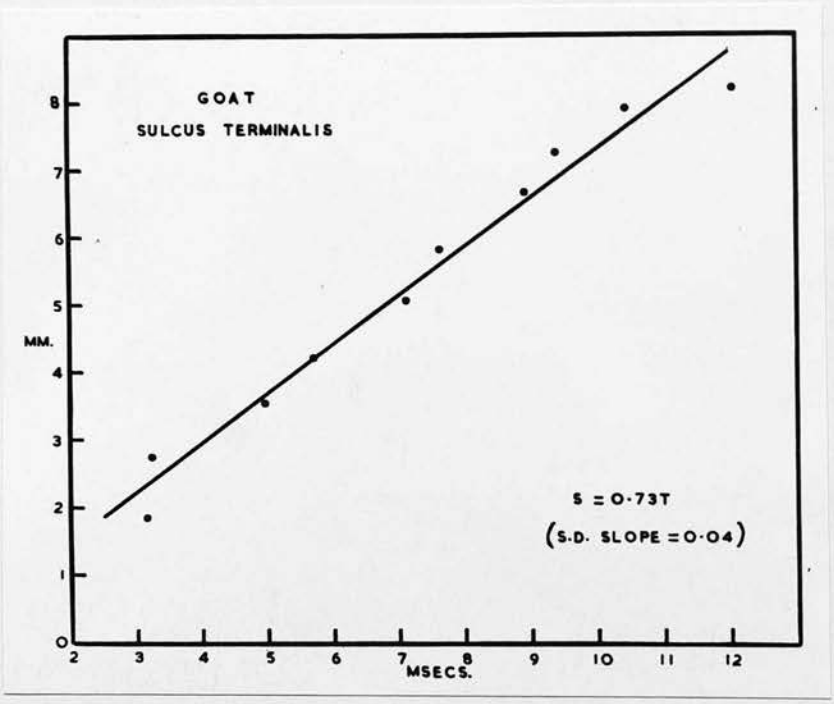


Fig. 41

Graph of conduction distance against time in a goat sulcus terminalis. Conduction velocity = 0.73 m/sec.

However little importance can be attached to this observation as nothing is known regarding the electrical behaviour of these transitional cells.

Conduction velocity in the sulcus terminalis

The conduction velocity was measured in the sulcus terminalis from 4 goat hearts, and one each from a dog, cat and rabbit heart.

The arrangement of the muscular fibres is extremely variable in these sulci and thus interpretation of the results of the conduction velocity measurements on them is rendered much more difficult. However, correlations between the histological structure and the conduction velocity results have been successful beyond expectation.

Fig. 41 shows the result obtained from a goat sulcus terminalis. The velocity in this strip, as indicated by the slope is 0.73 m/sec (S.D. 0.04). Taking into consideration the structural complexity of these tissues, the result is surprisingly good. Histological examination of this sulcus terminalis showed the presence of a stretch of fibres running longitudinally for more than 1 cm from the position where the stimulating electrodes were placed.

Fig. 42 shows the result obtained in another goat sulcus terminalis which was over 2 cm. long. Action potentials were recorded from 18 points on this specimen. As the graph shows, the first 8 points, up to a distance of about 10 mm from the cathode can be fitted with one regression line having a slope of 0.44 (S.D. 0.02), and the latter 10 points with another line having a different slope of 0.6 (S.D. 0.01). On the other hand, just one regression line can be fitted to

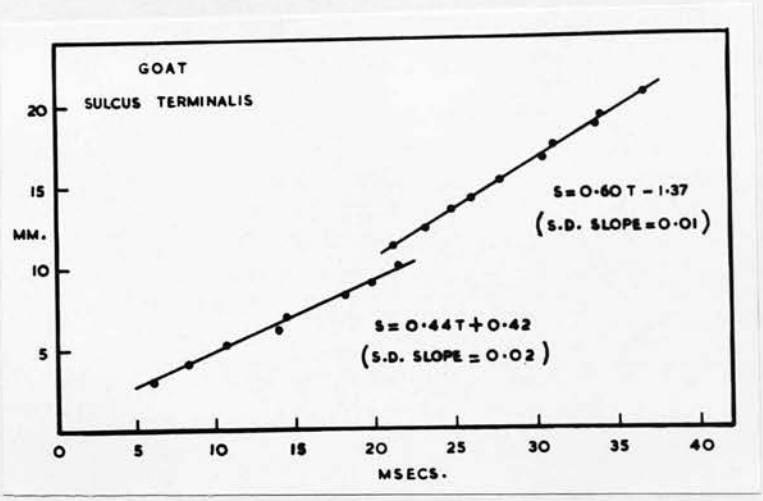


Fig. 42

Graph of conduction distance against time in a goat sulcus terminalis. Conduction velocity in the first half of the sulcus = 0.44 m/sec; in the latter half the conduction velocity = 0.660 m/sec.

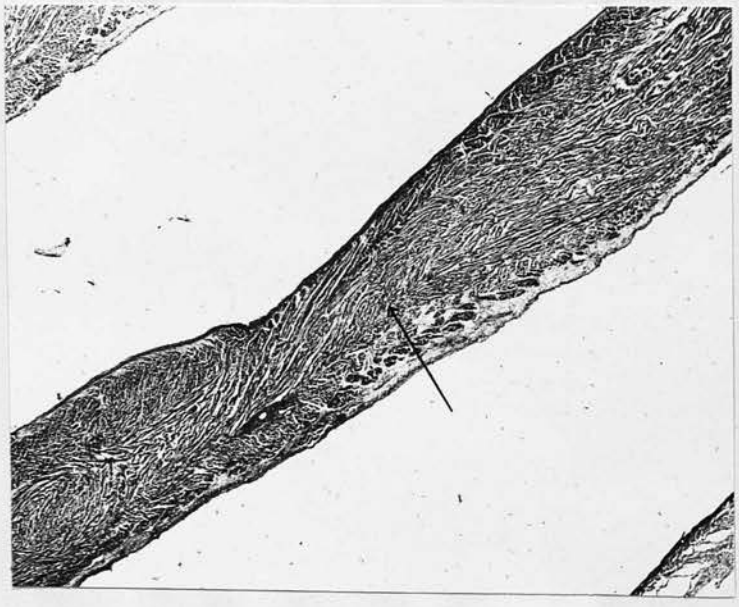


Fig. 43

Longitudinal section of the goat terminalis from which the result shown in Fig. 42 was obtained, showing the arrangement of the muscular fibres. The distance from the cathode which was just beyond the left lower edge of the photograph, to the point indicated by the arrow is ca. 10 mm. x 11. (Modified van Gieson)

all the 18 points, the slope in this case being 0.5 showing a greater S.D. of 0.06. Out of the two possibilities, better correlation with the structure can be made if the points are plotted along 2 different slopes as shown in the graph. Fig. 43 shows the microscopic structure of the sulcus. The position of the stimulating electrodes was just beyond the left lower edge of the photograph. One can make out the fibres nearer the electrodes sweeping out towards the periphery and at the point indicated by the arrow, the direction of the fibres from another group of fibres is longitudinal. This point of transition measured from the position os the cathode was found to be approximately 10 mm, i.e. about the point where the slope changes. The structural arrangement of the fibres can therefore explain why 2 slopes are obtained. The slope of the line through the first 8 points which indicates a slower velocity than the second slope can be accounted for by the slower spread of excitation in a direction oblique to the general fibre direction in the syncytium. The faster velocity found in the latter half of the sulcus is most certainly due to the measurement of impulses conducted along the main fibre direction.

In another sulcus terminalis shown in Fig. 44, the direction of the surface fibres can be seen easily even with the naked eye. The fibres nearer the stimulating electrodes (shown as large circles in the photograph), are seen running in a longitudinal direction whilst about the middle of the sulcus, the fibres can be seen running transversely across. Conduction velocity was measured deliberately across the length of the sulcus to study the effect of a change in the

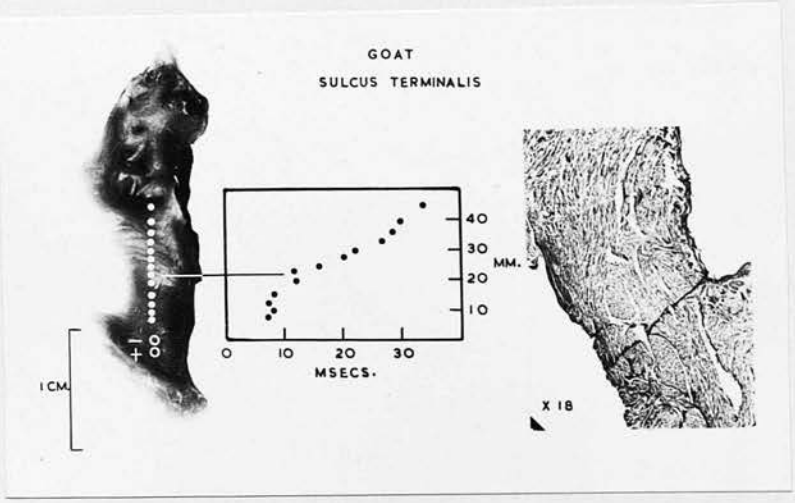


Fig. 44

Graph of conduction distance against time in a goat sulcus terminalis, correlated with the direction of the surface fibres. See text for discussion.

fibre direction on the conduction velocity measurement.

In the photograph, the position of the stimulating electrodes on the sulcus terminalis is indicated by the large white circles, the cathode being towards the recording electrode. The position of the points from which the action potentials were recorded are indicated by the white dots.

In the graph, the distance in mm. has been drawn on the ordinate on the same scale as the magnification of the photograph on the left, and the two have been aligned so that each point on the graph correspond to the white dot in line with it on the photograph. The conduction time, as usual, is plotted on the abscissa, on a suitable scale. In aligning the graph with the photograph of the sulcus, the distances were multiplied by 4, which is the magnification of the photograph of the sulcus terminalis. Thus the scale of the ordinate on the graph is 4 times the actual length on the tissue.

It is seen from the graph that there is a change in the slope at about 20 mm (i.e. 5 mm on the actual tissue), from the cathode. This point where the slope changes corresponds to the place where the fibres abruptly change direction. A horizontal line has been drawn between these two places to bring out this point more clearly. The change in fibre direction can be seen more clearly in the photomicrograph on the right. The slide had been turned over during photography and it is therefore a mirror image of the photograph shown on the left.

The first six points on the graph have a slope of ca. 0.6 and the other 7 points a slope of ca. 0.3. This finding

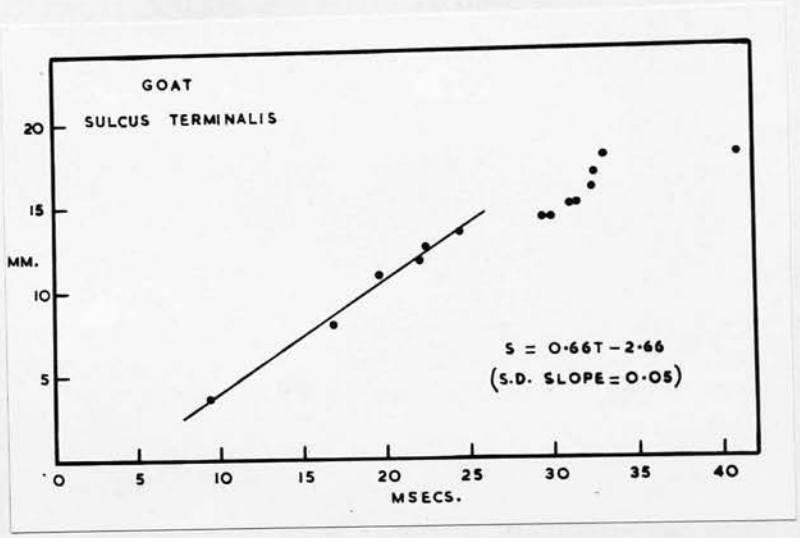


Fig. 45

Graph of conduction distance against time in a goat sulcus.
Conduction velocity = 0.66 m/sec.
See text for discussion.

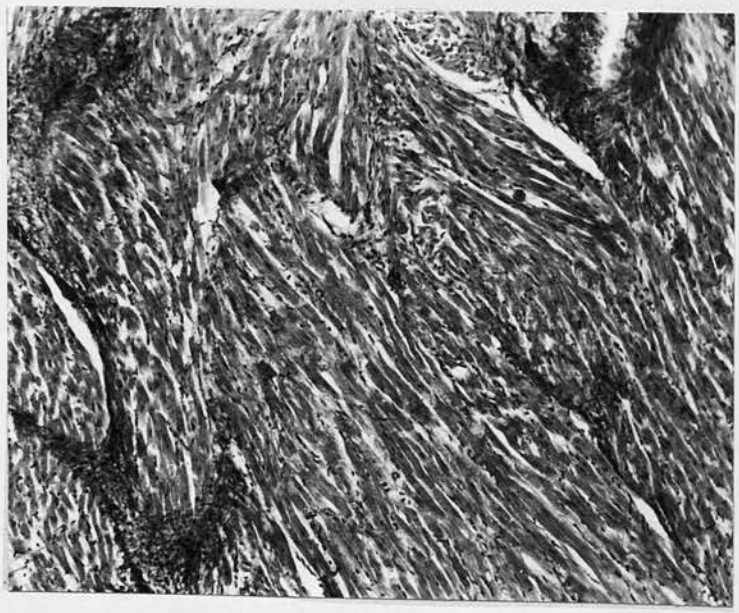


Fig. 46

Goat sulcus terminalis from which the result shown in Fig. 45 was obtained. The fasciculus on which the first six points were recorded can be seen in the right lower quadrant of the photograph. The abrupt break in the regular arrangement of this fasciculus can be seen as it is followed up.
x 100. (Modified van Gieson.)

can be explained from the structural arrangement of the fibres in the sulcus. The first six points were recorded from the longitudinal bundle which could be seen quite clearly through the endocardium. The latter 7 points were recorded from the fibres running obliquely; hence the slower velocity in these fibres.

The result of the measurements on the 4th goat sulcus terminalis is shown in the graph in Fig. 45. A regression line can be drawn through the first six points giving a fairly good fit (slope = 0.66; S.D. 0.05). But at a distance of about 15 mm from the cathode, the points fall away from the line, and irregular and varying conduction times are obtained for each point (see below). Fig. 46 shows the disposition of the fibres in the sulcus at the point where these irregularities begin. The position of the stimulating electrodes was somewhere beyond the part shown in the lower right corner of the photograph. The fasciculus along which the first six points were recorded can be seen in the right lower quadrant of the photograph. This bundle suddenly comes to an end, after which point, the fibres are seen to run in different directions. The distance from the cathode to this point of transition was found to be ca. 15 mm. The peculiar features of this graph can therefore be correlated with the histological findings.

To eliminate any bias, the correlation between the general direction of the fibres and the change in slope was checked independently by two observers in all these 4 sulci. In two of them (Fig. 42 and 45), the distance between the position of the stimulating electrodes and the change in

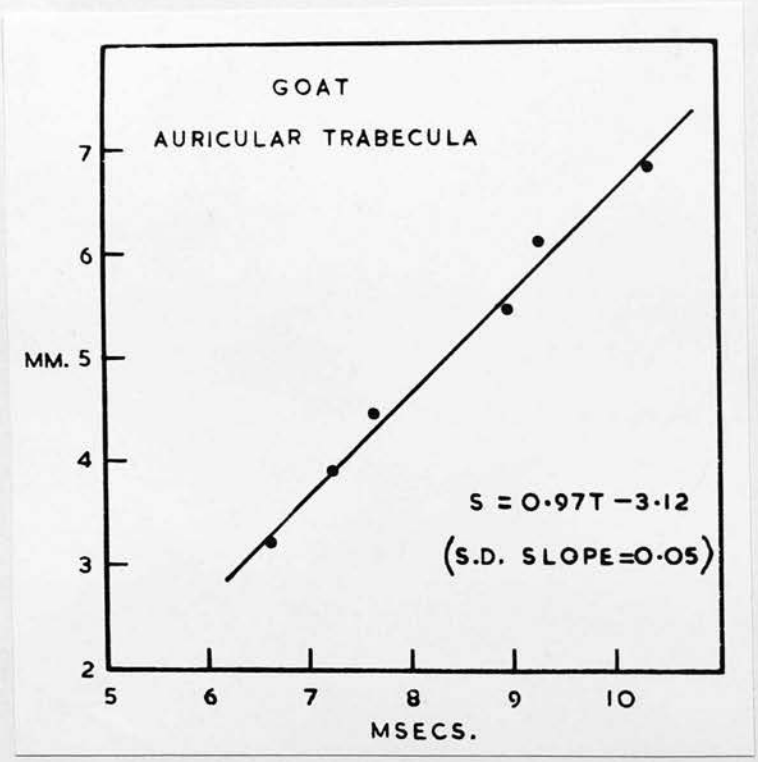


Fig. 47

Graph of conduction distance against time in a goat auricular trabecula. Conduction velocity = 0.97 m/sec.

structure was measured by one observer without having previously seen the graph. The distances measured were later found to tally with the distance from the position of the cathode to the point where the slope changed in the graph.

In the dog and rabbit sulci, the slopes were 0.51 (S.D. 0.06) and 0.51 (S.D. 0.05) respectively. The scatter of the points in both the graphs resembles that for the goat sulcus terminalis shown in Fig. 41.

The graph shown in Fig. 53A shows the result of the measurement in a cat sulcus terminalis. The points appear to scatter rather widely about the regression line as drawn. Closer inspection reveals that the points may be fitted with 2 lines, one drawn through the first 3 points and the other through the latter 4 points. Both these lines have approximately the same slope as the line drawn. A suggested explanation for the two parallel slopes is that the action potentials were recorded from two different bundles running side by side.

Conduction velocity in the auricular trabeculae

Measurements have been made on five auricular trabeculae - two from goats, two from dogs and one from a cat. The arrangement of the muscular fibres in these tissue samples has not been very regular. Consequently the points show a wider scatter than the results from papillary muscles.

Fig. 47 shows the result obtained from a goat trabecula. The conduction velocity is 0.97 m/sec (S.D. 0.05). In another auricular trabecula from a goat, a velocity of 0.84 m/sec (S.D. 0.03) was found.

In the dog auricular trabeculae, values of 0.41 (S.D.

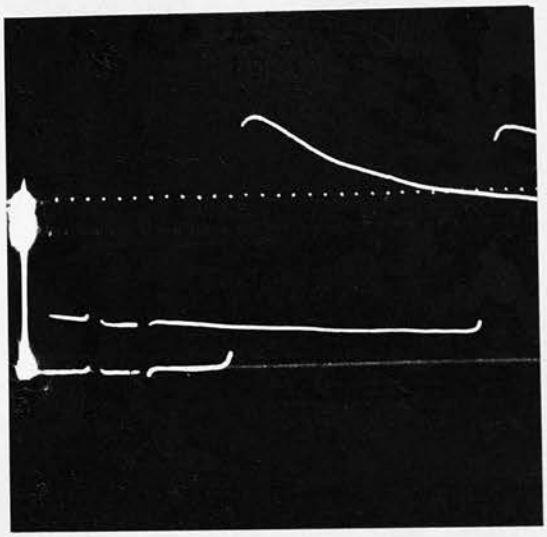


Fig. 48

Two action potentials from a single impalement in a rabbit sulcus terminalis, showing varying conduction times. The action potentials have been retouched. Time in msec.

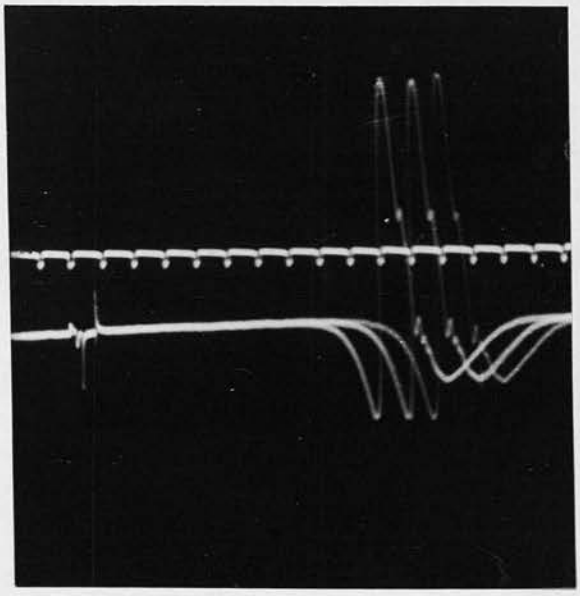


Fig. 49

Externally recorded action potentials from a goat auricular trabecula, to show the shortening of the conduction time on increasing the strength of the stimulus. Time in msec.

were obtained.
0.03) and 0.4 m/sec (S.D. 0.09)/ The velocity in the cat auricular trabecula was 0.58 m/sec (S.D. 0.03).

The results of all the conduction velocity measurements are summarised in Table X.

Varying conduction times

On a few occasions, in the course of recording from a single cell, the conduction time has been found to vary. Fig. 48 shows such an instance, observed in a rabbit sulcus. The change in conduction time is as much as 16 msec. That this is a true variation in the conduction time and not due to a change in the time base is shown by the constant position and duration of the stimulus artifact. Another example is seen in the graph in Fig. 45, in which the furthermost point has two conduction times, with a difference of about 15 msec.

There are several possibilities to account for this observation. One is that during contraction, the microelectrode slipped into another cell to which the conduction time is different. It is thought that this is not a likely explanation because on one occasion it was observed that while a cell was partially impaled, conduction to that cell varied, alternating most regularly between two conduction times. Within the next few beats, the microelectrode slipped into the cell completely, but the alternating conduction times were still kept up at precisely the same interval as before. This observation therefore shows that varying conduction times do occur even when recording from the same single cell.

A second possiblity was suggested by the following

observation. In a goat auricular trabecula, while recording with external electrodes the conduction times were found to change with the strength of the stimulus. Fig. 49 shows 3 externally recorded action potentials from this goat auricular trabecula, at 3 different strengths of stimuli, recorded on the same frame. The action potential furthest away from the stimulus artifact was produced when the strength of the stimulus was just above threshold. The conduction time became shorter as the strength of the stimulus increased. This finding is not surprising because increasing the strength of the stimulus would involve greater current spread from the electrodes and this could shorten the actual conduction distance by involvement of a new set of fibres with a more direct access to the microelectrode. In the present experiments, the strength of the stimulus used to stimulate the tissues was usually set at about 4 times the threshold strength, and it is therefore not likely that changes in the strength of the stimulus could have caused the changes in conduction times occasionally observed during a single impalement.

The third possibility which appears to be the most likely is that in some tissues, the impulse takes a slightly different pathway with each beat thus showing variations in the conduction time. This is corroborated by the observation that such changes in the conduction time, have been observed in the sulcus terminalis and in the auricular trabeculae where the direction of the fibres is irregular, and this would encourage the impulse to take different routes. This change in conduction time has been observed on one occasion in a

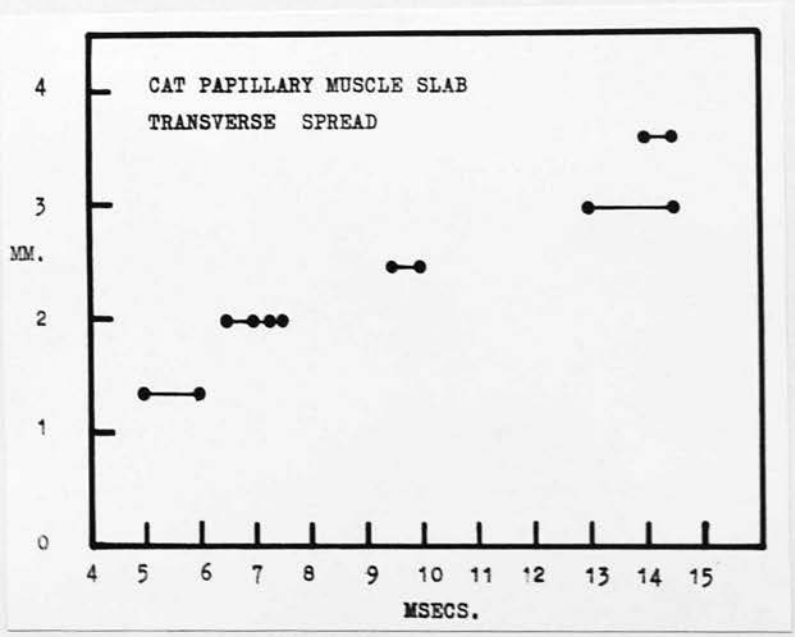


Fig. 50

Graph of conduction distance against time in a cat papillary muscle slab.
Measured in a transverse direction.
Rate of transverse spread = ca. 0.22 m/sec.

rabbit papillary muscle.

Spread of the impulse across the direction of the fibres

The velocity at which the impulse spreads transverse to the direction of the fibres was measured deliberately in 2 flat papillary muscles, one from a goat and the other from a cat. Fig. 50 shows the graph of conduction distance against time in the cat papillary muscle slab, the velocity being measured transverse to the direction of the fibres. As seen in the graph, for every conduction distance there are at least two conduction times. This finding is not unexpected as the pathway taken by the excitation wave across the fibres would be very irregular and a different pathway may be taken each time. This observations supports the suggestion made in the last section that the varying conduction times found in single impalements is due to the impulse travelling in different pathways for each beat.

The graph also shows the wide scatter of the points, also not unexpected. The velocity of transverse spread as indicated by the slope of a line drawn by eye through the points is ca. 0.22 m/sec.

In another papillary muscle slab from the goat heart, in which the transverse velocity was measured an approximate velocity of 0.2 m/sec was obtained.

The finding in these deliberate measurements of the transverse spread, that the transverse velocity is approximately half the longitudinal velocity lends weight to the correlations between the direction of the fibres and the change in the velocities, which have been previously made in the goat sulci.

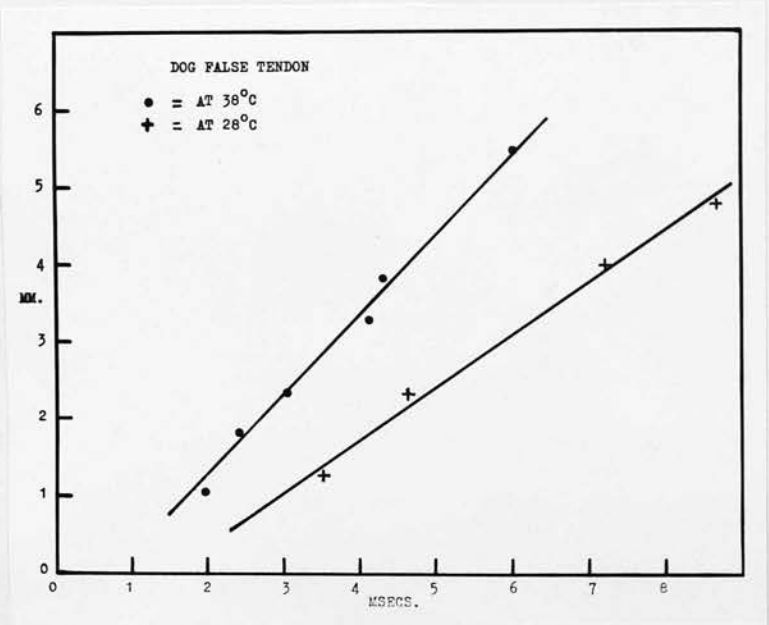


Fig. 51

Graph of conduction distance against time in a dog false tendon at 38°C and 28°C. The graph at 38°C is the same one as that shown in Fig. 36.
Slope of line at 38°C = 1.03 ± (.08)
Slope of line at 28°C = 0.67 ± (.03)

Q_{10} for conduction velocity

The Q_{10} for conduction velocity was measured in a few tissues. Fig. 51 shows the results of the measurement of conduction velocity at 38°C and 28°C in a dog false tendon. The false tendon was the one in which transitional cells were found. The velocity in this tissue at 38°C is 1.03 m/sec. At 28°C the velocity is 0.67 m/sec, giving a Q_{10} of 1.5.

Q_{10}s determined in a cat papillary muscle and a rabbit papillary muscle gave values of 2.0 and 1.5 respectively.

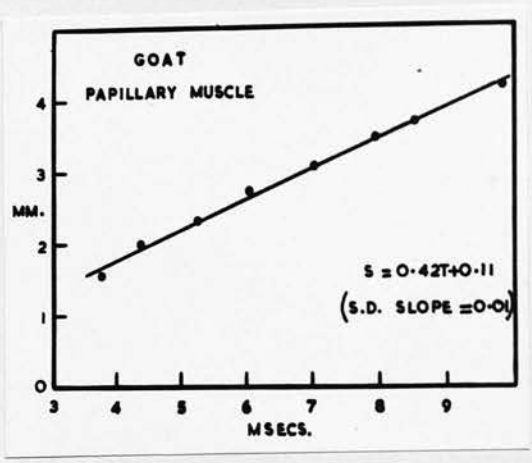
Effect of stretching the muscle on the conduction velocity

A single experiment was performed in which the effect of stretch on the conduction velocity was studied, to see whether the method of measuring conduction velocity which has been developed was suitable for studying the effect of various experimental procedures on the conduction velocity.

Fig. 52 shows the graphs of conduction distance against time in a goat papillary muscle at different degrees of stretching. The papillary muscle used for this purpose is shown in Fig. 40. As seen in the graphs in Fig. 51, the lines through the points recorded in each of the 4 series of measurements have given a good fit. This could be expected from the regular arrangement of the fibres of the muscle seen in Fig. 40.

The velocity in the unstretched muscle is 0.42 m/sec (Fig. 52A). On stretching it to 10% of its original unstretched length, the conduction velocity changed to 0.5 m/sec. On stretching to 41% and 61% of its original length, the velocities are 0.46 and 0.44 m/sec respectively.

A



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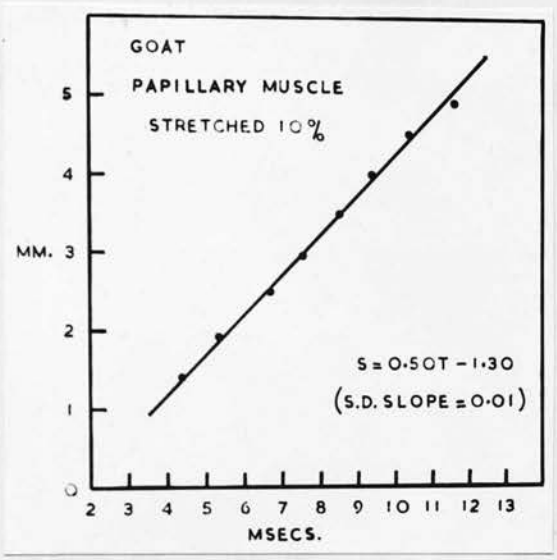

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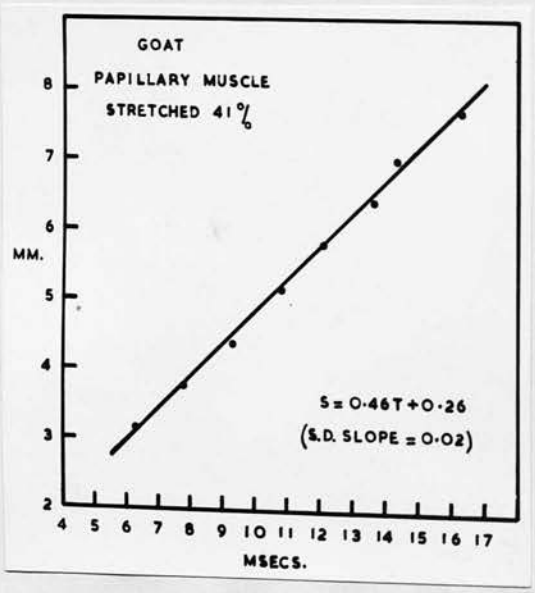

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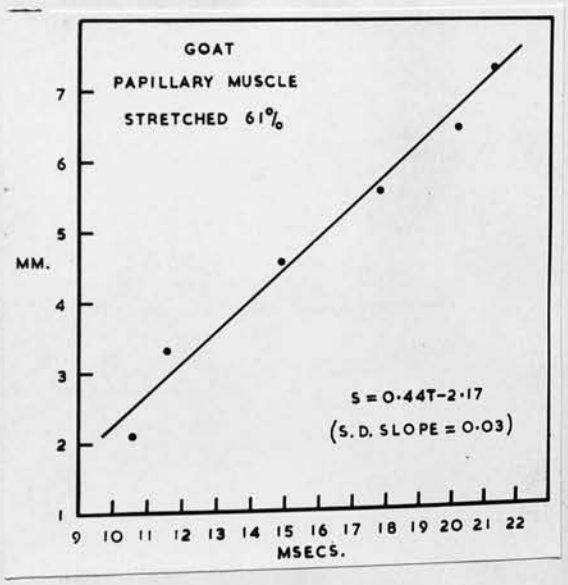


Fig. 52

Graphs of conduction distance against time in a goat papillary muscle, showing the effect of stretching the muscle on conduction velocity.

A. Unstretched. Conduction velocity = 0.42 m/sec.
B. Stretched to 10% of its unstretched length. Conduction velocity = 0.50 m/sec.
C. Stretched to 41% of its unstretched length. Conduction velocity = 0.46 m/sec.
D. Stretched to 61% of its unstretched length. Conduction velocity = 0.44 m/sec.

What the results mean is difficult to be certain from this one series of experiments. The slight increase in velocity at 10% stretch might have been due to the kinks in the muscle tissue being flattened out when it was stretched. Why the velocity is again decreased when it is further stretched is more difficult to understand. It might be that the stretching to these lengths damaged some fibres thus reducing the velocity. Many more experiments of this nature will be needed to study this interesting aspect of the subject. The results presented in this section merely indicate that by measuring the conduction velocity by the method developed and described in this thesis, changes due to the stretching can be followed with more certainty than by any other method used for measuring conduction velocity in the heart.

Effect of ACh on the conduction velocity in the sulcus terminalis

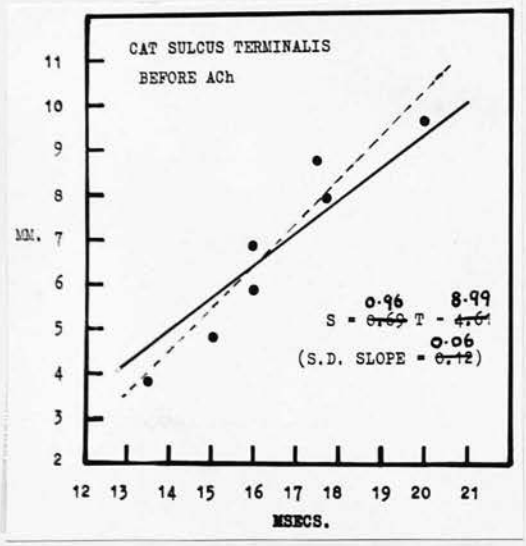
Various drugs have been claimed to alter the conduction velocity in some cardiac tissues. It was therefore of interest to test whether by using the present method the changes in the conduction velocity following drug action, could be followed with a greater degree of certainty than the other methods used. The effect of ACh on the conduction velocity in the sulcus terminalis was therefore investigated because of its physiological and pharmacological interest apart from the technical aspects.

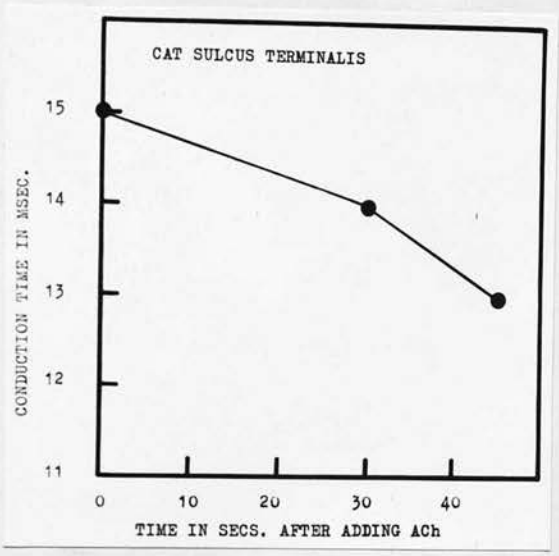
Fig. 53 shows the graphs obtained from the results of this study in a cat sulcus. The procedure was as follows: the conduction velocity was first measured before the

A

B

---- corrected regression line.





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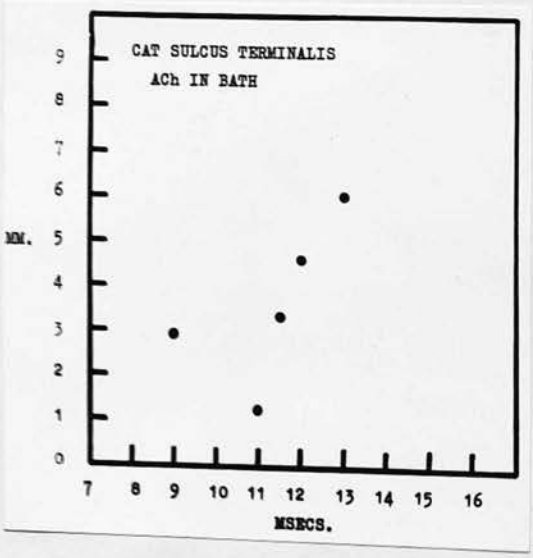

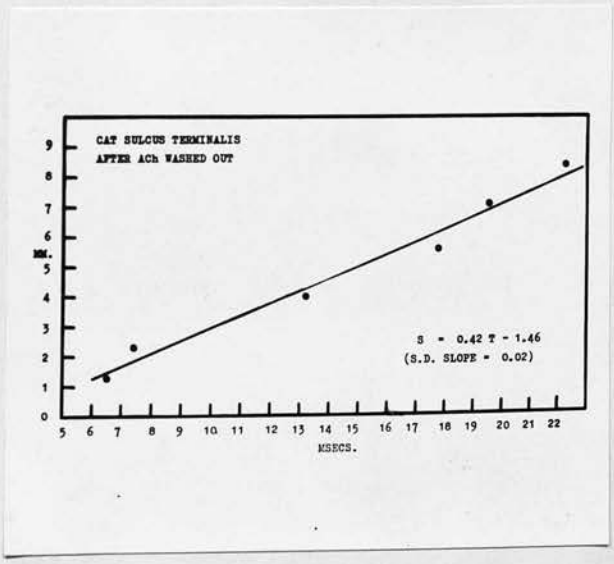

Fig. 53

Graphs showing the effect of ACh(10^{-8}) on the conduction velocity in the cat sulcus terminalis.

A. Graph of conduction distance against time. Control.
B. Graph showing the action of ACh on the conduction time of an impulse to a fixed point on the sulcus terminalis.
C. Graph of conduction distance against time during the action of ACh.
D. Graph of conduction distance against time after washing out with ACh.

addition of ACh to serve as a control. The microelectrode was then left in a cell at a known distance and the ACh added to the bath by means of a tuberculin syringe (final concentration of ACh in bath=1:10^{-8}), and the action potentials were recorded at 30 and 45 seconds after the addition of ACh. With the ACh in the bath, the action potentials were then recorded from a series of points, the total time for this manoeuvre taking 8 minutes. The ACh was then washed out, warm fresh Tyrode solution being run into the bath for 10 minutes. The conduction velocity was again measured after that period.

Fig. 53A shows the result of the measurement before ACh was added. This graph has been discussed previously on p. 95. The velocity was ~~0.69~~ 0.96 m/sec.

In Fig. 53B the change in conduction time to a single point followed for a period of 45 seconds is shown. Within a few seconds after the addition of ACh the conduction time is seen to get shorter. This shortening is more clearly seen in Fig. 54 showing the result of the action of ACh (10^{-8}) on the sulcus terminalis from another cat.

Fig. 53C shows the plots of the points recorded while the ACh was in the bath. Interpretation of this graph is difficult because all the 5 points do not fall about a line. Two possible regression lines can be fitted to these points, one passing through the 2nd, 4th and 5th points (counting from the left) and the other through the 1st, 3rd, 4th and 5th points. The slope of the first line is ca. 0.7 whilst that of the second is ca. 2.5. The first slope indicates that the conduction velocity is not changed while the second

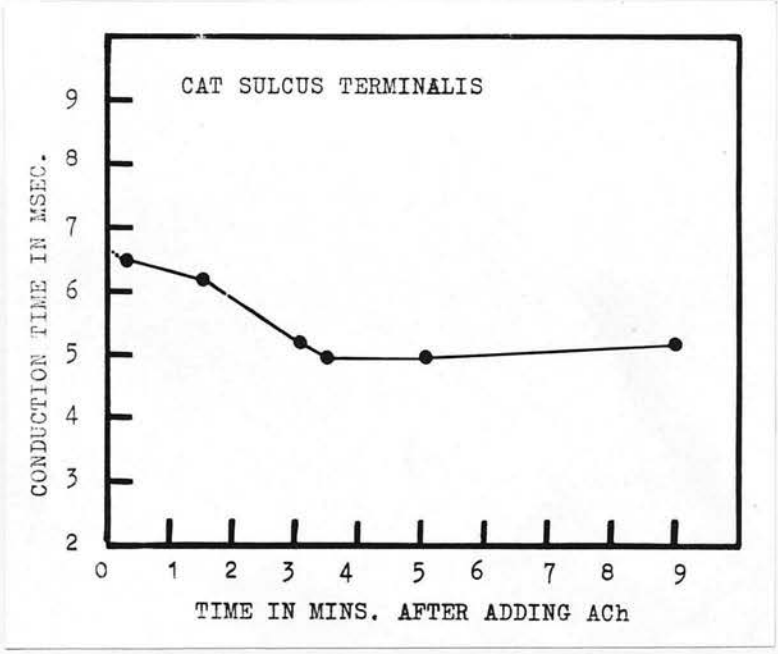

Fig. 54

Graph showing the action of ACh on the conduction time of an impulse to a fixed point on the sulcus terminalis of a cat.

indicates an increase in velocity by at least three-fold. Since it has been shown that the conduction time decreased within the first minute (Fig. 53B) it is more likely that the second slope indicates the true velocity. Since the actual recording of all the points while the ACh was in the bath took 8 minutes, the possibility that the ACh might have been destroyed during this period, thus leaving the conduction velocity unaltered, would have to be considered. This is not a likely possibility because as seen in Fig. 54, the conduction time remains short for as long as 9 minutes.

It must therefore be concluded that at this particular concentration ACh increased the conduction velocity in the cat sulcus.

The interesting finding however, was that after the ACh had been washed out, the conduction velocity slowed considerably, becoming much slower than that in the control (Fig. 53D). This observation might explain some of the conflicting results obtained by various workers.

Similar observations on the change in conduction time with ACh ($1:10^{-8}$) have been carried out on a cat papillary muscle. No change was observed.

DISCUSSION

Discussion of the Method

The important feature of this method is that the conduction velocity assigned to each tissue sample is a statistical estimate derived from a number of points. Hence all non-systematic errors should be cancelled out by the statistical treatment. A useful feature of this method is that the reliability of the conduction velocity value does not depend upon an exact knowledge of the point of origin of the stimulus, providing that this point does not change in the course of an experiment.

The results have justified the procedure of measuring the conduction velocity in selected small pieces of cardiac tissue instead of in whole hearts. With a good regular structure and a single type of excitable cell in the tissue sample, good results can and have been obtained consistently. Any departure encountered can be explained by an irregularity in either:

1) the distribution of the type of excitable cells in the tissue sample, or
2) the direction of the fibres in relation to the line of measurement.

Even in a simple conduction velocity measurement, one cannot be sure of obtaining a good result from any sample because of the variability in structural arrangement and cell composition of the tissue sample. A further difficulty is the fact that these variations are not apparent until after the conclusion of the experiment. This uncertainty of the base line measurement increases the difficulties when the

effect of drugs on the conduction velocity is being tested. One might go through the whole procedure of measuring the conduction before, during and after the action of a drug, and yet, because the interpretation of the control measurement is equivocal, the relation of the subsequent results to the action of the drug becomes difficult to interpret. This means that very many more experiments than is usually done with other methods will have to be performed before definite conclusions can be reached regarding the action of a single drug on the conduction velocity. However, much more weight can be attached to these results than those obtained by other methods.

There is no certain way of determining during the course of an experiment how the plot of the points for conduction distance against time will fall about a line. An indication can, however, be obtained by measuring the conduction times directly on the cathode-ray screen and plotting the points immediately.

The only other way of making sure that there is a reasonable chance of obtaining good results is to choose the tissue samples carefully. The selection of the tissue sample is thus of great importance in this method. Even with the best of selection, the fibre arrangement and cell constitution vary from part to part in the same and in different hearts. Subsequent histological examination of the tissue sample is thus an integral part of the method.

As Fig. 34 demonstrates, the use of intracellular microelectrodes is an advantage in the interpretation of the data. The results obtained in that experiment have been particularly

illuminating and many of the previous perplexing results have fallen into place when interpreted in the light of the results of that experiment.

One more factor which makes the method difficult, especially when the action of drugs is being tested is the time taken in which records from five or six points can be obtained is five minutes. Usually it takes about twice that time. This delay in obtaining records may be serious when the action of the drug being tested is transient.

The indirect method of measuring the conduction velocity in Purkinje tissue used by Lewis & Rothschild (1915, mentioned on p. 66) is liable to so many interpretations that the value of the results is greatly reduced. Considered in the light of the results obtained in the present study, it is very unlikely that the impulse spreads along the pathway assumed by them in their experimental situation (see p.67). The results of recent workers (e.g. Scher and his associates; Prinzmetal and his associates and others, see p. 17) who used intramural electrodes to measure the rate of spread through the ventricular wall at rights to the endocardium, would also seem to be artificial, since it is doubtful if their apparent outward spread of the impulse in the ventricular wall can be related uniquely to a true spread across the 'grain' of the muscle fibres. The more rational approach to the subject would seem to be to work out the architecture of the muscular walls and the distribution of the conduction system and from the results of accurate conduction velocity measurements in cardiac tissues, work out the probable pathway for the spread of excitation. Of course, in the final

analysis, direct measurements made on intact hearts will have to be considered, but there are still too many uncertainties in the various paramaters for the conclusions drawn from results obtained by direct measurements on whole hearts to be accepted with confidence.

Apart from its utility in the investigation of the spread of excitation in the heart, this method of measuring the conduction velocity in the heart would be of value in pharmacological experiments, as it enables one to establish a reliable base line before the action of drugs on this fundamental property of cardiac muscle is tested.

From the experience gained, certain additional precautions necessary to obtain good results by this method may be set out:

1) Selection of the tissue sample should be most rigid. If there is any suspicion that conducting tissue (in the case of cardiac muscle samples) or muscular tissue (in the case of false tendons) is present in the tissue samples, as observed macroscopically, they should be rejected.

2) The direction of the fibres in relation to the line of measurement should be carefully noted.

3) A scale drawing of the tissue sample as pinned out in the experimental bath should always be plotted, depicting the fibre directions and the exact position of the stimulating electrodes, any other unusual feature.

4) In pinning out the tissue sample, extra care should be taken to see that it is not twisted (see discussion regarding this on p. 109).

5) The action potentials recorded at the various points

TABLE X

Summary of the results of the measurements of conduction velocity in the heart.

The figures in brackets are the S.D. of the slope of the regression line. ca. in the brackets indicates that the line has been drawn by eye. The experiments giving values which are asterisked are discussed in detail in the text.

Animal	False Tendon	Papillary muscle	Sulcus Terminalis	Auricular Trabeculae
Goat	2.42 (.08) *0.43 (.01) *0.40 (ca.)	0.44 (.05) 0.42 (.01) 0.38 (.03) *(0.27 (ca.) *(1.20 (ca.)	0.44 (.02) 0.6 (.01) *(0.62 (ca.) *(0.29 (ca.) *0.66 (.05) 0.73 (.04)	0.97 (.05) 0.84 (.03)
Dog	2.40 (.05) 2.57 (.09) 1.03 (.08) *0.50 (ca.)	0.55 (.08) *1.45 (ca.) *0.7 (.03)	0.51 (.06)	0.41 (.03) 0.40 (.09)
Cat	-	0.8 (.04) 0.66 (.01) 0.62 (.04) 0.51 (.02) 0.43 (.04)	0.69 (.12)	0.58 (.03)
Rabbit	-	0.72 (.17) *0.67 (ca.) 0.48 (.04) 0.44 (.06) 0.33 (.06)	0.5 (.05)	-

should be taken on two sweeps, one on a slow sweep to record the total action potential and the other on a fast sweep suitable for the measurement of conduction time.

6) If the effect of drugs or any other experimental procedures are to be studied, the reliability of the base line of the control measurement should be checked immediately by measuring the conduction time approximately on the cathode-ray screen and plotting it on a graph against time.

Since all these precautions, together with the subsequent histological examination are necessary to obtain good results, it can be seen that this is not a simple or an easy method. However, taking everything into consideration, this seems to be the only accurate, and at the same time reliable method of measuring the conduction velocity in such a structurally complicated tissue as cardiac muscle.

Discussion of the Results

In Table X are summarised the results of all the conduction velocity measurements made in this study.

The results obtained in the goat heart show that the typical Purkinje fibres conduct at a much faster rate than the other muscular tissues in the heart. The velocity of 2.42 m/sec obtained in one false tendon agree with the result of Draper & Weidmann (1951) who found a velocity of 2.2 m/sec in goat false tendons.

The velocity in goat papillary muscle is about 0.4 m/sec. This value is in general slightly lower than the values obtained in the papillary muscles from other animals. The results obtained from the goat papillary muscles have been very good and in the graphs, the regression lines give very close

fits. It is just possible, however, that in pinning out the muscle in the experimental bath it might have become twisted. The measurement of the velocity would thus have been along obliquely running fibres, which would account for the slower velocity. It is, however, unlikely that all the muscles would have been thus twisted. The results have been consistently obtained and it is therefore believed that this value approximates the absolute velocity of longitudinal conduction in papillary muscles of goats.

Regarding the velocity in the sulcus terminalis of goats, it has already been discussed that where there are two slopes in a graph, the faster slope indicates the conduction along longitudinal fibres. Values thus obtained are: 0.73 m/sec; 0.66 m/sec; 0.62 m/sec; 0.6 m/sec. It is interesting to note that these values are fairly consistent despite their derivation.

In the two auricular trabecula from goats, higher velocities of 0.97 and 0.84 m/sec were obtained.

It is difficult to draw any definite conclusions from these few results, but it appears that conduction velocity in the hearts of goats, vary from tissue to tissue. The velocity increases in the following order: papillary muscle; sulcus terminalis; auricular trabeculae; Purkinje tissue. The Purkinje tissue conducts much faster than the other cardiac muscle tissues, 6 times faster than ventricular muscle and $1\frac{1}{2}$ times faster than auricular trabeculae. Conduction in the auricular trabeculae also appears to be definitely faster than that in papillary muscle - about twice as much. However, the difference in conduction velocity

between each of the three muscular tissues appear to be slight and it is difficult to decide from these results whether these differences are in fact real. More data is required. The differences in conduction rate between the sulcus terminalis and auricular trabecular, apparently the same kind of tissue, is also difficult to explain. An observation which might account for the difference in velocities in these tissues is that the diameter of the fibres in these tissues are slightly different (Table VI). Although there is some overlap in the range of their fibre sizes, it is seen that the bracket of the range itself increases as it passes from the ventricular, to the sulcus terminalis, to auricular trabeculae and to the Purkinje tissue, increasing in that order. This is shown up in the value of the mean diameters. However, it is uncertain whether these small differences in the fibre diameter between the cardiac muscular tissue would account for the different velocities obtained, though the large fibre sizes found in the Purkinje cells could certainly account for the high velocity of conduction found in them.

In passing, it may be mentioned that as far as is known, these values for the conduction velocity in the muscular tissues of the goat heart are the only ones so far reported. No figure has been found in the literature.

The results obtained in the dog cardiac tissues also show that conduction in the typical Purkinje fibres conduct much faster than the ordinary cardiac muscle. The velocity of 2.4 and 2.57 m/sec obtained in the present experiments are comparable to the value of 2.0 obtained by Draper & Weidmann

(1951) and Trautwein, & Gottstein & Federschmidt (1953) (2-3 m/sec).

The results obtained from the papillary muscles, sulcus terminalis and auricular trabeculae indicate that the velocities in these tissues are about the same. Nothing much can be said regarding the significance of these findings, because in addition to the paucity of the data, those obtained were not as good as the ones in the goat tissues. It is interesting to note, however, that the fibre diameters of the auricular trabeculae, sulcus terminalis and papillary muscles in this animal is of the same order (Appendix I, Table i).

The velocity of 1.03 m/sec obtained in the dog false tendon needs some comment. As transitional cells were found in this false tendon, and as the fibre diameter in these cells are, on the whole slightly larger than the muscular tissues of the heart , (15 μ S.D. 2.6), it is tempting to attribute the greater velocity to the larger fibre size in these transition cells. It seems unlikely, however, that the small increase in fibre diameter would account for the two-fold increase in velocity found in these cells as compared to those present in the tissues of the auricular trabeculae, sulcus terminalis and papillary muscles.

The velocities obtained in the cat papillary muscles have ranged from 0.43 to 0.8 m/sec. This is a difference of two-fold and it is unlikely that the actual range in these tissues is as great. It is necessary therefore to decide to which of these two limits the absolute longitudinal velocity would approach. The possibility that the faster

velocity is due to the presence of Purkinje fibres or transitional cells is ruled out because in none of these muscles were such cells found. The only other factor which could affect the conduction velocity measurements as performed in the present experiments is that of fibre direction. Any irregularity in structure would tend to decrease rather than increase the velocity. This, therefore, suggests that the velocity of 0.8 m/sec found approximates the absolute velocity rather than the velocities in the lower range. Can any explanation be sought for the finding of the slower rates? The arrangement of the muscular fibres in these tissues were regular and parallel and the regression lines fit closely. There is, therefore, no question of attributing a slower velocity to any irregularity in structure. There is, however, one other possibility, which has already been mentioned in connection with the slow velocity found in the papillary muscles of goats. That is, that during pinning out the papillary muscle in the experimental bath, the muscle might have become twisted and measurements made along the length of the muscle would actually be along obliquely running fibres.

The conduction velocity in the sulcus terminalis and auricular trabecula of cats are in range of values found in the papillary muscles. As in the dog, the diameters of the cells in the auricular trabecula, sulcus terminalis and ventricular muscle are about the same (Table VII).

The values of the conduction velocity results obtained in the rabbit papillary muscles and the sulcus terminalis are also within the range of values found in the cat heart.

Table X shows that the velocity in the muscular tissues of all the animals, with the exception of the goat auricular trabeculae, fall within the range of values found in a tissue for any single animal, for example the cat papillary muscle. It is remarkable that the fibre diameters of these tissues in the different animals are also similar. In the goat auricular trabeculae where slightly higher velocity have been observed, the range of the fibre diameters have also been observed to be slightly greater than the other cardiac muscle cells. However, as mentioned already, one cannot be certain whether this small difference in fibre diameter could account for the slightly higher velocity. It is interesting to note, however, that the fibre diameter approaches those found in the transitional cells of the dog false tendon in which a velocity of similar magnitude was obtained. Correlations between fibre diameter and conduction velocity cannot be carried safely further than this.

The danger involved in drawing any conclusions from comparisons made between the conduction velocities in the cardiac muscular tissues of the various animals and their fibre sizes is shown by a consideration of the results obtained in the Purkinje fibres of goats and dogs. In these animals, although the conduction is about the same in the Purkinje fibres, the fibre sizes are quite different.

It is hardly surprising that it is not possible in this complicated tissue to make any statement about an absolute velocity to be expected in fibres of a certain size. It is, however, important to establish that despite the complexity excitation does spread in a uniform manner in the longitudinal

direction of the fibres and it is interesting to find that the conduction rates in all myocardial tissue fall within the range 0.4 to 0.9 m/sec, despite the variations in size and species.

The results described under the sections on the effect of stretch and ACh on the conduction velocity in cardiac tissue have been presented merely to indicate the potentialities of the method. To elucidate the nature of the changes in conduction velocity due to each of these procedures would be a study in itself, which is beyond the scope of the present work.

PART IV

FINAL DISCUSSION, CONCLUSIONS AND SUMMARY

FINAL DISCUSSION, CONCLUSIONS AND SUMMARY

Hitherto the differences in the excitable tissues composing the heart have not been clearly recognised, and in the study of the heart as a pump little heed has been paid to the different properties of these tissues. The present work has established that the atrial, ventricular and Purkinje tissues have different properties, at least at the membrane level. As the animal gets smaller, the difference between these three tissues gets less prominent, until in the smallest animal from which action potentials from all three tissues have been obtained (viz. the rat), there appears to be little difference.

The rate of spread through the myocardial tissue appears to be of the same order in all animals. It certainly is in the goat, dog, cat and rabbit. If fibre size is any guide it would seem that the conduction velocity in the smaller animals would also be approximately the same. The differences observed, which become prominent as the heart increases in size are (1) the lengthening of the action potential and (2) the increasing prominence of the conducting system. Both of these changes can be related to the function of the heart as a pump.

In large hearts, the long action potential and hence the long refractory period would seem to ensure that under physiological conditions, it does not beat too rapidly as a rapid rate in a large heart would necessarily shorten its filling time and thus lessen its efficiency as a pump. The atria, having a shorter action potential than the ventricle would be more susceptible to rapid rates of stimulation but

as they do not contribute to the actual stroke volume, it probably does not matter. A further safeguard against too rapid stimulation of the ventricles by the atria is the interposition of the Purkinje fibres which have an even longer action potential than the ventricles.

The second feature which becomes noticeable as the heart increases in size is the prominence of the conducting system. A large heart with a large mass of muscular tissue would seem to require some system to activate it rapidly and systematically in order to co-ordinate its contraction and thus act efficiently as a pump. It is not surprising therefore that in continuity with the AV bundle there exists a system of large cells having a fast conduction rate, ramifying most extensively under the endocardium as well as in the myocardium itself.

It is difficult to decide whether the Purkinje fibre system developed to serve solely for rapid conduction and co-ordination of the ventricular contraction or whether it has some other function, probably of a metabolic nature and takes on the additional function of rapid conduction. Apart from the speed of conduction it certainly has one other important property, viz. that of spontaneous rhythmicity. Ventricular muscle, at least in vitro displays no spontaneous rhythmicity and any such activity can always be traced to the presence of Purkinje tissue in the sample.

If, as now appears, the ventricular muscle in large hearts is always activated by the Purkinje fibre system the problem of the spread of excitation in the ventricles becomes primarily one of an accurate knowledge of the distribu-

tion of this system.

In the ventricles of large hearts, with its extensive ramification of the Purkinje fibres, the spread of excitation will mostly be in this system and the actual pathway in myocardial tissue will be comparatively short. In small hearts, it seems doubtful whether a conducting system as such is present. This means that in these hearts, conduction will mainly be in myocardial tissue. This is supported by the fact that the duration of the QRS complex, which is regarded as the time taken for ventricular excitation is of the same order in large as well as in small hearts.

In a large heart, the maximum total excitation time of the ventricles may be estimated from the dimensions of the ventricles and the conduction rates in Purkinje tissue and ventricular muscle.

In a goat heart with a ventricular length of 5 cm, the maximum pathway taken by the impulse in the Purkinje tissue is 10 cm and with the excitation wave travelling with a velocity of 2.5 m/sec, the time taken in the Purkinje fibre network will be 40 msec. If the average rate of spread in the ventricles is taken as 0.5 m/sec, the time taken through the ventricular wall of thickness 0.8 cm will by 16 msec. Hence the total excitation time will be 56 msec. This is a generous estimate because the actual pathway in the Purkinje tissue would certainly be less than twice the ventricular length, and the rate of spread in the ventricular wall would be greater than 0.5 m/sec if the impulse travelled in the myocardial Purkinje fibres. If 50 msec is taken as an average figure for the total excitation of the

ventricles, it is seen that is is much shorter than the duration of the ventricular action potential which is about 240 msec. This means that the action potential must involve the whole of the ventricles for at least 80% of its duration. If as is believed, the T wave in the electrocardiogram represents the disappearance of electrical activity in the ventricles, the Q-T interval should approximate the duration of the action potential. That this is so has been demonstrated in the present study. The relationship is more close in large hearts where the difference between the total activation time and the total action potential duration is much greater.

From the above it can be seen that apart from the interesting aspects of the comparative study and the possible exploitation of the differences to explain the underlying mechanism of the "plateau" phase of the action potential, the present quantitative study has given a new perspective to the various problems associated with the co-ordination of the heart beat. It has not only opened a way to a better understanding of the control and co-ordination of the heart but also of its disorders for example in auricular and ventricular fibrillation. In the latter condition, the possible role of the Purkinje fibres should be considered.

SUMMARY

1. Three aspects of the spread of excitation in the heart have been studied, viz. the nature of the cardiac action potential, the velocity at which the impulse is conducted and the structural aspects.
2. With the use of a Ling-Gerard intracellular microelectrode, the action potentials from the cardiac tissues of goats, dogs, cats, rabbits, guinea-pigs, rats, a hamster and mice have been recorded and compared.
3. The magnitudes of the resting and action potentials are comparable in all these tissues.
4. The configuration of the action potentials have varied from tissue to tissue and from animal to animal. The auricular action potentials were the most consistent in shape from species to species. There was first a rapid initial phase followed by a regular but slower decline to the initial conditions. In the mouse the duration of the phase of membrane reversal is about 5 msec, the time to repolarise to 60 mV is about 25 msec and the action potential is 95% complete in about 50 msec. The corresponding figures in the goat are 95 msec, 200 msec and 250 msec respectively.
5. In the smaller animals, the ventricular action potential resembles the auricular both in shape and duration.
6. In large animals, the ventricular action potential differs from the auricular in that there is a much longer phase of membrane reversal giving the action potential a "plateau" phase.

7. The configurations of the action potentials in different animals have been correlated with their metabolic rates and with the function of the heart as a pump.

8. The total duration of the ventricular action potential correlates well with the Q-T interval in the electrocardiogram.

9. Histological study has shown the great variability and complexity of the architecture and cell composition of the heart tissues.

10. A method of measuring the velocity of spread along this complicated pathway has been developed.

11. The actual method of measurement is to record the intracellular action potentials from carefully selected excised tissue samples, at varying distances from the cathode and to plot on a graph the conduction distances against conduction time. The conduction velocity is obtained from the slope of the regression line.

12. An integral part of the method is the subsequent histological examination of the tissue on which the measurement has been made. Thus the structure and the results can be correlated.

13. The use of intracellular microelectrodes to record the action potential has greatly enhanced the value of this method. Thus in addition to making it possible for measurements to be made in very small pieces of tissue, the condition of the tissue and the cells being investigated can be determined at the same time.

14. Results of conduction velocity measurements on the cardiac tissues of goats, dogs, cats and rabbits, have been

presented. It has been shown that by a careful correlation with the structure, the results depend on (1) the regularity of the disposition of the fibres in relation to the line of measurement and (2) the purity of the cell type in the tissue sample. Any irregularity in the results can be explained by either of these two factors.

15. Values of the conduction velocities are given.

16. In the atrial and ventricular tissues the conduction velocities have ranged from 0.4-0.9 m/sec. In the Purkinje tissue of goats and dogs, the conduction velocity is about 2.5 m/sec.

17. The present quantitative study has opened the way to a better understanding of the problem of co-ordination of the heart beat.

APPENDIX

Appendix I

TABLE i

Diameters of the cardiac fibres from adult dogs (Data from 4 animals.)

Tissue	No. of fibres	Mean (μ)	S.D.	Range (μ)
Auricular	80	10	2.3	6-19
Atrial	50	12	3.0	9-20
Ventricular	111	11	3.4	6-22
Purkinje	42	22	4.4	13-32

TABLE ii

Diameters of the cardiac fibres of adult rabbits. (Data from 3 animals)

Tissue	No. of fibres	Mean (μ)	S.D.	Range (μ)
Auricular	30	11	1.7	8-14
Atrial	25	12	2.6	8-19
Ventricular	276	11	3.0	6-26
Purkinje	41	10	4.0	4-20

TABLE iii

Diameters of auricular fibres

Animal	No. of fibres	Mean (μ)	S.D.	Range (μ)
Rat	44	8	0.9	4-10
Hamster	16	9	3.4	4-13
Mouse	17	8	2.3	4-9

TABLE iv

Diameters of ventricular fibres

Animal	No. of fibres	Mean (μ)	S.D.	Range (μ)
Guinea-pig	38	13	3.9	9-21
Rat	17	9	2.9	8-12
Hamster	24	10	3.5	7-12
Mouse	35	6	1.9	3-9

Appendix II

Statistical data for the calculation of the regression line and the S.D. of the slope

Tissue	N	S(x)	S(y)	S(xy)	$S(y^2)$	$S(x-x_m)$	$S(x-x_m)^2$	m ± S.D.
Goat false tendon	6	12.84	28.04	66.4764	146.7592	0.84	2.7932	2.42±.08
Goat false tendon	6	59.2	21.34	270.7160	102.1556	41.2	421.74	0.43±.01
Dog false tendon	6	22.16	49.00	252.3650	584.5	17.31	30.665	2.4 ±.05
Dog false tendon	4	17.34	27.00	132.6650	219.5	1.34	6.9522	2.57±0.9
Dog false tendon - at 38°C	6	21.93	17.86	76.7628	65.2344	3.96	13.7714	1.03±.08
- at 28°C	4	24.06	12.34	85.3685	45.643	12.06	52.899	0.67±.03
Goat papillary muscle	5	55.20	13.89	162.37	42.7019	5.2	25.94	0.44±.05
Goat papillary muscle	6	52.01	12.65	115.1924	28.8021	4.01	17.3839	0.38±.03
Goat papillary muscle								
- unstretched	8	52.78	22.99	164.7786	71.5949	4.78	34.0982	0.42±.01
- stretched 10%	8	63.6	25.8	226.7648	94.0176	-0.4	43.4866	0.5 ±.01
- stretched 41%	8	90.57	43.49	530.806	254.2519	10.57	97.0891	0.46±.02
- stretched 61%	6	95.52	29.52	509.6602	162.7822	5.52	100.3328	0.44±.03
Dog papillary muscle	4	43.75	19.27	221.226	98.7621	3.75	22.7139	0.55±.08
Dog papillary muscle	5	48.6	15.28	157.0985	52.7172	13.6	49.2588	0.7 ±.03
Cat papillary muscle	6	29.29	18.66	99.1159	62.063	11.29	27.5491	0.8 ±.04
Cat papillary muscle	9	93.89	45.02	525.0324	261.8444	3.89	85.4553	0.66±.01
Cat papillary muscle	5	25.55	9.43	54.0096	21.4479	0.55	9.4579	0.62±.04
Cat papillary muscle								
- microelectrode	7	43.09	19.81	137.8726	64.2007	0.09	31.4523	0.51±.02
- external electrode	6	30.22	15.69	89.0197	46.1887	18.22	74.7638	0.51±.02

Appendix II (Cont.)

Tissue	N	S(x)	S(y)	S(xy)	$S(y^2)$	$S(x-x_m)$	$S(x-x_m)^2$	m ± S.D.
Cat papillary muscle	6	77.05	26.07	348.5279	117.8822	5.05	36.5937	0.43±.04
Rabbit papillary muscle	4	26.64	8.45	60.4321	21.1829	2.64	7.4922	0.72±17
Rabbit papillary muscle	5	19.72	7.8	33.8213	13.6752	-0.27	6.37	0.48±.04
Rabbit papillary muscle	6	47.8	15.2	127.86	41.64	5.8	21.22	0.44±.06
Rabbit papillary muscle	5	41.3	8.64	72.777	15.4382	1.3	4.61	0.33±.06
Goat sulcus (all terminalis points)	18	401.91	211.91	5559.0964	2986.8214	31.91	1511.4613	0.5±.06
1st slope	8	113.07	53.01	841.7968	392.1451	-46.93	486.2085	0.44±.02
2nd slope	10	288.84	158.45	4717.2996	2594.6763	88.84	1025.2528	0.6 ±.01
Goat sulcus terminalis	6	115.16	60.16	1253.3206	669.7192	25.16	253.177	0.66±.05
Goat sulcus terminalis	10	73.15	53.62	451.2455	331.8418	-6.85	85.1415	0.73±.04
Dog sulcus terminalis	7	58.5	27.83	263.202	127.3981	2.5	58.97	0.51±.06
Cat sulcus terminalis - Before ACh	7	115.75	47.69	813.955	351.1757	24.75	114.0625	0.96 ± 0.06 ~~0.69±.12~~
- After washing out ACh	6	86.7	28.63	502.489	174.4053	50.7	641.23	0.41±.02
Rabbit sulcus terminalis	5	45.19	13.69	129.4731	40.4785	5.19	16.7609	0.5 ±.05
Goat auricular trabecula	6	50.09	30.07	260.5685	160.0991	8.09	20.6919	0.97±.05
Goat auricular trabecula	7	38.17	25.10	149.6687	100.8816	3.17	16.6275	0.84±.03
Dog auricular trabecula	4	12.82	5.11	18.2645	7.3015	4.82	10.4634	0.41±.03
Dog auricular trabecula	5	79.72	34.07	731.7290	318.6313	29.72	589.2814	0.4 ±.09
Cat auricular trabecula	5	35.89	17.39	136.3573	67.8095	-4.11	23.4487	0.58±.03

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