

PREFACE

This investigation started as an inquiry into the involvement of sympathomimetic amines, including endogenous noradrenaline, in the production of ventricular fibrillation. The isolated perfused rabbit heart was chosen as a suitable preparation for study of the direct effects of treatments on cardiac muscle, free from interfering nervous and hormonal influences. It was soon realised that the methods previously described for inducing fibrillation in isolated hearts were unreliable, and so it became necessary to determine fibrillation thresholds to single pulses applied during the vulnerable period of the cardiac cycle. Once this method had been developed, and had been shown useful in that the thresholds were considerably changed when the potassium content of the perfusing solution was altered, it was then used to quantitate the effects of adrenaline, both alone and in conjunction with other agents such as chloroform.

My thanks are due to my supervisor, Dr. M.F. Murnaghan, who encouraged me to undertake this investigation, and insisted on a rigorous justification of each conclusion reached; also to Mr. John Robillard for his aid in the design and construction of the perfusion apparatus.

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ABSTRACT

An apparatus was constructed for perfusion of isolated rabbit hearts through their coronary vessels. This apparatus was arranged so that the solution perfusing the heart could be selected from three compositions available by turning a tap; changing took place with the minimum of undesired disturbance in temperature and perfusion pressure, and the new solution could reach the heart within a few seconds. Provision was made for varying the head of perfusion pressure, and for infusing drug solutions directly into the aorta. This infusion was at controlled rates, and could be done with less than one millilitre of drug solution. Force of contraction and electrical activity of the left ventricle were recorded on a Grass polygraph.

An initial series of experiments was carried out to investigate the effects of varying the potassium concentration of the perfusion fluid on the induction of persistent ventricular fibrillation by faradic stimulation of varying frequency at a fixed moderate current strength. A threshold concentration of potassium could be found for each heart, below and not above which persistent fibrillation could be induced; this threshold was not significantly altered by pretreatment of the animals with reserpine.

Attempts made to measure ventricular fibrillation thresholds for faradic stimulation at 30 pulses/sec were unsatisfactory. Instrumentation was then constructed to permit the determination of fibrillation thresholds for

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single electrical pulses delivered during the vulnerable period of late systole. They were determined by applying stimuli following every eighth normal beat: the stimulus was swept across the terminal 50 msec of ventricular depolarisation, and the strength increased on successive sweeps until fibrillation was produced. Ventricular fibrillation thresholds determined in this manner were consistent for any given heart. Of 60 hearts tested, 59 fibrillated on the first test, the mean threshold current being 1.82 ma, with standard error 0.24 ma, and the extreme values seen being 110 μ a and 22.5 ma. Of these fibrillations, 24 reverted to a normal rhythm within one minute; the other 35 continued until normal rhythm was restored following administration of concentrated potassium chloride solution.

Raising the potassium concentration of the perfusion fluid from its normal 5.6 mM to 8 mM raised the fibrillation threshold to an average of four times its control value, while a reduction to 4 mM lowered it to 33% of the control.

Infusion of adrenaline in amounts sufficient to produce maximal acceleration of rate and mild arrhythmias failed on its own to produce marked changes in the fibrillation threshold. When carbachol or chloroform was included in the perfusion fluid in a concentration producing marked effects, the fibrillation threshold was not altered: infusion of previously ineffective amounts of adrenaline now lowered this threshold to less than a quarter of its previous value.

Increased perfusion pressure raised the fibrillation threshold slightly. Administration of procaine caused a rise in this threshold in spite of a reduction in the coronary flow which would normally have been associated with a slight fall.

INTRODUCTION

Experimental Ventricular Fibrillation

Ventricular fibrillation, first described by Hoffa and Ludwig (1) in 1850, and named 'mouvement fibrillaire' by Vulpian (2) in 1874, is considered by many as an important cause of sudden death. It is comparatively well known as a terminal event in degenerative heart conditions, but is often overlooked as a factor in a seemingly normal heart. It shows similar external signs to cardiac arrest, and is in fact functionally the same; the pumping action of the heart ceases, the pulse disappears, the blood pressure collapses and the circulation stops. The resulting peripheral anoxia leads to irreversible damage to the sensitive tissues of the brain if the fibrillation is not rapidly arrested. Correct diagnosis is important, as a common and reasonable treatment for genuine cardiac arrest (intracardiac injection of adrenaline) is likely to aggravate the situation. No good drug treatment to revert ventricular fibrillation is known. The related, but much less serious, atrial fibrillation has been treated with success by such 'antifibrillatory' drugs as quinidine and procaine amide, but their actions on the ventricles are not necessarily similar.

Experimental investigations of ventricular fibrillation may be said to date from 1884, when Kronecker and Schmey (3) found that it could be produced by stabbing the heart, usually about a third of the way down the interventricular

groove. MacWilliam (4) extended this observation, and demonstrated that it was the ventricular muscle which was involved, while the atria could continue to beat normally. He also showed that it was more difficult to produce and maintain fibrillation in the hearts of small animals (small species, or young) than in those from larger. Further, he noted that he could markedly lower the fibrillation threshold for any stimulus, mechanical, electrical or chemical, although he did not describe the particular conditions required for this lowering, nor quantitate the effects.

The problem of unwanted ventricular fibrillation, triggered by trauma, led Wiggers (5) into a long series of investigations that laid much of the foundations for more recent work on the experimental induction of this arrhythmia, especially the idea of the 'vulnerable period' of late systole. Meanwhile, Levy (6) had initiated a series of investigations into the liability of adrenaline to precipitate ventricular fibrillation in cats anaesthetised with chloroform. The causal relationship of these two factors was shown, and later work (7,8) developed this further, with the double substitution of cyclopropane for chloroform, and of dogs for cats. Thus two lines of investigation were opened up: one, using electrical stimulation to [certainly] induce fibrillation; and the second, testing whether arrhythmias resulted as the outcome of a particular challenge, such as the administration of a

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sympathomimetic amine to an animal under the influence of cyclopropane.

Electrical Induction

In the investigations of the electrical induction of ventricular fibrillation, several questions had to be answered. The early investigators (1,2,4) had used either 'galvanic' or 'faradic' currents, but always of prolonged duration. The first question, then, was as to what duration of current was necessary. A lead was given in 1930 by the work of Andrus, Carter and Wheeler (9), who were able to produce fibrillation in atria by delivering a single induction shock, suitably placed in the relative refractory period, during stimulation of the vagi. The next major advance was the work of Ferris, King, Spence and Williams (10) in 1936, investigating the effects of 60 cycle alternating current on the heart. They measured thresholds to 3 second bursts, and confirmed MacWilliam's observations on the relationship between the size of the animal and the ease of producing a persistent fibrillation. Thresholds with 25 cycle alternating current were not much greater than those with 60 cycle, but direct current ('galvanic') stimulation was much less efficient. Further examination of the effects of shortening the duration of the stimulus burst indicated that with durations of 35-120 msec (2-8 cycles), the placing of the shock in the cardiac cycle was important; fibrillation occurred most readily if the stimulation

coincided in time with the T wave. Lower thresholds were obtained when the stimulus burst was longer than the cardiac cycle (time per beat).

By 1940, Wiggers and Wégria (11) had developed the single pulse method, driving the heart at a regular rhythm and inserting induction or condenser shocks of determined strength at various intervals after systole. They further clarified the presence of a 'vulnerable period' in late systole; it was only when a stimulus was applied during this vulnerable period that fibrillation could be induced. Provided the shock was properly placed, and was considerably shorter than the cardiac cycle, the current strength was much more critical than the duration of the stimulus. Having quantitated their method using mechanically interrupted direct current stimuli 10 to 30 msec in duration, Wiggers and Wégria (12) proceeded to show that procaine raised the ventricular fibrillation threshold without preventing the occurrence of fibrillation. Wégria and Nickerson (13) then investigated the effects of other drugs: they showed that papaverine and quinidine raised the fibrillation threshold, their effects being well maintained; adrenaline produced a rise in threshold which was only transient. However, if the papaverine or quinidine were administered in a dose sufficient to cause a fall in the blood pressure, the expected rise in the fibrillation threshold did not occur, and indeed a decrease might appear.

While intensively studying the excitability of cardiac muscle, Hoffman, Gorin, Wax, Siebens and Brooks (14) further delineated the vulnerable period. These workers used electronic pulse generators to control their stimuli, and cathode ray oscilloscope recording. Using pulses 1 to 3 msec long, they observed that the duration of the vulnerable period depended on the strength of the stimulus. With very high currents (their maximum being 30 ma), the vulnerable period extended over 15 to 25 msec; its range narrowed as the current was decreased, until the minimum current producing fibrillation was effective at only one point of the cardiac cycle. This point generally fell in the later half of the vulnerable period at the highest current.

Siebens, Hoffman, Enson, Farrell and Brooks (15) demonstrated that the injection of adrenaline or noradrenaline caused a transient decrease in the threshold for diastolic extrasystoles: the concomitant rise in potassium concentration accounted for part of the effect, halving the amount of catecholamine required to produce a given change. Further work by Hoffman, Siebens, Cranefield and Brooks (16) showed that this drop lasted only two to three minutes, even during a catecholamine infusion lasting for 10 minutes. A similar, but greater, drop was seen in the ventricular fibrillation threshold; this was as transient.

Like Wiggers and Wégria, Hoffman and his collaborators were using close bipolar stimulating electrodes. Their results with these showed that excitation of only a part of

the total ventricular myocardium could, under suitable circumstances, precipitate ventricular fibrillation. Wiggers (12) had shown that with this type of stimulation, ventricular fibrillation started following a run of three to six extrasystoles, all but the first being re-entrant in origin. Hoffman et al. (14) found that on occasion a shock well above the ventricular fibrillation threshold failed to elicit any response: this was later shown (Crane-field, Hoffman and Siebens, 17) to be an artefact arising from an anodal block.

The primary purpose of all this work being the delineation of the vulnerable period, it had all been done with artificially driven hearts to avoid the variations in timing of the refractory and vulnerable periods with varying lengths of the cardiac cycle. In 1957, Shumway, Johnson and Stish (18), concentrating on the threshold value, developed instrumentation which made it possible to use the method with spontaneously beating hearts. Using a thyatron trigger circuit responding to the QRS deflection of the electrocardiogram, and irresponsive for a reasonable time thereafter (to avoid spurious triggering), they were able to place their stimulus at a chosen point in the respiratory cycle of heart rate. They regularly stimulated with a 10 msec pulse just before the peak of the T wave, and were able to measure consistent thresholds. They noted that multiple extrasystoles could occur at current strengths less than those producing fibrillation, but accepted only the

latter response as significant. Again using close bipolar electrodes (10 mm separation), they could demonstrate variations in the fibrillation threshold for different areas of the ventricles.

vanTyn and MacLean (19) finally made the method fully reliable. They returned to the use of a variable timing in the cardiac cycle, sweeping a 10 msec stimulus by 5 msec steps through the 60 to 90 msec preceding the end of the T wave. They showed that more consistent thresholds were obtained with widely spaced (up to 35 mm) than with close bipolar electrodes: there was too much tendency for local short-circuiting in coagulated tissue to occur if the electrodes were within 4 mm of one another. The importance of allowing sufficient recovery time between stimuli was also brought out: successive shocks had to be at least 4, preferably 8 to 16, beats apart to obtain consistent results.

All this work on the vulnerable period and single pulse ventricular fibrillation thresholds had been carried out on dog hearts in situ; in order to permit multiple determinations to be carried out on a single preparation, it was necessary to defibrillate, usually with a very powerful countershock, as soon as fibrillation had been observed.

Adrenaline and Anesthetics

From the time of Levy (6) on, many workers questioned whether the rise of blood pressure produced by an adrenaline injection was linked to the induction of ventricular fibrillation. The link became more likely when Garb and Chenoweth (20) reported that injections of adrenaline or noradrenaline would produce fibrillation in cats sensitised by hydrocarbon inhalation, while isoprenaline injections would not. [This report, an abstract, does not give any doses.] Isoprenaline is known to differ from adrenaline and noradrenaline in causing a fall rather than a rise in blood pressure.

Moe, Malton, Rennick and Freyburger (7) then demonstrated that Dibenamine, which blocks the pressor but not the cardiostimulating effects of adrenaline, protected dogs under cyclopropane anesthesia against the arrhythmic sequelae of an adrenaline injection. Using a blood pressure regulating system, they showed that either the abolition of the pressure response, or Dibenamine blockade in the presence of a raised blood pressure, would markedly but not completely antagonise the induction of idioventricular rhythms by adrenaline. The fibrillatory response was abolished.

Nickerson and Nomaguchi (8) further separated these effects: they showed that after an injection of adrenaline in sensitised dogs, fibrillation always appeared before the blood pressure rise was complete. Larger doses of Dibenamine

were needed to block the arrhythmic effect than to block the pressor response. [This result would have been more convincing if noradrenaline had been used as a challenge: mere abolition of the pressor response to adrenaline (without going over to the depressor 'adrenaline reversal') is obtained at a certain partial blockade of the vasoconstrictor receptors, such that the remaining pressor effects just balance the depressor.] More significant was their observation that it was possible to induce and abolish arrhythmias (but not fibrillation) by varying the mean blood pressure around a critical 'threshold' level.

These results were confirmed by Dresel and Sutter (21), who were able to convert a bigeminal rhythm during an adrenaline infusion into either sinus rhythm or multifocal ventricular tachycardia by mechanically lowering or raising the blood pressure respectively. Similar shifts of blood pressure at a higher rate of adrenaline infusion failed to convert multifocal ventricular tachycardia into fibrillation. Stimulation of the cut vagi could raise the threshold dose of adrenaline required to produce arrhythmias, but did not affect the dose needed to precipitate fibrillation.

The influence of hydrocarbons on the arrhythmic activity of adrenaline was well demonstrated by Riker, Depierre, Roberts, Roy and Reilly (22). Using cats, they injected a standard dose of adrenaline (30 μ g/kg), and observed the arrhythmic effects. Under barbiturate

anesthesia alone, 79 tests failed to produce a single fibrillatory response; if Petroleum Ether (a mixture of volatile hydrocarbons) was added to the inspired air, 49 fibrillations were obtained in 98 tests. With double this dose of isoprenaline, no ventricular fibrillation was obtained in 10 trials without, or in 9 trials with, the Petroleum Ether.

Linenthal, Zoll, Norman, Gibson and Shustari (23) tested adrenaline and isoprenaline in dogs with an experimental heart block. They constructed response curves for the idioventricular pacemaker over a dose range from 10 ng/kg to 100 µg/kg. With doses 10 to 100 times those giving a 30% acceleration (the maximum obtained being about 70% of the basal rate), both drugs were reported to have produced multifocal ventricular tachycardia and ventricular fibrillation.

Ventricular Fibrillation in Isolated Hearts

The isolated heart, perfused through its coronary circulation in the manner originated by Langendorff (24), offers many advantages for experimental studies, notably the facility with which the composition of the perfusing fluid may be altered. Further, in investigations of ventricular fibrillation, an isolated heart may be allowed to continue fibrillating for a prolonged time; in the whole animal, fibrillation must be arrested quickly, or the animal dies and the experiment is ended.

MacWilliam (4) was able to obtain fibrillation in isolated blood-filled ventricles. Of more interest is the statement by Wiggers (5) that he had been able to induce ventricular fibrillation by a single shock in isolated cat hearts, perfused with a saline solution.

In 1955, Dirken, Gevers, Heenstra and Huizing (25) were investigating various defibrillating procedures on isolated rabbit hearts. They ordinarily induced fibrillation by faradic stimulation with 60 cycle alternating current of 10 volt strength in 100 msec long bursts; injecting 200 μ g of adrenaline was found to produce the same result. At about the same time, Grumbach, Howard and Merrill (26) reported that they had been able to induce ventricular fibrillation in isolated rabbit hearts by administering high concentrations of calcium, and noted that the amount of calcium needed depended on the potassium concentration of the perfusing solution. Further, they obtained spontaneous

ventricular fibrillation within five minutes if they perfused the hearts with a potassium-free solution. This result was confirmed by the work of Melville and Mazurkiewicz (27) shortly afterwards; they were able to reversibly abolish the fibrillation by adding potassium to the perfusing fluid.

In 1957, Armitage, Burn and Gunning (28) reported a series of experiments with isolated rabbit hearts. They stimulated the ventricles for 5 minutes with 1 ma pulses at frequencies rising from 3 to 15 per second, gradually increasing the frequency until fibrillation was produced. Having stopped the stimulation, they observed whether the fibrillation persisted or not, noting that if fibrillation continued for more than 5 minutes, it did not spontaneously revert to normal rhythm within half an hour. Increasing the potassium concentration of the perfusion fluid decreased the proportion of hearts in which they could induce a persistent fibrillation; at 1.4 mM potassium concentration, all hearts could be induced to fibrillate persistently: indeed 3 of the 4 tested fibrillated spontaneously; at 5.6 mM (the normal concentration), 11 of 28 persisted; while at 11.2 mM, they were unable to induce any fibrillation. They were thus able to show variations in susceptibility to fibrillation, but their results are complicated by the low current strength used, and also because they insist on referring to hearts as 'fibrillating' and 'not fibrillating' when they mean 'fibrillating persistently' and 'failing to fibrillate

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persistently'.

Further work by the same group (29) showed that if the sodium chloride concentration in their perfusion solution was halved [sodium reduced by 40%], all hearts tested would fibrillate persistently. This paper also mentions that there was a 2-3% greater potassium loss from the hearts when fibrillating than when beating normally; this slightly greater loss is proposed as the cause of the fibrillation. Such a change, however, is to be expected as a result of the fibrillation, as the cardiac muscle is depolarised for a larger proportion of the time - each fibre refiring as soon as it is repolarised - than during normal rhythm, when there is a significant diastolic interval.

With Goodford (30), a decline in the significance of the results from the Oxford laboratories begins. He no longer varied the frequency of the stimulation while attempting to induce fibrillation. Having adjusted the potassium concentration to a level where he no longer got [persistent] fibrillation, "the fluid was next changed to one containing adrenaline". No mention is made of any antioxidant preservative to protect the adrenaline in a solution of pH 7.3, through which gaseous oxygen was vigorously bubbled. It is likely that some of the adrenaline reached the heart; for he was able to produce a persistent fibrillation, under his earlier stimulation conditions, during perfusion with his adrenaline-containing solution.

Kärki (31), again using a [new] fixed frequency of stimulation, was able to increase the incidence of persistent fibrillation by lowering the potassium or sodium concentrations, or by raising that of calcium. Variations in the calcium level were most effective when the potassium was reduced from its normal 5.6 mM to 1.4 mM. Milton (32) returned to a variable stimulation frequency, but only over the range of 9.7 - 20 pulses/sec (103 - 50 msec pulse interval). He found a lower incidence of persistent fibrillation at 32 C than at 37 C, even when using currents as high as 10 ma.

Lowered potassium levels, therefore, had been shown to render the isolated heart more susceptible to fibrillation; at the same time, adrenaline was implicated, under suitable conditions, in the production of ventricular fibrillation in vivo, and could produce fibrillation in vitro. Admittedly, large amounts of adrenaline had been used; but no systematic investigation of the minimum amount needed to induce fibrillation had been made. The question thus arose as to whether any link existed between the effects of potassium and those of adrenaline; some evidence from experiments on isolated atria had suggested such a connection.

Armitage (33) had shown that in isolated atria or isolated perfused hearts treated with quinidine (a reputedly antifibrillatory drug), reducing the potassium concentration to a half or a quarter of normal revived the depressed contractility; Kumar and Sheth (34) had shown that

adrenaline also could revive atria depressed by quinidine, and that the action of acetylcholine in doing this (Briscoe and Burn, 35) was due to the liberation of a catecholamine.

Graham (36) showed that the stimulant action of acetylcholine on isolated atria was augmented by lowered potassium levels, this action also being due to catecholamine - probably noradrenaline - release, as further demonstrated by Kottegoda (37) as well as Kumar and Sheth (34). Thus Graham's results could be interpreted as either sensitisation to noradrenaline by low potassium levels, or release of more noradrenaline; in both cases, an increased sympathomimetic response appeared.

The Isolated Saline-perfused Heart

The ventricular muscle of the mammalian heart is normally supplied with nourishment through the coronary circulation. Its metabolic requirements are too high, and its thickness too great, for simple diffusion from the internal and external surfaces to suffice. Although MacWilliam (4) had used a ventricular sac preparation, tying a ventricle onto a cannula and filling the lumen with defibrinated blood, it was the work of Langendorff (24) in 1895 that laid the foundations for more recent work on isolated mammalian hearts. Langendorff found that by perfusing defibrinated blood into the aorta of an excised heart, under a moderate head of pressure, the semilunar valves were closed, and the blood flowed through the coronary vessels to emerge into the right atrium and eventually be expelled through the cut pulmonary artery.

Defibrinated blood was not the most convenient perfusing fluid, and by 1901, Locke (38) had commenced using a saline substitute of known composition, which was free from the vasoactive constituents of serum. Ringer (39) in 1883 had developed the first satisfactory saline solution for maintaining the activity of isolated frog hearts. His solution contained sodium chloride for osmotic effect, empirically balanced quantities of calcium and potassium salts (preferably the chlorides), set to maintain the contractions of the heart at a steady level, and sodium bicarbonate as a buffer, to prevent wide shifts of acidity.

In 1895, Locke (40) emphasised the necessity of using highly purified water, preferably distilled in an all-glass still, for physiological experimentation.

For his experiments on isolated perfused rabbit hearts, Locke (38) considerably modified Ringer's original solution. The sodium chloride level was raised to correspond with the greater tonicity needed for mammalian tissues; the potassium and calcium levels were adjusted to match those of rabbit serum; d-glucose was added to provide energy; and the solution was oxygenated to provide for the higher oxygen demand of the active tissues. Using perfusion pressure heads of 22 to 30 mm Hg, he could record for periods of 7 to 8 hours.

In carrying out experiments on the utilisation of glucose by rabbit hearts, Locke and Rosenheim (41) modified their apparatus to allow for recycling of the solution. The fluid entering the aortic cannula was warmed just previously in a heat exchanger. These authors noted that if the perfusion pressure was kept below 45 mm Hg, long lived preparations could be obtained; higher pressures led to the rapid development of edema and consequent mechanical obstruction and poor flow of the perfusion fluid. Gunn (42) further improved the apparatus, using a modified Liebig's condenser as a heat exchanger. He stressed the necessity of keeping the stub of the aortic cannula that projected beyond the jacket as short as possible to minimise alterations in temperature of the perfusing fluid with variations in the

flow through the coronary vessels.

Unfortunately, a commercial apparatus (C.F. Palmer) became available which disregarded Gunn's principles, especially in having an exposed cannula some 120 mm long between the exit of the heat exchanger and the heart. Bell (43, pp.220 - 224), describing the use of this apparatus, suggests using perfusion pressures of 50 to 70 mm Hg, well above Locke's recommended maximum. Injections of drugs were to be made at least 70 mm above the aorta, with a dead volume of some 3-5 ml of perfusion fluid through which they could be spread. The problem of temperature changes due to varying coronary flows led Saxby (44) to devise an ingenious arrangement in adding a 'bleed off' to maintain a minimum basal flow at all times.

Meanwhile, Anderson and Craver (45) had produced an elegant Pyrex apparatus for perfusing cat and rabbit hearts. The temperature control problem was met by using a fairly short cannula, and a temperature-controlled jacket around the heart itself. Changing between perfusion fluids of different compositions was not very convenient with this apparatus; only a single heat exchanger was provided. The perfusion pressure was quite low, being about 30 mm Hg.

In 1956, McEwen (46) introduced a significant addition to the composition of his perfusing solutions. Using a basic Locke formulation, with 25 mM bicarbonate to maintain a pH of 7.3 when gassed with a 5% carbon dioxide - 95% oxygen mixture, he added a small amount of phosphate (1 mM: not

enough to have any buffering action in the presence of the 25 mM bicarbonate), and also 13 mM sucrose. The presence of the sucrose made it possible for him to perform experiments of some 9 hours duration on a preparation consisting of the isolated rabbit heart with its vagus innervation.

Single Pulse Induction of Ventricular Fibrillation

Wiggers and Wégria (11), Hoffman et al. (16), and vanTyn and MacLean (19), have outlined the basic requirements for inducing ventricular fibrillation with single electrical stimuli, and for measuring the threshold current which will do this. Necessary for the induction of fibrillation is the application of a stimulus of adequate strength within a relatively limited 'vulnerable period' late in electrical systole. In order to keep the strength of the stimulus required within reasonable bounds, vanTyn and MacLean point out the necessity of a good separation of the stimulating electrodes (more than 10 mm in the dog) to prevent local short-circuiting through cauterised tissue. While the vulnerable period is shortest at just threshold strengths, and lengthens somewhat with higher currents, most of the lengthening is probably due to better persistence of a local excitatory state until the true vulnerable time after ventricular depolarisation arrives. Stimuli applied following repolarisation, or in the early stages of depolarisation, have never been found to evoke fibrillation.

To measure thresholds accurately, it is necessary to apply a pulse of known current strength at the moment when the tissue is 'most vulnerable', i.e., at the time of the apex of the vulnerable period. The most satisfactory way of doing this is to use the technique of vanTyn and MacLean, repeatedly sweeping a pulse of known strength through the period of vulnerability, gradually increasing the strength on successive sweeps until fibrillation is produced. By using a pulse 10 msec long, and sweeping by 5 msec steps, all parts of the expected vulnerable period are covered by the stimulus. A reasonable and steady interval between test pulses has been shown to be necessary; this is preferably 8 to 16 beats.

Defibrillation

From the time of MacWilliam (4), investigators have been rediscovering the fact that ventricular fibrillation is more likely to revert spontaneously to a normal rhythm in small hearts than in large. One of the main reasons that Wiggers (47) gives for his choice of dogs as experimental animals is that with these, fibrillation almost never reverted spontaneously; with the assurance that ventricular fibrillation, once induced, would persist, he was then able to test methods for producing defibrillation. He obtained good results by injecting potassium salts into the heart to depolarise the muscle completely, and then reviving it with subsequent administration of calcium salts. Although this

method worked well, it could not be repeated very often on the same animal without producing marked ionic imbalance.

Dirken et al. (25) investigated various defibrillating procedures on isolated rabbit hearts. In order to obtain only persistent fibrillation, they perfused at a temperature of 39 C: their general results suggest that non-persistent fibrillation became commoner as the perfusion temperature was lowered. These investigators found that by cooling the heart to 24 C, pouring cold saline over it, they could abolish the fibrillation; rewarming then restored the beating with a normal rhythm. Since they had set their hearts in an inverted position (base down, apex up), it was easy to pour the cold saline over the ventricles. Dirken et al. could not get consistent defibrillation with injections of potassium chloride solutions; they were troubled by the reappearance of the fibrillation as the potassium was washed out.

General Plan of the Investigation

The isolated rabbit heart, perfused with a saline solution such as that of McEwen (46), offers many advantages for experimental studies in cardiac physiology and pharmacology. Although the physiological responses may be expected to be somewhat less constant than in vivo, this is more than compensated for by the removal of hormonal and nervous controls, so that the direct effects of various treatments on the heart may be evaluated. Further, the composition of the perfusing fluid may be varied widely without introducing secondary effects as would occur with a heart in situ.

The reports from the Oxford laboratory (Armitage et al., 28, 29) had shown that persistent ventricular fibrillation could be induced in isolated perfused rabbit hearts by faradic stimulation under suitable conditions. Their results suggested some dependence on the potassium concentration of the perfusing fluid, but as they did not give enough details of their experiments to determine at which concentrations any given heart had been tested, it was not possible to construct more than an approximate dose-response curve relating the cumulative proportion fibrillating persistently to the potassium level.

Thus the first step was to quantitate more exactly the variations in ease of induction of ventricular fibrillation with alterations in potassium concentration, and then to test the effects of a number of agents on this response.

Among these agents were:

Adrenaline. The results of Goodford (30), together with those of Siebens, Hoffman et al. (15, 16) indicated that adrenaline might facilitate the induction of ventricular fibrillation, at least for a brief period, although Wégria and Nickerson (13) had found it to increase the fibrillation threshold. Most of this work was done with hearts in situ, where blood pressure responses and vagal effects could alter the response. Goodford's results are complicated by the weak experimental techniques described.

The work of Graham (36) also suggested that variations in potassium concentration could alter the response to catecholamines. If the effects of low potassium were in any way mediated by these amines, then depletion of the cardiac stores by pretreatment of the experimental animals with reserpine (Bertler, Carlsson and Rosengren, 48) should alter the response to varying the potassium level.

Synergists with adrenaline. Many workers have shown that adrenaline can produce arrhythmias, but Riker et al. (22) made a sharp distinction between the ability to produce arrhythmias and that to cause fibrillation; they found that sensitisation with a mixture of hydrocarbons was necessary before adrenaline would precipitate fibrillation. The classic example of a sensitiser is chloroform (Levy, 6).

Dresel (49) makes the interesting claim that vagal activity can cause A-V nodal blocks which increase the

propensity of adrenaline to produce moderate arrhythmias in vivo; he never investigated the effects on fibrillation.

High pressure. This has been implicated, in conjunction with adrenaline, as a predisposing factor in the production of arrhythmias and fibrillation since the time of Levy (50). Panisset, Carle and Beaulnes (51) have recently presented a series of experimental results which they claim show that raised perfusion pressures, of up to 70 mm Hg, make ventricular fibrillation more easily inducible in the isolated rabbit heart.

Procaine. This seems to be a more tractable anti-fibrillatory agent than quinidine, which is directly depressant to cardiac muscle. Wiggers and Wégria (12) had shown it to raise the ventricular fibrillation threshold in vivo, and a less readily hydrolysed derivative, procaine amide, is in therapeutic use as an anti-arrhythmic drug.

METHODS

Perfusion Solution

McEwen's solution (46) was used as the perfusing medium for these experiments. Batches were mixed freshly on the morning of the experiment, using strong stock solutions of the saline constituents; these were made in integral molar strengths. Solutions of sodium, potassium and calcium chlorides, and of sodium dihydrogen phosphate, were presumed stable. Sodium bicarbonate solution (1 molar, almost saturated) was pregassed with 100% carbon dioxide; with this precaution, no trouble was experienced with precipitation of calcium carbonate during preparation. d-Glucose was preweighed in vials, and was dissolved by addition to the bulk of the solution. A 2 molar (68.4% w/v) solution of sucrose was prepared at frequent intervals, and kept refrigerated.¹ The final mixed solution was oxygenated with a mixture of 95% oxygen and 5% carbon dioxide.

A typical recipe for the preparation of a batch of the solution is given in table 1, and the final concentrations of the components in the gassed solution in table 2. For those experiments where the effects of several potassium concentrations were to be examined, a potassium-free stock

¹Preparation of stock sucrose solution, 2 molar: 200 ml of glass distilled water were heated in a 500 ml Erlenmeyer flask, using an electric hot-plate. 342.3 gm sucrose was added, while stirring. The last of the sucrose was rinsed in, and the mixture boiled gently for half an hour, with a cotton plug in the mouth of the flask. It was then allowed to cool, and made to 500 ml with boiled distilled water.

was first made, and then the potassium added as a 2 molar stock of the chloride, using 2.8 ml/litre for the normal solution. The dilution by a factor of about 1/300 so occurring was considered insignificant, being less than the limits of measurement of the final volume of the stock.

TABLE 1. PREPARATION OF McEWEN'S SOLUTION

For 8 litres of solution, take:

Glass distilled water			6 400	ml
Sodium chloride	NaCl	5M	212	ml
Potassium chloride	KCl	2M	22.4	ml
Calcium chloride	CaCl ₂	1M	17.6	ml
Sodium dihydrogen phosphate	NaH ₂ PO ₄	1M	7.2	ml
d-Glucose	C ₆ H ₁₂ O ₆		16	gm
Sucrose	C ₁₂ H ₂₂ O ₁₁	2M	52	ml
Sodium bicarbonate	NaHCO ₃	1M	200	ml
Glass distilled water to make			8 000	ml

Mix: gas with 5% CO₂ / 95% O₂ for 10 minutes.

All batches of solution for perfusion were filtered through Whatman No. 4 or Reeve Angel No. 202 filter paper before use.

TABLE 2. COMPOSITION OF McEWEN'S SOLUTION

Concentrations in Millimolar

Na ⁺	158.4	Cl ⁻	142.0
K ⁺	5.6	H ₂ PO ₄ ⁻	0.4
Ca ⁺⁺	2.2	HPO ₄ ⁻⁻	0.5
d-Glucose	11	H ₂ CO ₃	2.9
Sucrose	13	HCO ₃ ⁻	24.5
Oxygen	2	pH	7.3

Perfusion Apparatus: Series 1

The first 19 experiments, which formed series 1, were carried out using the apparatus illustrated in fig. 1.

The McEwen's solution was contained in a Mariotte bottle A, which served to set the head of pressure for the perfusion. From here, the solution passed to an open tube B, where it was oxygenated, and then through the filter C (a wad of glass wool) over a siphon into the warming column D; this was assembled from standard spherical-joint chemical glassware (obtained from Ace Glass Inc., Vineland, N.J.). From below upwards, it consisted of:

- a helical-tube (Graham) condenser;
- a straight-tube (Liebig) condenser;
- an adapter with a long inner tube descending below the surface of the solution in the column: this formed the descending limb of the siphon mentioned above;

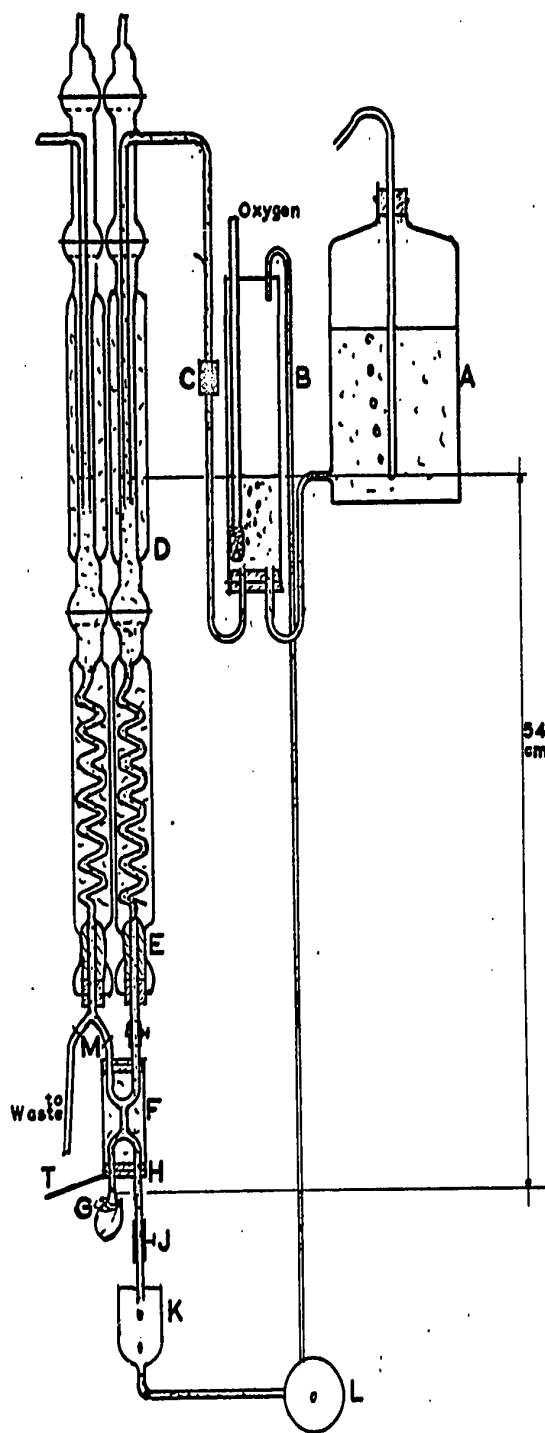


Figure 1. Perfusion Apparatus for Experiments of Series 1.

All parts shown on right-hand column, for normal solution. Second column supplied in same way with solution of alternate composition. Bleed off from Saxby bypass at H returned by pump L to oxygenation tube B.

a reducing adapter, with a long rubber tube (not shown) attached, to enable suction to be applied during filling of the apparatus.

At the lower end of the column, the 'dead volume' of fluid was reduced by inserting a polymethacrylate liner E. The lowermost joint was closed by a rubber stopper with a glass tube projecting from it, and this tube was joined to the 'Junction Box' F by a short length of rubber tubing. The column for the normal McEwen's solution had a simple tube; the second column, for the solution of altered composition, had a Y-tube M to facilitate emptying and refilling it as necessary during the course of an experiment.

The Junction Box F consisted of two Y-tubes joined stem-to-stem, enclosed in a warming jacket of large-bore tubing closed at each end by rubber stoppers. The limbs of the upper Y-tube served as inlets from the two warming columns. Of the limbs of the lower Y-tube, one led to the glass aortic cannula G, which was inserted into the lower closure H of the jacket; the second permitted the perfusion fluid to escape through a rubber tube into a funnel K. The rate of escape was controlled by a long screw-adjusted clamp J (Harvard Apparatus Company: Microflow Drip Regulator). This long-bodied clamp, with a stepwise adjustment, provided the fine control necessary to achieve the optimum effect. By this means, a constant basal flow could be maintained through the column in the manner suggested by Saxby (44) to lessen the variations in temperature of the

perfusion fluid which can occur in an apparatus of this kind containing partially unjacketed pathways. The fluid escaping here was collected in the funnel K, and returned by a Nylon cam pump to the oxygenation tube B. It was this recirculation that necessitated the presence of the glass-wool filter C.

A needle thermocouple temperature probe T was inserted between the ends of the junction box Y-tube and the aortic cannula. Also inserted at this point was a fine polyethylene tube to serve as a drug injection cannula; this passed down inside the aortic cannula to its orifice, thus ensuring that injections made through it would pass as rapidly as possible into the heart.

The reference junction for the thermocouple probe was placed in water in a Dewar flask at approximately room temperature (22 - 25 C), with an accurate mercury-in-glass thermometer to check the actual value of this temperature, which did not change noticeably over the duration of any one experiment.

Temperature control of the perfusion fluid was derived by circulation of water from a Labline water bath, of ± 0.5 C constancy, through the junction box and condenser jackets. The temperature of the fluid entering the aortic cannula, as measured by the probe T, was displayed on a mirror galvanometer, and read from its calibrated scale.

Although by no means ideal, this apparatus served until the succeeding improved version was ready.

Perfusion Apparatus: Series 2

Fig. 2 illustrates the general form of the perfusion apparatus employed in series 2, which comprised all the experiments on ventricular fibrillation thresholds to single electrical pulses. As can be seen, there were three warming columns, any one of which could be connected to the heart by the selector tap F. These three were arranged in line: the central one served to provide the normal or control conditions, and the outer pair were used for the variations. These outer columns could be completely drained during the course of an experiment, and be refilled with a new solution.

As in the previous arrangement, the Mariotte bottle A provided a solution reservoir and pressure reference; the latter was the lower end of the air inlet tube, which gave an absolute reference pressure of the day's atmospheric pressure, or a gauge pressure of zero. The gauge pressure was the important one, as the heart itself was exposed to the same atmospheric pressure as the bottle. Again, the solution passed from its reservoir to the oxygenation tube B. The Mariotte bottle and the oxygenation tube for each of the side columns were fastened to a sub-frame, which could be raised or lowered as a unit to vary the perfusion pressure. The normal head of pressure from the reference point of the bottle to the orifice of the aortic cannula was 540 mm, as used by Goodford (30). This head of McEwen's solution gave a pressure of 40 mm Hg; other heads were then

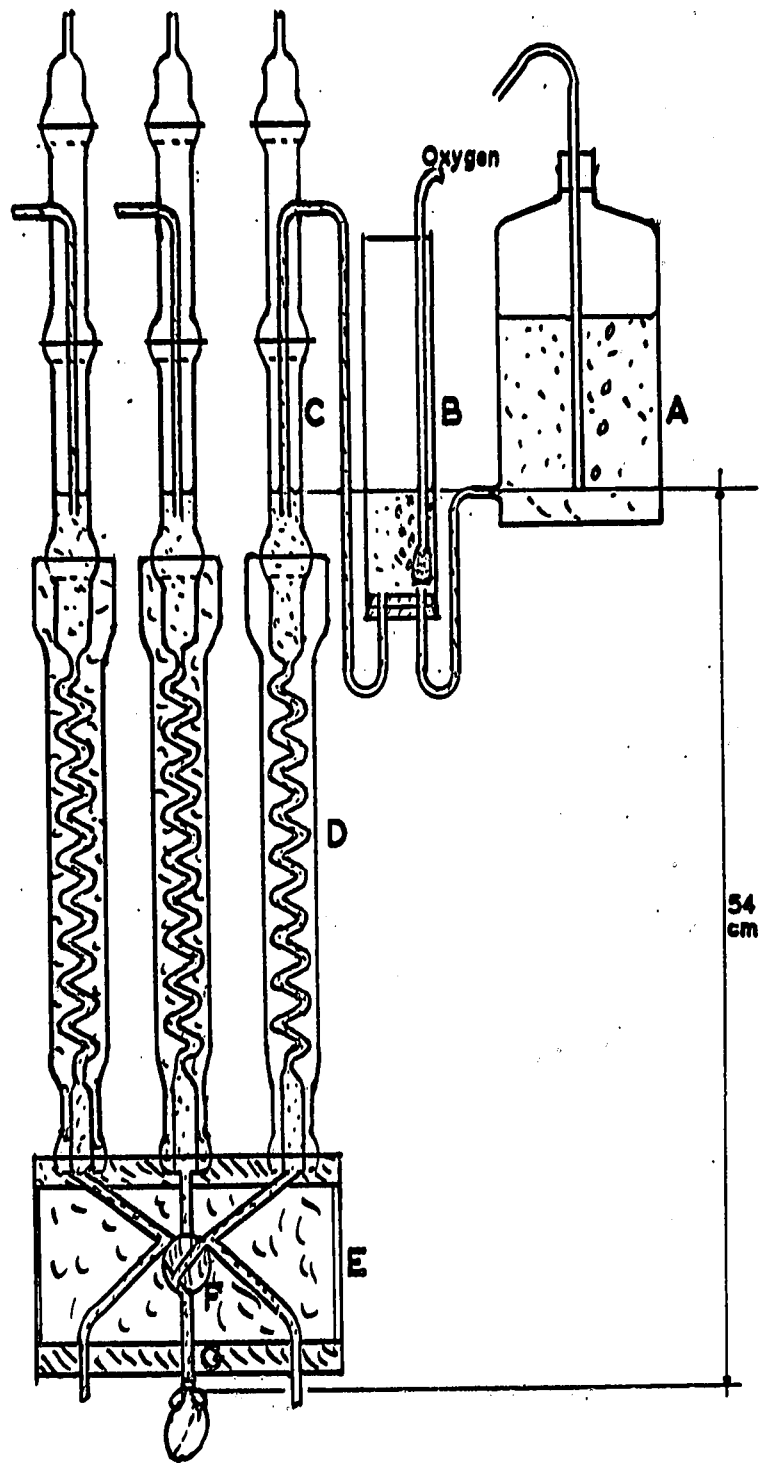


Figure 2. Perfusion Apparatus for Experiments of Series 2.

Reservoir and oxygenation shown for only one of the columns, but similar in the other two. Normal head of pressure indicated (54 cm McEwen's = 40 mm Hg).

expressed as mm Hg, and the main frame of the apparatus was marked with reference points for a convenient series.

In the tube B, a vigorous flow of the 95% oxygen - 5% carbon dioxide mixture was introduced through a sintered glass diffuser. The flow of this oxygen mixture was normally set at 300 ml/min for each section in use; this was well in excess of the minimum requirement. The gas mixture passed from its storage tank through a two-stage regulator, where its pressure was reduced to 100 mm Hg (2 lb/in²) gauge. At this pressure, it passed through a flowmeter, and then through the distributing manifold to the individual oxygenating tubes. Each of these final lines, of rubber tubing, could be controlled (a) with a spring clamp as an on/off control, and (b) with a screw clamp, to regulate the flow. By fixing the body of this clamp to the frame of the apparatus, a much finer control could be obtained than if it were hanging loosely.

From the oxygenation tube B, the solution passed through a siphon into the main warming column C. The flexible connection here was of transparent Nalgon polyvinyl chloride tubing; that from the reservoir to the oxygenation tube was either of the same material or of surgical latex. The entry of the siphon into the column and its descending limb were formed by a glass tube passing inside the main body of the column to a level below that of the solution inside.

The column was assembled from standard chemical glassware (again from Ace Glass Inc.), with $\frac{3}{8}$ 35/25 joints. The upper section was unjacketed, and contained a simple spacing piece below the siphon entry adapter, and a reducing adapter at the summit; again, a length of rubber tubing was attached for applying suction when priming the siphon. Between the siphon entry and the uppermost piece, extension sections were inserted when needed to provide for higher heads of pressure. The lower section of the column was a helical-tube (Graham) condenser D, of 250 mm nominal length, with completely jacketed joints at both ends. This provided sufficient heat exchanging capacity at all flow rates below 40 ml/min, a flow never attained under the usual experimental conditions.

The lower ends of the three similar columns joined a polymethacrylate 'switch box', which included the selector tap and the cannula. This box had thick upper and lower faces, machined for the various connections required, and thinner side and end walls. Some difficulty was found initially in making a leak-proof joint between the glass spherical joint and the inlet in the box: the solution used is shown in fig. 3A, a cross section through one connection.

A cylindrical recess was milled into the upper face block H, with a small step as shown. A Neoprene O-ring J was placed on the horizontal face of this step, and the lower end of the condenser D pressed against it by the U-shaped spherical joint clamp K.

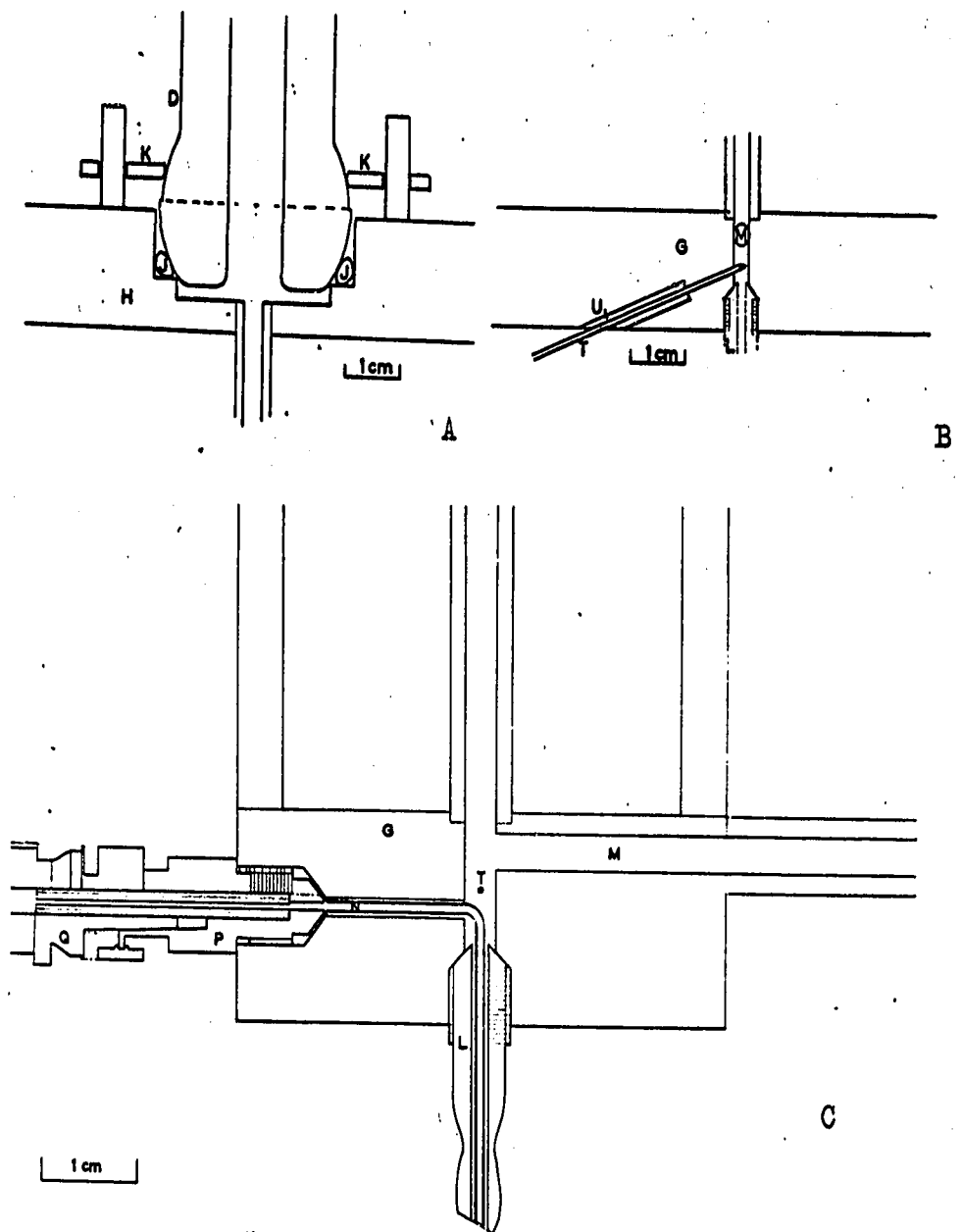


Figure 3. Construction Details from the Perfusion Apparatus of Figure 2.

- A. Method of sealing spherical glass joint of condenser D into block H, using Neoprene O-ring J and clamp K.
- B. Seal of needle thermocouple probe T with Teflon sleeve U, showing tip of probe in relation to aortic cannula L.
- C. Section at right angles to that of figure 3B, showing insertion of Nylon aortic cannula L into block G, and infusion tube N with adapter P.

From this level, the solution flowed to the selector tap F, which had a Teflon plug in a polymethacrylate sleeve. Below this, a single tube led to the lower block G, in which the aortic cannula was inserted. The two side columns had drainage tubes running from their entry into the selector tap barrel.

The machined Nylon aortic cannula L screwed into the block G. Somewhat above this, a bypass tube M was included in the construction, in case a Saxby-type (44) temperature stabilisation proved necessary. In fact, this was not needed, but the bypass was still useful for withdrawing air bubbles during the filling of the apparatus.

Just above the upper end of the cannula socket, the needle thermocouple probe T and the infusion cannula N were introduced. Fig. 3B shows the method of sealing the thermocouple needle by passing it through a Teflon sleeve U, formed to give a very close fit. The combination of the water-repellent surface and the low coefficient of friction of the Teflon enabled the needle to be passed through while the aqueous solution did not infiltrate the narrow annular clearance zone.

The infusion cannula N was a length of 1.09 mm outside diameter polyethylene tubing (Clay-Adams No. PE 20). Fig. 3C shows how this passed down the centre of the aortic cannula L, and was cut off flush at its tip. At the other end, the cannula N was secured by the male section P of a Clay-Adams Plastic Tubing Adapter; the block G was machined to the

inside dimensions of the female part of the adapter. The tip of the adapter section P thus sealed the flared end of the polyethylene tubing. A three-way tap Q, with a male Luer-Lok connection, was inserted in the adapter. As this pair of fittings had an internal volume of some 80 μ l, the combined lumens were stuffed with three concentric layers of polyethylene tubing (PE 20 inside PE 160 inside PE 205), reducing the total dead space from the plug of the tap to the tip of the cannula N to about 5 μ l. A drainage tube was connected to the lateral inlet of the tap: after a drug infusion or injection, the system was flushed in reverse by allowing some McEwen's solution to flow from the aorta back through the cannula and out through this drainage tube.

An array of funnels and tubing below the apparatus collected the various drainage streams and conducted them to a single waste receptacle. The whole apparatus was mounted on a frame of Dexion angle, which proved very useful for the attachment of ancillary sections for convenient operation of an experiment.

The temperature of the perfusion fluid was maintained by the circulation of water from a thermostatically controlled water-bath through the switch box and then the jackets of the helical-tube condensers. With the complete enclosure of the pathways of the perfusion fluid from its entry into these condensers, the temperature of the fluid entering the aortic cannula showed no variations with varying flow rates, or when changing from one column to

another.

The water bath consisted of an uninsulated 13 litre container, with a 500 watt heater controlled by a bimetallic thermostat (American Instrument Company, Model 4-235F) through a relay unit. The circulating pump also provided stirring of the contents of the bath. The temperature was monitored by a mercury-in-glass thermometer, divided to 0.1 C. No periodic variations in bath temperature could be detected by this thermometer, suggesting that the temperature of the water in the bath varied by less than 0.02 C. With this temperature constancy, the reference junction for the thermocouple probe was inserted in this bath.

Before starting an experiment, the main column was filled with McEwen's solution, and the selector tap opened just enough to permit the fluid to drop slowly from the tip of the cannula.

Langendorff Heart Preparation

Rabbits (*Oryctolagus cuniculus*) were stunned by a blow on the head, the throat cut and the thorax opened. The pericardium was then incised, the heart lifted up and removed by cutting the great vessels behind it. Most of the blood was flushed out by massaging lightly in some 150 ml of McEwen's solution in a bowl at room temperature (23-28 C), and the organ transferred to a second bowl of this solution; this bowl was then carried to the perfusion apparatus.

On reaching this, the aortic wall was secured by a clamped pair of forceps (still suspending the heart in the solution), its trunk freed of surrounding tissues, and the arterial ligament cut. The aorta was then cut just proximal to the commencement of the arch, and the aortic stump slipped over the perfusion cannula; a linen thread ligature previously placed loosely around this was tightened, and perfusion was begun by allowing the fluid to flow freely.

The blood remaining in the coronary vessels was rapidly diluted and washed out before it had time to coagulate; within less than a minute, the perfused fluid, originally pink, became colourless. Once perfusion had been started, any air bubble was flushed out by briefly opening the bypass outlet, and the injection cannula filled by 'back flushing'. Meanwhile, the ligature was properly knotted, relooped and retied to secure the aorta, and the superfluous venous tissue trimmed off. The recording attachments were then made.

It was important to make sure that a sufficiently long stump of the aorta be left so that the tip of the cannula barely entered the region of the aortic sinuses, and the aortic valves could close. The normal time between stunning and commencement of perfusion was about 100 to 150 seconds; between excising the heart and starting perfusion, from 40 to 70 seconds.

Recording: Series 1

The heart was anchored in position by securing the left ventricle with a clip (C.F. Palmer: Frog Heart Clip) attached to the frame of the apparatus by an electrically insulated rod. Another clip was fastened to the right ventricle; a linen thread connected this clip to a strain-gauge myograph (Grass model FT-10), passing over an aluminium pulley on the way. A fine platinum wire was also connected to this clip; this served as one of the stimulating connections, the other going to the clip in the left ventricle. Electrical activity was led off from two bipolar electrodes, one being applied to the atria, and the second to the surface of the right ventricle, usually near the apex. These electrodes were connected through a switch, so that activity could be recorded from either pair, or from one pole of each.

At the start, the electrical activity and the output of the myograph were amplified in conventional D.C. amplifiers, and displayed on the screen of a dual-beam cathode ray

oscilloscope, whose face could be photographed. When the Grass polygraph became available, this, with its integral preamplifiers, was used to make permanent records of the experiments; the dual beam oscilloscope was then connected to the polygraph so that it could be used as a convenient monitor, especially of the detailed shape of the electrogram.

Electrical stimulation with 750 μ sec long pulses was applied from a Grass S-4 stimulator through the clip electrodes already mentioned. A 100 ohm resistor was connected in series in one lead, and the voltage drop across this resistor due to the stimulating current was monitored on a second oscilloscope. In this way, the strength of the stimuli could be measured, at 10 ma for each volt across the resistor.

Attempts to produce persistent fibrillation were based on the method of Armitage et al. (29). The minimum stimulus current at which the ventricles would follow a rate of 5 pulses/sec was determined; the current strength was set at twice this value, and the frequency slowly increased up to and beyond the fastest rate the ventricles would follow. Fibrillation, if it occurred, generally appeared when the frequency of the stimulation had been raised to or slightly above this maximal follow rate. Stimulation was continued for a total of 5 minutes, then stopped, and the record observed to see whether fibrillation, if present, persisted. Long persisting fibrillation was arrested, when

so desired, by administering a small volume of concentrated (1.4 M) potassium chloride solution through the intra-aortal injection cannula.

Recording and Stimulating: Series 2

The recording and stimulating arrangements for the experiments of series 2 are shown in fig. 4. A Grass FT-03 strain-gauge myograph was used to record the contractile activity of the heart. The armature of the myograph was connected by a Nylon rod to an aluminium pulley which served as a bell crank; this rod ran at a slight descending angle, to reduce the risk of perfusate reaching the myograph itself. A second point on the pulley, 90° removed from the point of attachment of the Nylon rod, was connected to the apex of the heart. Initially, this connection was a heart clip similar to those used in the experiments of series 1; later, a B&S #22 platinum wire hook, impaling the thickest part of the ventricular wall at the apex, was used.

Recording electrodes were applied to the left ventricular wall and to the right atrium. Bipolar electrodes proved awkward: movement artefacts were too frequent; so hook electrodes of B&S #26 platinum wire were finally used. The atrial electrodes were inserted, one in the auricular appendage, and the second above the entry of the superior vena cava; the ventricular were equally spaced from base, apex, and one another, about 5 mm lateral to the interventricular groove.

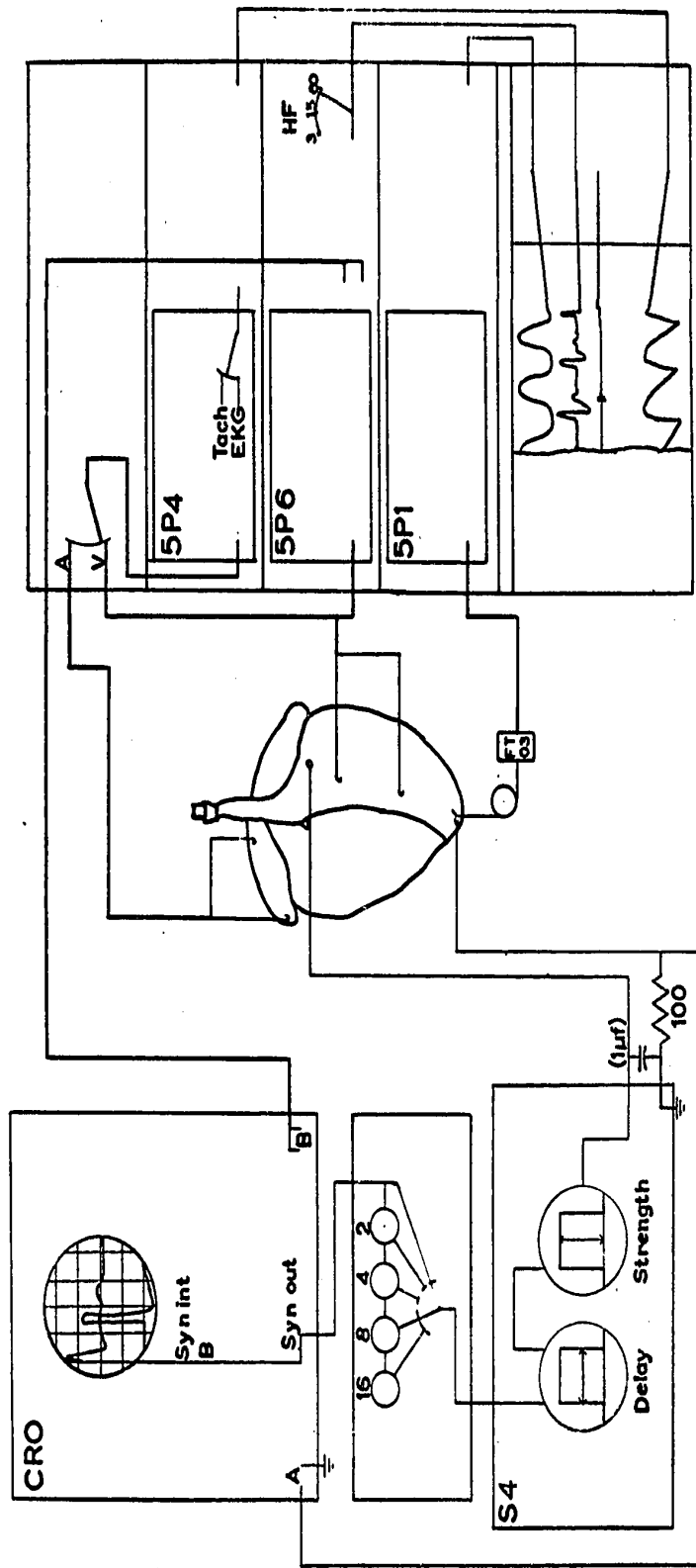


Figure 4. Block Diagram of the Recording and Stimulating Connections. Force and electrical activity recorded on polygraph; electrogram also monitored on oscilloscope. Stimulator activated through counter on every 8th beat; output current measured on second channel of oscilloscope.

One stimulating electrode was clipped to the base of the left ventricle: it was necessary to be sure that the left auricle did not touch it, and that it did not occlude the left descending coronary artery. The second stimulating electrode was at the apex. Initially, the apex attachment was used; later, a separate lead was clipped close to this point.

Channel 1 of the polygraph was used to monitor the mechanical activity. The myograph was connected to the preamplifier input in a bridge circuit, and its output signal then amplified. In the majority of cases, a diastolic tension of 50 gm weight was applied; this gave almost maximal force under the Starling's Law relationship.

Ventricular action currents were picked up as the potential difference between the two recording electrodes, then amplified and recorded by channel 2 of the polygraph. The amplified signal was also fed to one input of the dual-beam oscilloscope and displayed; its 'QRS' deflection was used to trigger the sweep in synchrony with the heart beat.

A switch allowed the selection of whether ventricular or atrial electrical activity was applied to the input of the third polygraph channel. This channel provided the option of recording either the normal electrogram (used for the atrial activity) or a 'tachogram', showing the rate and regularity of beating; this was composed of a series of deflections linearly proportional to the time between successive input impulses. The latter was more normally

used.

The 'QRS' complex of the ventricular electrogram triggered the oscilloscope sweep. An output pulse, synchronous with the start of the sweep, was passed through a scale-of-two counter; on the start of every eighth sweep, the output of the counter triggered the Grass S-4 stimulator. After a set delay, the 10 msec long output pulse was generated. The voltage drop across the 100 ohm series resistor in the stimulating circuit was applied to the second channel of the oscilloscope, its position in the cardiac cycle monitored, and its strength measured by applying Ohm's Law (i.e., 1 volt was equivalent to 10 ma). The 1 μ f capacitor placed across the output terminals of the stimulator was needed to produce a square current pulse; without it, the initial peak was so sharp as to render accurate measurement of its height impracticable.

The delay being initially set at a low value (50 to 60 msec), the current strength was set to a suitable point on the scale used. This consisted of steps equally spaced on a logarithmic scale, with 20 steps for a factor of 10 in current strength, or 6 steps for a factor of 2. (See table 3). The delay was then increased by steps of 5 msec following each stimulus until normal (diastolic) extrasystoles were obtained. The end of this sweep was usually 20 to 30 msec after the expected vulnerable time.

TABLE 3. VALUES FOR CURRENT STRENGTH,
OVER ONE DECADE

1.0	1.12	1.25	1.4	1.6	1.8
2.0	2.25	2.5	2.8	3.2	3.6
4.0	4.5	5.0	5.6	6.3	7.1
8.0	9.0	10.0			

After a completed sweep at one current strength, this was increased by one, two or three steps (according to the conditions of the particular experiment), and a new sweep made. This process was continued until ventricular fibrillation of at least one second duration was observed. The minimal current inducing fibrillation was taken as threshold, and the delay time at which this occurred as the vulnerable time.

If the fibrillation did not spontaneously revert to normal rhythm, it was arrested by slowly injecting intra-aortally a small volume (about 0.1 to 0.5 ml) of 2M potassium chloride solution.

During stimulation, the rise time constant of the electrogram amplifier was set at 0.1 sec, and the decay time constant at 0.2 sec. This reduced the amplitude of the pen record, but not of the oscilloscope trace, and was done to damp the pen excursions due to stimulus artefacts.

A method for continuously monitoring the coronary flow had not been built into the apparatus. Hence, such measurements as were considered necessary were performed on

a spot basis. The perfusion fluid flowing from the heart was collected for 30 seconds, and this volume measured. Doubling the figure so obtained gave the coronary flow per minute.

Drugs and Treatments

All chemicals used for the preparation of the McEwen's solution were of reagent chemical grade. At one stage, some trouble was encountered from impurities in the sodium chloride, both in this investigation and in others in progress in the same department. In the isolated perfused heart, the trouble was manifested by the development of irregularities in the sinus rhythm after some 2-3 hours perfusion. Fisher Reagent grade sodium chloride was found to be fully satisfactory.

Levels of potassium. The various concentrations of potassium used were selected from a set equally spaced on a logarithmic scale, thus giving equal steps of potassium voltage. For convenience, the potassium levels were indicated on a pK^+ scale, analogous to the pH scale for hydrogen ions; on this scale, the normal potassium concentration of 5.6 mM had a pK^+ value of 2.25.

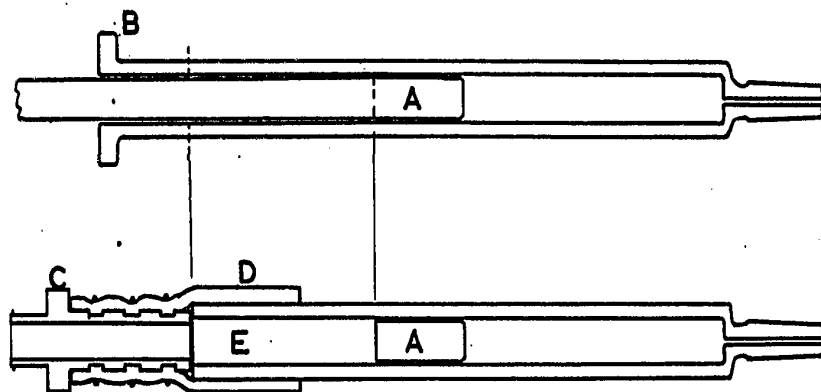
Reserpinisation. For depletion of the cardiac stores of catecholamines, injections of reserpine (dissolved in 1 N acetic acid) were given in the same manner as used by Burn and Rand (52): the rabbits were injected intraperitoneally with 1.5 mg/kg 45 hours before, and 5 mg/kg 23 hours before

starting the experiment.

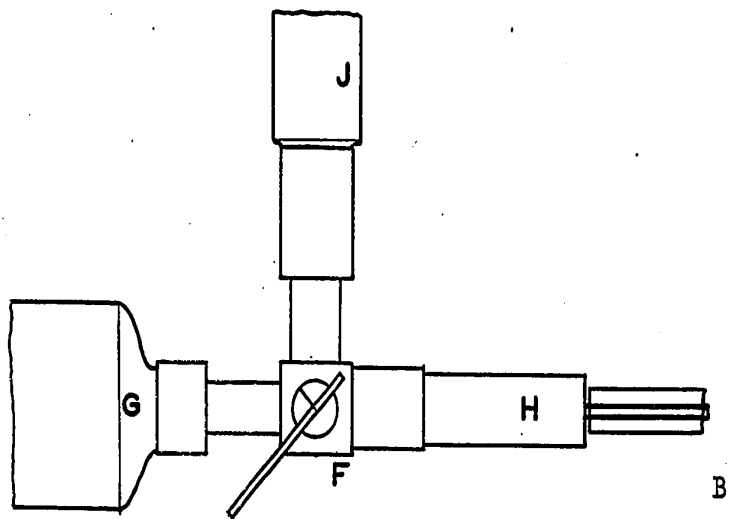
Infusions and injections. Wherever a quantitative estimate of drug effects was sought, the drug in question was infused at a known rate. Manual injection was only used for defibrillation.

The infusion rates were set with an electrically driven pump (C.F. Palmer: The Continuous Slow Injector): this had a screw-driven ram, with six selectable speeds; with a 20 ml syringe, these gave infusion rates ranging from 25 to 800 μ l per minute, with a factor of 2 between successive rates. From this syringe, a reinforced tube, of PE 160 polyethylene tubing jacketed by 1/16 in wall polyvinyl chloride tubing, led to the direct inlet of the three-way tap fastened to the infusion cannula.

In a number of cases, small volumes of concentrated solutions were to be infused. In order to reduce the total volume of such solutions needed from 5-10 ml to about 1 ml or less, a special small volume infusion syringe was set up. Fig. 5 illustrates this. A standard 1 ml tuberculin syringe with a solid glass plunger was taken. A 15 mm length of the plunger tip was cut off and used as a piston A; the outer lip B of the syringe barrel was removed, and a female Luer socket to tubing adapter C was connected with a piece of close-fitting polyvinyl chloride tubing D. As is well known, this tubing adheres firmly to glass; the seal to the metal adapter was increased by binding with copper wire bands.



A



B

Figure 5. Infusion Arrangements for Small Volumes.
 A. Standard 1 ml syringe, before and after modification.
 B. Connections of infusion syringe G and auxiliary syringe J to adapter H for reinforced tube.

The space E behind the piston was filled with distilled water. All air bubbles were removed, and then the reinforced tube, also filled with water, was connected to the adapter C. At the infusion pump, a three-way stopcock F was placed between the infusion syringe G and the adapter H at the end of the reinforced tube; a small (2 or 5 ml) syringe J was attached to the side arm of the stopcock. This syringe could then be used for manual control, e.g., filling of the infusion syringe by withdrawing the piston A; the main syringe G then gave the controlled infusion rate.

In a few early experiments, stock solutions of drugs, made up in 1 mM hydrochloric acid solution, were kept in the refrigerator: in the majority of the experiments, however, a small amount of concentrated solution was made up during the experiment, and further diluted as required. All solutions for infusion contained sufficient sodium chloride to bring their nominal osmolarity to 320 mOsm.

Adrenaline and isoprenaline contained about 10 μ M hydrochloric acid to prevent alkaline oxidation; reasons for the choice of this particular level of acidity are given later.

The drugs used in this part of the investigation were: Adrenaline. Epinephrine Bitartrate (1-adrenaline hydrogen d-tartrate), Nutritional Biochemicals Corporation. Carbachol. Carbachol (carbamylcholine chloride), British Drug Houses.

Chloroform. Chloroform (trichloromethane), ACS Reagent,
Fisher.

Procaine. Procaine Hydrochloride (diethylaminoethanol
p-aminobenzoate hydrochloride), U.S.P., Merck

Statistical Methods

If the data for any parameter showed a large range, with a ratio of 3:2 or greater between extreme values, they were usually transformed to a logarithmic scale before statistical calculations were carried out. When this was done, the final arithmetic mean of the logarithms was taken, and retransformed to the original scale of measurements to give a geometric mean (GM). Only where the range of the original data was quite narrow was an arithmetic mean (AM) normally calculated.

Statistical methods were normally those of Fisher (53), with tests of significance being evaluated from the tables of Fisher and Yates (54). The normal approach used to determine the significance of particular effects was the technique of the Analysis of Variance. This uses the Variance Ratio for the actual significance tests. Standard deviations (SD) and standard errors of means (SE) were calculated where required. Where the description being made was one of a population, the standard deviation was normally shown in the tables; if the question was one of the significance of means or differences, then the standard error was used. Significance levels given are the decimal

(rather than percentage) values of the conventional P, the probability of as large a difference being obtained if the null hypothesis that both samples were drawn from the same population was true.

When standard deviations or standard errors were obtained on a logarithmic scale, they were reconverted to the best approximations on the scale of the original measurements. Where this gave intractable or absurd results (as with the population of thresholds), then the standard deviation on the logarithmic scale was expressed as its logical equivalent, the factor on the original scale to be applied to the mean (as multiplier or divisor) to find the appropriate points on each side.

In the tests of 2 x 2 tables made, the chi-square test was not appropriate, as there was always an expectation of less than 5 in some cell; instead, Fisher's Exact Probability test (53, p.96) was used: this is based on the possibilities obtainable under the binomial distribution.

EXPERIMENTAL PROCEDURES AND RESULTS: SERIES 1 .

Potassium Levels

The hearts were initially perfused with the normal (5.6 mM) potassium solution. After a half-hour stabilisation period, stimulation was started at a frequency of 5 pulses/sec, and the current gradually increased. The minimal current at which the ventricles would just follow this rate was recorded as threshold. The current was then set at twice this value, and the frequency of the stimulation increased. The amplitude of the mechanical record decreased as the frequency was raised; at a certain point, the ventricles failed to respond to every pulse: the size of the response following a missed beat was greater, due to the longer interval preceding it. The maximal rate at which the ventricles would follow each pulse was noted: this was recorded as the 'maximal [follow] rate' (Dawes, 55). As the frequency was further increased, the record became very irregular, until the ventricles started to respond only to every second pulse, and the record became regular once more.

When ventricular fibrillation appeared, it was normally at a rate somewhat above the maximal follow rate. Occasionally, fibrillation started before a maximal follow rate had been determined: on other occasions, carrying the stimulation frequency as high as 20 pulses/sec produced at the most a transient fibrillation, or even none at all. The presence of ventricular fibrillation was judged by the

appearance of the mechanical record: the criterion was the absence of any regular oscillation, as was always seen even at the maximal follow rate, and the presence of irregular movements of extremely small amplitude. The electrical record was less clear during stimulation; large stimulus artefacts interfered too much. The atria were unaffected, and continued to beat regularly.

Stimulation was continued for a total of 5 minutes, and then stopped. Fibrillation, if it had been induced, could be affected in one of three ways:

(a) it would stop immediately, and the ventricles revert to a normal sinus rhythm;

(b) it would continue for a short time before reversion occurred;

or (c) it would persist indefinitely.

A dividing line had to be drawn between (b) and (c); taking into account the results from the Oxford laboratory (28, 29, 30, 31, 32, 56), this was originally drawn at 5 minutes after the end of stimulation. Observation was continued for at least 5 minutes, and often to 15 minutes; the latest reversion seen was less than 80 seconds after the end of the stimulation, so that, in fact, a division at 2 minutes was sufficient. Again using the criterion of the Oxford workers, a positive result was recorded only where ventricular fibrillation occurred and persisted [(c) above]; this was denoted as 'persistent fibrillation'. All other outcomes of the stimulation were recorded as negative.

At the end of the observation period in the case of persistent fibrillation, the ventricles were arrested by injecting a small amount of a 1.4 M solution of potassium chloride into the aorta; they then resumed a normal sinus rhythm as the excess potassium was washed out.

The next step was to perfuse the heart with a solution of changed potassium concentration. The selection of the new concentration depended on the result of the previous test: if this had been positive, the potassium concentration was increased; if negative, it was decreased. The attempt to produce persistent fibrillation was then repeated at the new potassium level. The whole process was continued, changing the potassium concentration by successive steps of 0.2, 0.1, and 0.05 on the pK^+ scale; the direction of each change was determined by the outcome of the preceding test. Thus, two potassium concentrations were determined: at one, a negative result was obtained, and at the second, 0.05 pK^+ units higher, a positive. The latter was considered as the threshold pK^+ for induction of persistent fibrillation. One advantage of the pK^+ scale here was the fact that at all values above the threshold, a positive result should occur; on a direct concentration scale, the logical threshold would be one for non-persistence of fibrillation.

Implicit in the preceding section is the question whether such a threshold could be determined. It could. Table 4 shows the results of seven experiments on hearts from normal rabbits. These results are consistent with those

of Armitage et al. (28), and of Kärki (31).

TABLE 4. THRESHOLD pK^+ FOR PERSISTENT VENTRICULAR FIBRILLATION

Normal		Reserpinised	
Expt No.	Threshold pK^+	Expt No.	Threshold pK^+
5	2.15		
6	2.30		
7	2.30	8	2.30
10	2.40	9	2.30
11	2.30	16	2.50
15	2.15		
17	2.40		
Mean	2.29		2.37
SD	0.10		0.11
Difference of means		0.08	
SE		0.07	

As the threshold current for driving and the maximal follow rate had both been determined, a check was made whether these were altered by varying the potassium concentration. No significant effects could be detected; the appropriate 't' test in each case only achieved a significance level of 0.1.

With the normal potassium concentration, the (geometric) mean threshold for driving was 1.7 ma, with a

standard deviation of a factor of 1.4x; for the maximal follow rate, the mean frequency was 8.8 per second, with a standard deviation of ± 0.7 .

The mean temperature recorded at the aortic cannula was normally between 36 C and 36.8 C; fluctuations in any one experiment were seldom more than ± 0.4 C.

The results from the first three experiments with hearts from reserpinised rabbits are also shown in table 4. As can be seen, they fit quite well among the normal values; no clear-cut difference appears. The threshold for driving and the maximal follow rate also failed to show significant differences from the controls.

Faradic Stimulation Thresholds

At this stage, 10 experiments had supplied only 10 figures. This slow rate of accumulation of data suggested that an enormous number of experiments would be needed to quantitate the effects of even the most powerful agents. As for smaller effects, they would surely be confounded with the differences in response between the individual hearts. A search for a better method was thus started.

Injections of calcium salts, as used by Grumbach et al. (26), had already been tried. The major problem here was the careful control of the injection rate that was necessary, and so this method was laid aside until the necessary instrumentation could be developed.

The next simple method seemed to be the determination of the minimal faradic current needed to produce fibrillation. Szekeres and Lénárd (57) claimed to have obtained consistent results by this method. Following their description, the heart was stimulated with 10 msec pulses at a frequency of 30 pulses/sec, and the current strength gradually increased until ventricular fibrillation was produced. Fibrillation did occur, but there was little correlation with the current strength at the moment of onset. It seemed that the rate of increasing the current was important; however, perusal of both the paper of Szekeres and Lénárd, and that of DiPalma, Lambert, Reiss and Schults (58) [who had been investigating atrial fibrillation], failed to disclose any details of this significant factor.

Not only did fibrillation thresholds vary within any one experiment: in many cases, no fibrillation was obtained at relatively high currents (e.g., 1.4 ma), and a short while later, a much lower current (400 - 500 μ a) would produce fibrillation. The major drawback to this method, as to that of Armitage et al. (28), was that often, even under constant stimulation conditions, fibrillation appeared only after a delay of a minute or more. This introduced an uncertainty as to whether the measured threshold was the true one or an overestimate. Further, it was difficult to say whether the investigation was of the induction of fibrillation, or of its persistence, if and when induced.

EXPERIMENTAL PROCEDURES AND RESULTS: SERIES 2

Single Pulse Fibrillation Thresholds

The only unequivocal method of inducing (or failing to induce) fibrillation now seemed to be the single pulse method originated by Wiggers and Wégria (11), and most recently improved by vanTyn and MacLean (19).

While preparing for these experiments, the improved version of the perfusion apparatus was assembled, making for better temperature control and easier changing between perfusion solutions. With the complete jacketing of the path of the perfusion fluid until its entry into the aortic cannula, temperature fluctuations measured at this point were less than 0.1 C at most flow rates, or on switching between different solutions. The temperature of the external surface of the heart was checked on occasion, using a stirrup thermocouple probe; the temperature here was within 0.6 C of that of the fluid entering the aorta.

All the remaining experiments, whose results are to be given in the succeeding sections, were performed using this apparatus and the procedure to be described.

As the measurement of current strength was important in these experiments, the accuracy of the monitoring system was checked from time to time. The absolute sensitivity of the cathode ray oscilloscope was set using a 1.35 volt mercury cell as reference, and the value of the series resistance was checked with an ohmmeter. This resistance was within 1%

of its nominal value. The attenuator of the oscilloscope was checked by noting that a full scale deflection on one scale gave the correct deflection on the next lower sensitivity. This always held true, to within the resolution of reading the instrument.

In a number of cases, ventricular fibrillation occurred while the heart was being set up on the apparatus, seemingly triggered by the manipulation, particularly the insertion of electrodes; when this happened, the heart was defibrillated in the usual manner (by injection of potassium), and the experiment continued.

During the first half hour after starting the perfusion, the force of contraction of the ventricles gradually increased to a stable level. The ventricular electrogram, monitored on the oscilloscope screen, often had a monophasic contour initially; as the tissues around the electrodes 'healed', it gradually became biphasic, resembling a normal electrocardiogram, and showing typical 'QRS' and 'T' waves. The S-T segment, however, was often displaced from the isoelectric line; such displacements seemed without particular significance.

From time to time, irregularities in the spontaneous rate were observed. These fell into two classes: first, an alternating rhythm, alternate beats differing in duration by 10-20 msec; and secondly, alternate periods of rapid and slow beating, each phase often being several minutes in length. In both these cases, the heart was electrically

driven through the atria at a rate just fast enough to prevent escape.

Once the force and rate of the heart had stabilised, the first measurements were made. The coronary flow was first recorded. Then the stimulator delay was set at about 150% of the R-T interval as measured on the oscilloscope, and the stimulation started (one pulse after every eighth normal beat) at a current strength of about 100 μ a. The current strength was gradually raised until extrasystolic responses appeared, with stronger than normal contractions following the subsequent compensatory pauses. The current strength was then varied to find the diastolic threshold value which just produced these responses. This was generally of the order of 200 - 400 μ a, and could be measured to an accuracy of 2%. It was not noticeably altered by responses after the first.

The delay was then set at a value of about 50% of the R-T interval (to an integral multiple of 5 msec), and the stimulation started at the nearest step on the standard scale to the diastolic threshold value. The delay was increased by 5 msec after each pulse until a total of 50 msec had been swept over. If no extrasystoles had appeared, the sweep was repeated with a current strength three steps higher on the scale. The appearance of an extrasystole following a pulse showed that this pulse had been applied outside the apparent refractory period corresponding to its strength. If such an extrasystole only appeared late in the

sweep, the three step jump in current strength continued. When the apparent refractory period suddenly shortened, as shown by a much earlier appearance of the first extrasystole, the change was decreased to two or one steps, as this shortening normally indicated an approach to the fibrillation threshold. The first stimulus of a sweep was always delivered at least 10 msec before the end of the last determined apparent refractory period.

The 'dip' phenomenon, described by Brooks, Hoffman, Suckling and Orias (59, p.82) appeared on occasion. This was manifested by the successive appearance during a single sweep of a response, its disappearance at a slightly greater delay, and return to persist. The time of the early response was generally close to the vulnerable time at that stage; the current strengths at which the phenomenon appeared were close to the fibrillation threshold.

At a certain current level, one pulse (which might fall at the end of, or outside, the apparent refractory period) initiated ventricular fibrillation; this always started abruptly, and could be recognised on the myogram and the oscilloscope, and to a lesser extent on the highly damped electrogram. The tachograph record showed a typically irregular tracing. Fig. 6B shows such an initiation of fibrillation: also shown for comparison, fig. 6A, is the extrasystolic response obtained on the previous sweep. This was the first stimulus of the sweep falling outside the apparent refractory period for this current strength, and

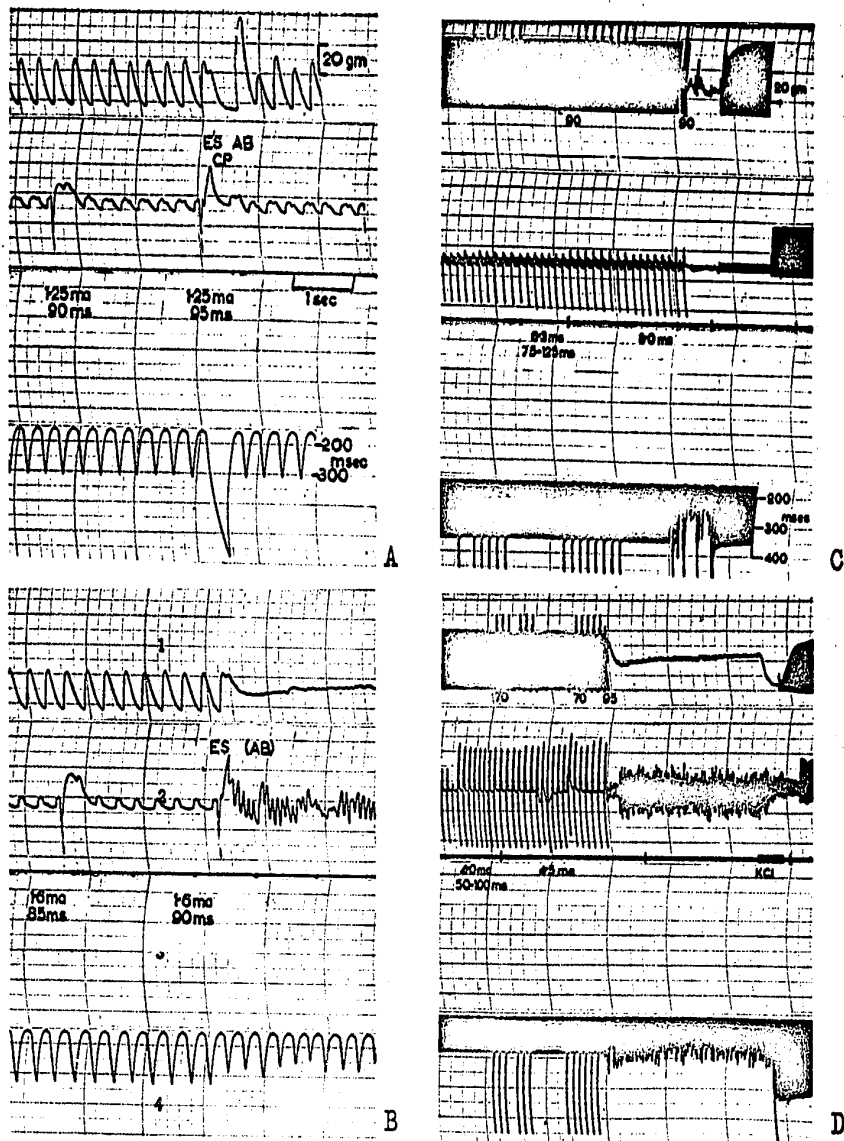


Figure 6. Induction and Patterns of Ventricular Fibrillation.

Polygraph records show, reading down: Ventricular force; ventricular electrogram, highly damped, with stimulus artefacts; time; current strength and delay from R wave; and cardiogram. Scales the same in A & B, also in C & D, which have time scale in minutes.

- A. Extrasystolic response ES at subthreshold current, with augmented beat AB following compensatory pause CP.
- B. Induction of fibrillation at threshold, with initial extrasystolic response and expected time of next beat.
- C. Induction of non-persistent fibrillation, and spontaneous reversion.
- D. Induction of persistent fibrillation, and defibrillation with KCl.

the latency from the stimulus (end of the apparent refractory period) to the extrasystolic response ES (end of the effective refractory period) can be seen; this response is visible on both the damped electrogram and the myogram. The compensatory pause CP, and the following augmented normal beat AB, are also evident.

On the next sweep (fig. 6B), it can be seen how ventricular fibrillation started immediately following the extrasystole, although a small electrical response can be seen at the expected time of the augmented beat; this may be due to the fact that fibrillation, initiated in the left ventricle between the stimulating electrodes, took some time to spread into the right ventricle. The atria meanwhile continued to beat normally.

Fibrillation, once initiated, could be divided into two classes: persistent and non-persistent. Persistent fibrillation continued indefinitely; non-persistent reverted spontaneously to a normal rhythm. As the myogram was normally used to recognise the onset of fibrillation, there was some doubt about durations of less than one second. Hence, an arbitrary distinction was made, and only a fibrillation which persisted for more than one second was considered to have occurred; shorter bursts were considered as three or four multiple ventricular extrasystoles.

If a short non-persistent fibrillation was obtained, further stimuli were applied. In some cases, a second stimulus of the same or higher current strength would

produce a persistent fibrillation: in other cases, only non-persistent fibrillations were obtained, even with far stronger stimuli. If a persistent fibrillation was obtained at the same current strength as the non-persistent, the threshold was recorded as being for the persistent: if only at a higher current, then both thresholds - for non-persistent and for persistent fibrillation - were recorded.

After the observation period, persistent fibrillations were arrested by slowly injecting 2M potassium chloride solution until complete ventricular depolarisation and arrest was produced, and then allowing the excess potassium to be washed out, and the normal rhythm to resume. The usual volume required varied between 0.1 and 0.5 ml. Occasionally, a second injection was required if the fibrillation resumed after recovery from the first; very often, this was due to not having had complete arrest with this injection.

A rest period of at least 15 minutes was allowed between successive determinations of the ventricular fibrillation threshold. Once an approximate value was known, the next determination could be started with a current strength about 5 steps below the previous threshold value, and this increased by single steps between the sweeps.

Typical records of non-persistent and persistent fibrillations are shown in figs. 6C and 6D respectively. The dividing line between these two classes was finally set at 60 seconds: only 5 of the 175 non-persistent fibrillations lasted longer than 30 seconds, so that no significant error

was introduced by this division. Further, the force of contraction always fell off following a prolonged period of fibrillation, and the fall seemed greater, the longer this period.

Table 5 summarises the distribution of the durations of the non-persistent fibrillations. The duration as such was of little significance; the only consistency was of non-persistence or persistence.

TABLE 5. DURATIONS OF NON-PERSISTENT FIBRILLATIONS

Seconds	Number	Seconds	Number
1	16	9	2
2	39	10	0
3	29	11	3
4	17	12	3
5	15	13 - 17	11
6	8	18 - 22	7
7	7	23 - 30	7
8	5	Over 30	5

These durations seemed to fall into two classes, an initial Poisson distribution from 1 to 10 seconds, and a second, more spread out range, with the median value at 20 seconds.

The first ventricular fibrillation thresholds determined for each heart were grouped according to the

persistence or non-persistence of the fibrillation, and then the figures in each group analysed in conjunction with the weights of the animals from which the hearts were taken. These data are summarised in table 6.

TABLE 6. STATISTICS OF INITIAL VENTRICULAR FIBRILLATION THRESHOLDS

	Non-Persistent	Persistent	P: Difference in means
Number in group	24	35	
Mean weight (AM)	2.12 kg	2.36 kg	< 0.05
SD	0.51	0.37	
Mean threshold (GM)	2.5 ma	1.5 ma	< 0.02
SD (factor)	3.3 x	2.6 x	
P: correlation, weight/log threshold	< 0.01	> 0.3	

Examination of the overall results showed that all 8 hearts from rabbits weighing less than 1.75 kg gave non-persistent fibrillations initially. Application of Fisher's Exact Test for 2 x 2 tables (53) indicated that the probability of such an asymmetric distribution arising by chance was 1/3015. On the other hand, although all 3 initial thresholds below 450 μ a were for persistent fibrillation, the probability of this distribution was 1/5

Thus there was evidence, supported by the just significant difference in mean weights, that the hearts from lighter rabbits were more likely to show non-persistent fibrillation. The correlation between weight and logarithm of the fibrillation threshold in the non-persistent group was positive; the test for difference in the mean thresholds was corrected for the effects of this correlation. The significance of this difference indicates that, for hearts from animals of the same weight, the fibrillation threshold tended to be higher where only non-persistent fibrillation was found. None of these effects was sufficiently clear-cut to be of much use in predicting the result with any given heart.

The overall range of the initial ventricular fibrillation thresholds was from 110 μ a to 22.5 ma. Only 1 out of 60 hearts tested failed to fibrillate initially at a current of 45 ma, the maximum stable output of the stimulator.

Control Experiments

A number of control experiments showed that ventricular fibrillation thresholds could be measured for at least 7 hours. The values obtained fitted consistently into one of two patterns, either a steady level, or, less often, a gradual increase over the whole experimental period. It was not possible to say why one pattern or the other appeared in a given heart. As the experiment progressed, a heart that originally gave only non-persistent fibrillation might later give persistent; the reverse conversion seldom occurred.

The pattern of a typical experiment is shown in fig. 7. Here, both the highest non-fibrillating, and the lowest fibrillating, currents have been plotted to show how closely the fibrillation threshold could be estimated. An atypical first value as shown occasionally appeared; succeeding thresholds were always more consistent. Because a gradual drift in the value of the threshold could occur, estimations of the effects of the various treatments used were always made with reference to preceding and following control determinations.

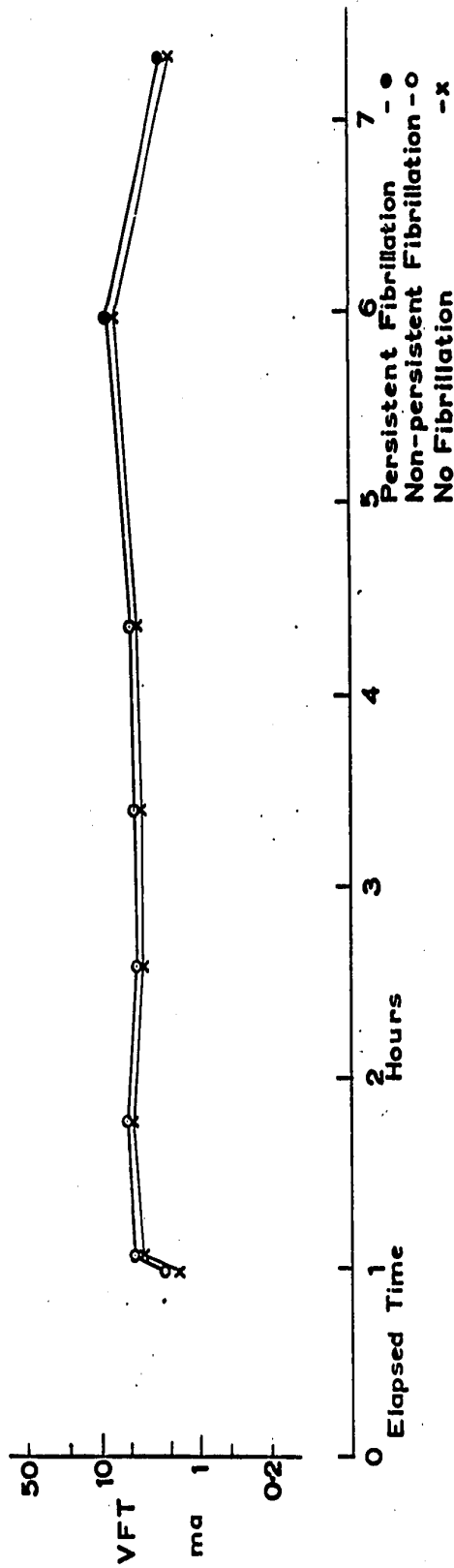


Figure 7. Consistency of Ventricular Fibrillation Thresholds (VFT).
 Ordinate: current strength in ma (log scale); abscissa: elapsed time since excision of heart. Crosses show highest current at which no fibrillation was produced, and circles, lowest current at which fibrillation occurred, i.e., fibrillation threshold. Persistence or non-persistence of the fibrillation is indicated by closed and open circles respectively. (Experiment 39)

Effects of Changing Potassium Levels

Potassium concentrations from one-half to twice normal (2.8 to 11.2 mM) were tested. Initially, attempts were made to duplicate Goodford's experiments with adrenaline (30) by finding a potassium concentration just high enough to produce non-persistent rather than persistent fibrillation. This was not as easy as it seemed, and in some cases the potassium concentration needed was as high as 11.2 mM. Such high concentrations were in themselves toxic, producing sinus arrhythmias and irregular beating. The subsequent administration of adrenaline aggravated these arrhythmias.

The next step was to investigate the effects of variations in the potassium concentration on the magnitude of the ventricular fibrillation threshold. For these experiments, control fibrillation thresholds were always determined before and after perfusion with the solution of altered potassium concentration. The results were later plotted as in fig. 8. A logarithmic scale was used for the ventricular fibrillation threshold strength (in practice, to facilitate the calculations, the logarithms of the thresholds were used for plotting); this was plotted against the elapsed time since killing the rabbit. The assumption was made that changes in the logarithm of the fibrillation threshold would take place, to a first approximation, smoothly with time, and that interpolation between determinations would thus be justified. This assumption was supported by a sufficiently large number of the experiments.

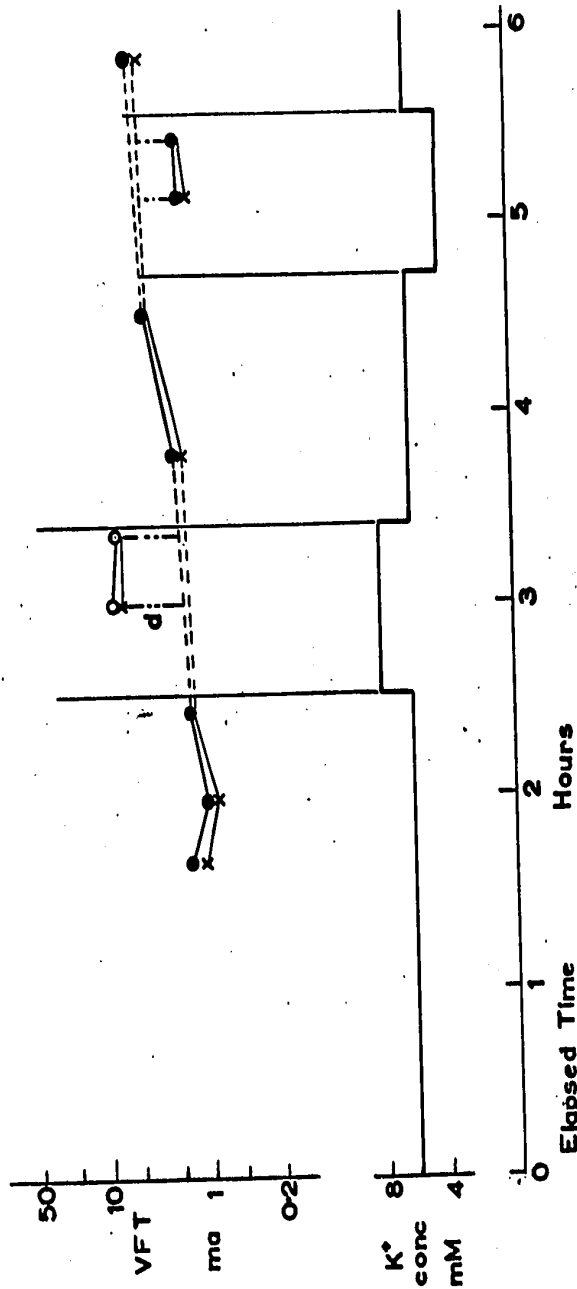


Figure 8. Effect of Potassium Concentration on Ventricular Fibrillation Thresholds.

Symbols as in figure 7. Also included: potassium concentration of perfusing fluid (log scale). Dashed line indicates interpolation; broken line, as at \bar{d} , the measured change in threshold. Highest non-fibrillating current not marked by symbol if it was only one step below measured threshold. (Experiment 87)

The changes in fibrillation threshold resulting from the alteration of the potassium concentration were thus estimated from the interpolated control values; where the threshold was not closely bracketed (to one step on the current strength scale), the minimal possible change was recorded. The change was measured on the logarithmic scale (i.e., as the logarithm of the ratio of test to control threshold), and these logarithms averaged. The mean value was then converted to a [geometric] mean ratio. The same procedure was used for all treatments tested.

A concentration ratio of 1.4 ($2^{1/2}$) was normally used to avoid secondary effects from highly unphysiological potassium levels: thus the high potassium concentration was 8 mM, and the low, 4 mM. Changes in rate and force of contraction of the heart occurred following the changes between the various perfusion fluids, but were transient, and the original rate and force were reattained after some 10 to 15 min. Normally, about half an hour was allowed as a stabilisation period before determining the first fibrillation threshold at the new potassium concentration. No significant changes in diastolic thresholds were seen. The ventricular fibrillation threshold was raised with the high potassium concentration, and fell with the low. Such conversions between persistence and non-persistence of fibrillation as were observed were in the expected directions: persistence becoming non-persistence as the potassium level was raised and vice-versa. The overall

results of changes in the fibrillation threshold are shown in table 7, together with those from other treatments. Tabulated are: the number of tests made; the mean change in the logarithm of the fibrillation threshold and its standard error; the significance of the 't' test for difference from zero; and the change in the threshold expressed as a ratio, fractional ratios indicating decreases, and non-fractional, increases.

TABLE 7. ALTERATIONS IN THE VENTRICULAR FIBRILLATION THRESHOLD (VFT) PRODUCED BY VARIOUS TREATMENTS

Treatment	Number of Tests	Mean Change \pm SE in Log_{10} (VFT)	P : Difference from Zero	VFT ¹ Change Ratio
High Potassium (8.0 mM)	6	+0.61 \pm 0.02	< 0.001	4.1
Low Potassium (4.0 mM)	10	-0.47 \pm 0.09	< 0.001	1/3.5
Adrenaline 8 nMole/min	8	-0.11 \pm 0.04	= 0.05	1/1.3
alone 32 nMole/min	8	+0.23 \pm 0.17	> 0.2	1.7
Carbachol alone	6	+0.03 \pm 0.04	> 0.5	1.1
Carbachol + Adrenaline	4	-0.74 \pm 0.12	< 0.01	1/5.5
Chloroform + Adrenaline	4	-0.59 \pm 0.07	< 0.01	1/3.9
Raised Pressure (56 - 80 mm Hg)	11	+0.38 \pm 0.09	< 0.01	2.4
Procaine (60 - 150 μ M)	5	+0.24 \pm 0.08	< 0.05	1.75

¹VFT Change Ratio: Ratio of VFT under treatment to its control value.
1/3 denotes a reduction to one-third of the control level.

The Effects of Adrenaline

The first infusions of adrenaline were made using a vehicle of 160 mM sodium chloride solution containing 1 mN hydrochloric acid to inhibit oxidation. Infusions of small quantities (1 nMole/min) produced the expected acceleration of sinus rate which the ventricles followed. Larger infusion rates (4 nMole/min) gave, as anticipated, ventricular extrasystoles with some atrio-ventricular block. In some cases, however, unexpected sinus arrhythmias appeared: these were found to occur at the higher infusion rates. Calculation of the possible shift in pH of the McEwen's solution due to the acid in the infusion showed that this was extremely small, being less than 0.02 units under the worst possible conditions. Intensive examination of the constituents of the infusion solution showed that these sinus arrhythmias only appeared when infusing more than 100 μ l/min of solutions with a pH less than 4. Hence, the composition of the infusion solution was modified so that the pH of the final mixture was always above 4, and normally close to 5. Provided that this solution was kept enclosed, as in the infusion syringe, no noticeable oxidation (to yield pink adrenochrome) was observed; flushings from the side-arm of the three-way tap (fig. 3C, Q), collected in an open beaker, reacted quickly with the air to give a noticeable colour.

An attempt was made to find a rate of adrenaline infusion that would not of itself produce significant

arrhythmias, although giving as large an accelerative response as possible. Preliminary testing indicated that the accelerative response to adrenaline could best be described as a gradual approach to a fixed maximum rate as the infusion rate was increased. The higher infusion rates produced a progressive series of pacemaker shifts: these were well delineated by concurrent recording from the atrial and ventricular electrodes at moderate paper speeds.

Control observations showed a PR interval of some 100 msec, and an RT duration of about 150 msec. As the adrenaline was infused at successively increasing rates, a regular sequence of changes occurred.

Initially, the T wave became deeper, and the RT segment depressed, until a single R+T deflection, some 100 msec in duration, appeared on the ventricular electrogram. Meanwhile, the PR delay shortened slightly. Some ventricular extrasystoles could appear, these giving complexes of reversed polarity in the electrogram. The next step was the development of an atrioventricular nodal rhythm. This was shown by the almost simultaneous depolarisation recorded from atrial and ventricular electrodes with no change in the shape of the ventricular electrogram. Then an idioventricular pacemaker appeared: the ventricular complexes were reversed in polarity, but all of the same shape. The atria were now beating out of synchrony with the ventricles, and at a fractionally slower rate. Both atria and ventricles showed a beat interval of about 250 msec,

slightly slower than the maximum rate achieved under the adrenaline acceleration. On a few occasions, ventricular fibrillation developed suddenly; multifocal ventricular tachycardia was never seen. Occasionally, during adrenaline (or isoprenaline) infusion, electrical stimulation triggered, instead of fibrillation, a ventricular flutter: this was characterised by a pattern of similar electrical complexes occurring at approximately 100 msec intervals, together with rapid oscillations of small but regular amplitude in the mechanical record. Such a flutter was never seen to arise without the electrical stimulation. On many occasions, the onset of the arrhythmias due to the adrenaline infusion came after the infusion had been stopped; this was particularly likely for the idioventricular rhythm.

The accelerative action was well maintained during a 5 minute infusion, but the initial augmentation of force soon passed off; the force of contraction often fell below its previous control level, taking some 20 to 30 minutes after the end of the infusion before it regained its previous value.

Fig. 9 shows the overall accelerative effects of the adrenaline infusions. Results were plotted only from experiments where two or more infusion rates were used; two experiments, in which more extreme infusion rates than usual were tested, are indicated separately at the two ends of the graph. It can be seen that the mean maximal rate attained

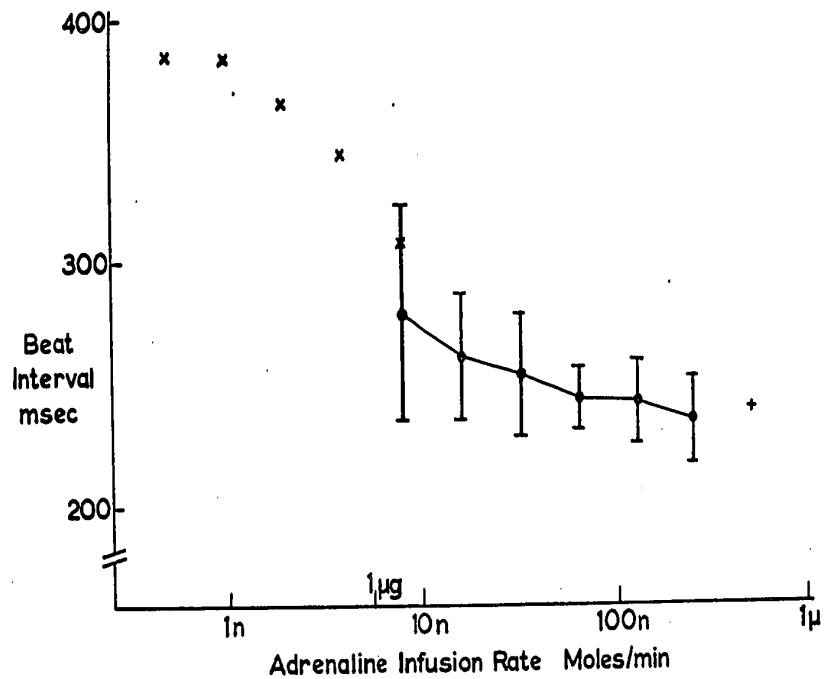


Figure 9. Direct Effects of Adrenaline on Heart Rate.
Mean \pm SD of maximum absolute rate reached (as time between beats).
Abscissa: infusion rate of adrenaline in moles/min; rate of 1 μ g/min also indicated.
x: values from experiment 67, with low infusion rates.
+: additional value from experiment 108.

corresponded to a beat interval of some 240 msec; the decrease in the standard deviation with increasing dose indicated that the maximal effect of adrenaline was a maximal absolute rate, rather than some function of the control rate.

The first tests for changes in the ventricular fibrillation threshold were made with an infusion rate of 8 nMole/min (1.5 µg/min). This produced a mean acceleration to 280 msec/beat, and was chosen as giving a well marked response with the minimum of arrhythmia; the worst seen was a coupled bigeminal rhythm similar to that described by Dresel, MacCannell and Nickerson (60). When clear cut changes in the fibrillation threshold were not obtained with this level, the infusion rate was increased to 32 nMole/min (6 µg/min). This occasionally produced runs of ventricular extrasystoles: the next higher rate (64 nMole/min) would often give ventricular tachycardia.

Taking into account the results of Wégria and Nickerson (13) and of Hoffman et al. (16) showing the transient effect of adrenaline on the ventricular fibrillation threshold of the dog heart in situ, the determinations of the fibrillation threshold in the present investigation were started at the end of the first minute of the infusion, when the increase in rate was nearly complete. Most thresholds were determined within 3 or 4 minutes, many within 2, and no correlation was seen between the size of the changes and the time after the start of the infusion when the threshold was determined.

With both infusion rates, only small changes in the fibrillation threshold were found, as shown in table 7. At 8 nMole/min, the mean change from the control was only two steps on the scale used: application of the 't' test showed this difference to be just significant at the 5% level; if this change was genuine, it was still very small. With infusions of 32 nMole/min, the overall average effect was a rise: three experiments showed a slight fall in the fibrillation threshold, the maximum change being by four steps on the scale; one showed no change; and one, marked increases of threshold to 5-10 times its control value on three separate tests. These increases were the largest changes in fibrillation threshold seen with adrenaline infusions.

If the ventricular fibrillation threshold was not significantly altered during adrenaline infusions, the vulnerable time was. As had been expected, in the control tests the vulnerable time showed a certain, although not close, dependence on the length of the ventricular action potential. Thus some shortening was expected with the shortened action potential during adrenaline infusions: this in fact took place, and table 8 summarises the effect. The control vulnerable times were taken both before and after the infusion: no significant difference was found between these pairs. The mean change in each case was calculated, and then these averaged.

TABLE 8. EFFECT OF ADRENALINE ON THE VULNERABLE TIME

		msec
Control	\pm <u>SD</u>	84 \pm 15
During infusion of adrenaline	\pm <u>SD</u>	61 \pm 15
Mean change	\pm <u>SE</u>	-22 \pm 3
P: Change		< 0.001
Number of tests		11

The Influence of Carbachol

Dresel (49) had suggested that in the dog heart in situ, atrioventricular blocks produced by vagal action could facilitate the production of ventricular arrhythmias by adrenaline. Carbachol (carbamylocholine) was chosen as a suitable stable agent to mimic vagal action.

It was necessary to make a preliminary determination of the level of carbachol needed to produce well marked effects; with a concentration of 0.5-1 μ M in the perfusing fluid (90-180 ng/ml), the rate was slowed below one beat per second, yet it was still possible to drive the atria at their original rate. The P-R interval was not much altered by the carbachol when the atrial rate was allowed to drop: if the rate was maintained constant by driving, then when carbachol was added to the perfusing fluid, the interval from the stimulus artefact to the ventricular depolarisation

increased by 20 - 30 msec from its original duration of some 100 msec. With high enough concentrations of carbachol, this delay would become a 2:1 block; decreasing the driving frequency would restore the 1:1 ratio of atrial to ventricular beating.

No significant changes in the ventricular fibrillation threshold were produced during perfusion with carbachol, even in combination with the electrical driving of the atria. When adrenaline was then infused at 32 nMoles/min, a marked fall in the fibrillation threshold was seen. This occurred even in an experiment (shown in fig. 10) where infusion of the same amount of adrenaline before the carbachol was added produced almost no change. Although the control fibrillation threshold rose during the second hour, it thereafter remained constant. During the perfusion with the carbachol-containing solution, tests while adrenaline was being infused produced fibrillation at the current strengths shown; the true threshold may have been even lower, so the measured fall is a minimum fall. Another experiment gave consistent decreases in fibrillation threshold of 16 steps on the scale used, i.e., to 1/6.3 of its control value. Table 7 again summarises the results obtained with the carbachol treatments.

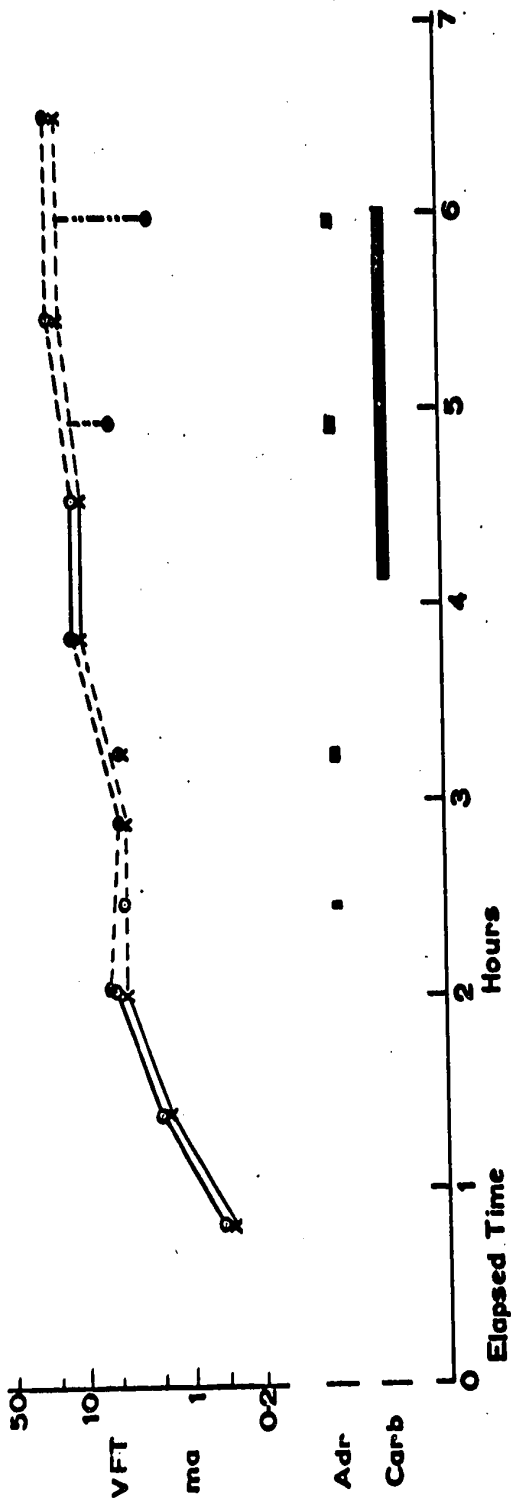


Figure 10. Effects of Adrenaline and Carbachol on Ventricular Fibrillation Thresholds. Symbols as in figure 8. Time and duration of adrenaline (32 nMole/min) and carbachol (1 μ M) administration shown by bars. (Experiment 106)

Chloroform and Adrenaline

Enough chloroform was added to the McEwen's solution to give a final concentration of 1 mM (120 µg/ml), and the mixture shaken to dissolve as much as possible. The spontaneous rate and the force of contraction both decreased slightly when perfusion with this solution was started, but they returned to the control levels within 10 minutes. As with carbachol, chloroform did not itself alter the fibrillation threshold, but when adrenaline was then infused, there was a considerable fall, as shown in table 7.

Effects of Raised Perfusion Pressure

Panisset et al. (51) had suggested that a high perfusion pressure would facilitate the induction of ventricular fibrillation in the isolated rabbit heart. Pressures from 56 to 80 mm Hg were tried: no fall in threshold was found, but rather a rise. The same results were obtained when the threshold was determined during continuous perfusion at a given pressure, or when the perfusion pressure was alternated between normal (40 mm Hg) and raised on alternate sweeps in a single determination. There was so little difference between the results for the different raised pressures that they were all pooled to give the entry in table 7.

Procaine: Coronary Flow

Addition of procaine to give concentrations from 60 to 150 μM (15 - 35 $\mu\text{g/ml}$) in the perfusing fluid resulted in a moderate rise in the ventricular fibrillation threshold to a little less than twice its control value, as shown in table 7. These doses of procaine reduced the coronary flow by up to 30%, but had no noticeable effect on the rate or force of the heart. Transient drops of a similar magnitude in the coronary flow, occurring on other occasions in the absence of procaine, were usually accompanied by temporary falls of two to four steps (20 - 30%) in the threshold, which disappeared when the flow returned to normal.

In one test with 215 μM procaine (50 $\mu\text{g/ml}$), no fibrillation was obtained with stimuli as high as 45 ma, at a time when the control threshold was about 6 ma; with this dose, a marked decrease in the spontaneous rate was seen.

DISCUSSION

Composition of the Perfusion Solution

McEwen's solution was chosen to preserve as far as possible the conditions of Armitage et al. (28, 29) and the other workers in the Oxford laboratory, so that the results from this investigation could be compared with theirs. It is a development of the original Locke formulation, as already mentioned (p.26).

The use of stock solutions of as many components as possible was convenient, since 8 litres of the solution were needed for each experiment, and the dissolving of the solid sodium chloride and sucrose could be quite time-consuming. The use of stock solutions of inorganic salts is quite old, going back to the time of Ringer (39), and comment seems required in relation to only three of the components.

d-Glucose: solutions of glucose were prepared and kept in the same manner as with the sucrose solution (footnote, p.33), but contamination by microorganisms was evident within a few days. Hence, glucose was weighed and added in powder form.

Sucrose: in contrast, solutions of sucrose showed no contamination when kept for more than two weeks, provided care was taken in decanting the portion used for preparation of the McEwen's solution. This was probably due to the fact that sucrose is not as readily available a substrate for the growth of microorganisms as is glucose.

Bicarbonate: sodium bicarbonate as such is not an absolutely essential constituent of the final solution, but serves rather as a convenient source of fixed base without fixed acid. Many sodium-containing alkalis would serve; hydroxide, carbonate, or bicarbonate; the final free acid concentration, and pH, is determined by the partial pressure of the carbon dioxide passed through the solution.

The major advantage of using sodium bicarbonate solution saturated with carbon dioxide is to produce the most acid conditions practicable during the mixing of the McEwen's solution, as this will lessen the tendency for calcium carbonate to be precipitated.

Construction of the Perfusion Apparatus

The principal objective in constructing an apparatus for the perfusion of the isolated mammalian heart through its coronary circulation is the presentation of a reasonable flow of the perfusion fluid at a controlled temperature and head of pressure. This is most simply achieved using the apparatus of Gunn (42): a bottle elevated above the heart provides the pressure, and the fluid flows from this through a vertical tube to the heart, passing on its way through a heat exchanger which raises it to the desired temperature. This specification seems simple enough: closer examination brings out some practical difficulties. The development of a final useful apparatus from this initial specification may be considered under several headings.

Controlled pressure head. A simple bottle acting as reservoir for the perfusion fluid would give a gradual fall in the pressure head as the fluid flowed out. Hence, the standard procedure was adopted: the use of a Mariotte bottle, where air enters at a constant level to replace the fluid lost. Since the Mariotte bottle has only this one connection with the atmosphere, it is not practicable to oxygenate the solution in it, so this oxygenation must be carried out elsewhere. One solution, commonly used (Bell, 43), is to oxygenate at the open top of the heat-exchanger tube. This was considered inadvisable, and a subsidiary oxygenation tube was used as described (p.40). Since the fluid entered this tube from below, the setting of the adjacent Mariotte bottle regulated the fluid level in it. Vigorous oxygenation could be carried out here with no risk of interfering with the operation of the heat exchanger tube proper.

Heat exchanger. The vertical tube leading from reservoir to heart has two functions: containing the column of fluid representing the pressure head, and bringing this fluid to a controlled temperature before it enters the heart. It is often desirable to keep the volume of fluid in this column as small as possible, especially in experiments where several variations of composition are to be used.

Although Gunn used a simple straight-tube condenser as a heat exchanger, circulating water at the desired temperature through the jacket so that it warmed the fluid

descending the inner tube, this is not the most satisfactory pattern. The conduction of heat from the periphery of the tube to the centre is likely to be poor unless turbulent flow conditions are established, and the surface-to-volume ratio (which is a measure of the probable efficiency of heat transfer) of this configuration is rather low. Most later investigators have preferred to use a helically coiled heat exchanger, which gives a much greater surface area for the same overall height, and at the same time can promote better mixing of the fluid flowing through it. Gunn did have one good idea in using a condenser: temperature control is much simpler if water from a large exterior bath, with a good thermostatic control, is circulated through a jacket around the heat exchanger tube proper than if attempts are made to control the temperature in a small jacketing bath.

Attachment of the heart. Once again, a return to Gunn. The simplest method of attaching the heart is to insert a cannula into the lower end of the heat exchanger, and to tie the aorta on this. Given the modern availability of helical-tube condensers with a jacket enclosing the ends of the inner tube, a rubber stopper may be inserted in the lower end of this inner tube, with the aortic cannula in turn inserted in the stopper. This is satisfactory if fluid of a single composition is to be used: however, if variations in the composition are to be investigated, then some means of changing between different batches of fluid must be provided.

Gunn's solution to this problem was to provide a Y-tube above the heat exchanger. This meant that the total volume of fluid in the heat exchanger had to be replaced before the new fluid could reach the heart, and this replacement could well take several minutes, since it was limited by the flow through the coronary vessels. A better approach is to use several heat exchangers in parallel, and provide the selection at their lower ends. Now, the simple insertion of the aortic cannula is no longer possible. The first solution adopted (for series 1 of experiments) was to provide an exposed manifold and a further heat exchanger before the aortic cannula: regulation of the pattern of supply was accomplished with ordinary clamps applied to rubber tubing sections of the manifold. Inclusion of the manifold within the heat exchanger jackets would have been desirable, but this would have made the regulation of the flow more awkward.

A more practical approach, used for the experiments of series 2, was a tap connecting the chosen source with the heart, this tap and its connections being enclosed in the jacket system. Manufacture of such a structure from glass would have been involved, and the lubrication of the glass-to-glass junction in the tap could have led to contamination of the perfusing solution, so a polymethacrylate box and tap barrel with a Teflon plug (needing no lubrication) was constructed.

A glass cannula would have been difficult to attach to this, and as the polymethacrylate was easily machined, it was decided to use a Nylon cannula screwed into the base block of the 'switch box'. Using a 1/4 in - 32 thread (the same as that used on the Clay-Adams Plastic Tubing Adapters), a good seal was obtained. The Nylon cannulas proved a great improvement over the glass ones used in series 1; the aorta slipped very smoothly over the Nylon, with no tendency to stick, and yet once the linen thread ligature had been tightened, a perfect seal was made.

Ancillary attachments. It is desirable to know the temperature of the perfusion fluid as it enters the heart. Again, with a perfusion apparatus using only one solution pathway, it is possible to place an ordinary thermometer in the lower part of the heat exchanger. Even this has its disadvantages, chiefly the obstruction to free flow presented, and the slow response time of the ordinary mercury-in-glass thermometer. Further, it is not very easy to find accurate sensitive thermometers which are small enough to fit within the limited space available.

With the use of a tap close to the final cannula, there is no room for any thermometer of a normal pattern. Thus the use of some type of rapidly responding temperature transducer such as a thermocouple or thermistor is required. As a thermocouple probe was readily available for this investigation, it was inserted as described (p.44) with its sensitive tip just above the aortic cannula.

Another necessity is the provision of some means of administering drugs other than by including them in the perfusion fluid. This is important with substances such as adrenaline which are unstable in the environment (pH 7.3, high oxygen content) provided by the perfusion fluid; it is also convenient when investigating a wide range of dosages, allowing administration of varying quantities or concentrations without the need to mix large batches of a modified perfusion fluid, and then fill one of the columns.

It was always possible to fill the connecting tubing, up to and including the bore of the tap in the final three-way stopcock (fig. 3C, Q), with the drug solution before starting administration, but there was a 'dead volume' extending through the last part of the adapter and infusion cannula. When this was reduced to less than 5 μ l by the 'stuffing' procedure described (p.45), it was no longer necessary to compensate for it when administering infusions.

Recording Methods

Brooks et al. (59) claim that the ideal way of recording the electrical activity of cardiac muscle is the application of flat non-polarisable electrodes to undamaged areas of the myocardium with a minimum of pressure. This is admittedly true. On the other hand, vanTyn and MacLean (19) showed that localised myocardial damage in dogs had little effect on the ventricular fibrillation threshold unless the stimulating electrodes were so close that there was a short-circuit current path between them passing wholly through the damaged tissue. As the object of the present investigation was the measurement of fibrillation thresholds rather than the recording of the exact pattern of cardiac activity, local damage was considered less important than facility of recording. Hook electrodes of light platinum wire, which would remain fixed in place, were therefore used. These were applied so as to be in the most constant position from heart to heart with reference to the stimulating electrodes. Although larger stimulus artefacts were obtained with this in-line arrangement than with a cross-wise position, it was preferred to use the activity from a constant region as timing reference for the stimulating pulses.

The best recording of the contractile force of the ventricular muscle is generally considered to be with a technique similar to the Walton strain-gauge arch (Boniface, Brodie and Walton, 61). The method actually used, measuring the pull of the apex towards the fixed aorta, was certainly

not a physiological measure, but did give the comparative information sought.

The general shape of the electrogram could be seen on the polygraph record, although the high frequency response of this instrument fell off sharply above 60 cycles/sec; the finer details were visible on the face of the oscilloscope. This oscilloscope monitoring was more important for the measurement of the stimulus current. No commercial ink-writing instrument claims a maintained high frequency response much beyond 200 cycles/sec, due to the inherent inertia of the pen system; thus peaking or rounding of the stimulus pulse produced by the highly complex nature of the impedance presented by the heart is only apparent on oscilloscope records. As mentioned, a compensating capacitance was needed across the output of the stimulator to prevent extreme peaking. The value used was a compromise: ideally, a complicated bridge circuit, or a constant current generator, of very high output impedance, would be needed to produce a truly flat-topped stimulus pulse.

The traditional method of recording heart rates is to count the number of beats occurring in a fixed interval of time, often one minute. This seems reasonable enough; but counting beats leaves an uncertainty arising from their discreteness; the values obtained must proceed by integral steps. This is even more so if the number of beats is counted over 30 or 15 seconds; then the observable rates move by steps of 2 or 4 beats/min, making it difficult to

examine small yet significant changes.

If, on the other hand, the time between individual beats is measured, then the accuracy of this measurement depends only on the measuring instrument; time being a continuous quantity, it admits of as fine subdivision as desired. Again, by considering the time per beat as the time for one complete cardiac cycle (including systolic activity and diastolic pause), the durations of the various subdivisions can easily be related to the whole. Thus the fact that the tachograph record showed time per beat rather than beats per minute was considered an advantage, and this basis was used for the measurement of heart rates in the present study.

Logarithmic and Linear Scales

There is a fundamental fallacy involved in the automatic use of linear scales in the measurement of biological parameters. Fundamental to the Normal Distribution is the fact that the parameter being considered may take any value between positive infinity and negative infinity: a fortiori, negative values are to be regarded as reasonable. In contrast, a large number of biological parameters are better considered as being able to assume any positive value. Now, the logarithm of a positive number (taken to any base) can possess any real value between the limits of the positive and negative infinities: it thus possesses the fundamental property required for the Normal

Distribution to hold.

Further, the normal distribution requires that the sources of variation be of the same magnitude at all values of the variate: in the case of most biological measurements, it seems more likely that the absolute accuracy and consistency are proportional to the value measured; again, the logarithm of the measurement will possess the required equality of variability.

For these reasons, the normal approach in this study has been to consider distributions as being normal on a logarithmic scale. Logarithms were normally taken to the base 10, and a convenient origin was assumed; e.g., currents were measured as decimilliamperes, so that all the logarithms would be positive. This selection of a base and origin is equivalent to the shifts of scale and origin normally employed in statistical manipulations, and likewise was used only for convenience in calculation, having no effect on the final results.

If the range of the measurements, considered in terms of their absolute values, is a small proportion (say 3:2 for a moderate sized sample), then the logarithmic distribution approximates the linear quite closely, and the latter can often be used.

The Production of Ventricular Fibrillation

Refractory periods. The classically defined absolute and relative refractory periods of an excitable tissue are from their definitions unmeasurable in practice. The absolute refractory period being defined as the time during which no stimulus, however strong, will excite the tissue, this raises the point that there is always a limit to the strength of stimulus useable: if this is not inherent in the design of the stimulus source, it certainly is reached when the heating effect of the power dissipated by the stimulating current causes sufficient damage to the tissue to permanently change its properties. Further, Brooks et al. (59, p.72) occasionally found cases where the absolute refractory period of cardiac muscle almost disappeared with strong enough stimuli. On the other hand, the end of the relative refractory period being defined as the time when the threshold no longer falls with increasing delay but remains constant, this raises the problem of how small a difference in threshold can be measured; quite small variations in the minimum change accepted can lead to large alterations in the duration of the estimated relative refractory period.

Dawes and Vane (62), during an investigation of the refractory state of isolated atria, made a significant observation, confirming the results of Orias, Brooks, Suckling, Gilbert and Siebens (63). This is the fact that although increasing the strength of the testing stimulus

allowed the first effective stimulus to be delivered earlier in the refractory period, the time of appearance of the second response stayed almost constant; most of the shortening of the refractory period, measured as the delay between the reference and testing stimuli, may be explained by the better persistence of the local excitatory state arising from the latter.

It seems therefore preferable to talk of the 'effective' and 'apparent' refractory periods: the effective refractory period being the minimal temporal separation between two evoked responses, and being determinable in cardiac tissue, as shown by Orias et al., and by Dawes and Vane, and the apparent refractory period being measured by the time of the earliest stimulus of a given strength which will evoke a propagated response, and being a function of the strength of this stimulus. Apparent refractory period determination is fairly simple, the delay time of the first effective stimulus from the previous response being measured, but effective refractory period determination requires care that the artefact arising from a strong testing stimulus does not interfere with the recording of the second response.

The vulnerable period and production of fibrillation.

It is generally agreed by workers in this field (Wiggers and Wégria, 11; Hoffman et al., 14; vanTyn and MacLean, 19) that a single electrical stimulus can produce ventricular fibrillation only if it falls within the vulnerable period,

which forms but a small part of the cardiac cycle, even with the strongest stimuli. In order to induce fibrillation with a single pulse, it is thus necessary to have some means of timing it so that it falls within this vulnerable period. Nowadays, the most practical method is to use electronic devices to deliver the stimulus at a determined interval after ventricular depolarisation, as was done by Hoffman et al., Shumway, Johnson and Stish (18), vanTyn and MacLean, and in the present investigation. The low frequency of these test stimuli, not more often than one every eighth beat, was merely to aid in determining the fibrillation threshold, and would not be so necessary if the only objective were to produce fibrillation.

It is very likely that the same principle of delivering a stimulus within the limited vulnerable period also holds for the faradic induction of fibrillation. This is not quite as easily achieved in practice as might seem possible. If a fixed frequency of stimulation is adopted, then, as seen in the experiments of series 1, if this frequency is less than the maximal follow rate, the ventricular muscle will respond to each stimulus, while with higher frequencies, it will tend to follow a submultiple (e.g., every second or third pulse) which falls below the maximal follow rate. In the latter case, the ineffective stimuli fall at constant points in the cardiac cycle so established. With the 30 pulses/sec stimulation frequency used by Szekeres and Lénárd (57), the ventricles will probably respond to every fourth stimulus at

a rate of 7.5 beats/sec, or 133 msec/beat; the other stimuli will then fall at 33, 67 and 100 msec after the effective ones; the first of these will certainly fall too soon to have any effect; the second and third could fall in the vulnerable period, but could also fall one on either side. The results of Hoffman et al. (14) from the dog heart in situ showed a total vulnerable period of 25 msec with the strongest stimuli, and less with lower strengths. The duration of the vulnerable period will hardly be greater, and probably less, in the rabbit and cat hearts with their higher rates and shorter cardiac cycles. The results obtained in this investigation showed a mean vulnerable time of 84 msec, with a standard deviation of 15 msec, for the isolated perfused rabbit heart. With these values, it is likely that stimulation at 30 pulses/sec will result in a pulse falling within the vulnerable period only occasionally, and then often well before or after the time of maximum vulnerability. Thus it will be uncertain whether any 'threshold' for fibrillation so measured will be the minimum current strength required. In many cases, it may not be possible to induce fibrillation at all.

Use of 60 cycle/sec alternating current, as by Ferris et al. (10) is considerably better: this frequency of alternating current actually gives 120 pulses/sec, so that there is a stimulus every 8 msec in the significant region from 50 to 100 msec, and at least one is sure to fall in the vulnerable period.

If, on the other hand, the stimulus frequency is varied from slightly below to slightly above the maximal follow rate, then the situation is different. As described in the results of series 1, at stimulation frequencies just above the maximal follow rate, the response of the ventricular muscle is quite irregular, and many of the responses may not arise directly from the stimuli; it is at this point that the random relationship of the stimuli to the actual ventricular responses is most pronounced, and thus there is the greatest possibility of a pulse falling in the vulnerable period. That this in fact is probably so is shown by the observation that fibrillation, when produced in series 1, did generally start when the stimulation frequency was just greater than the maximal follow rate.

In their original studies, Armitage et al. (28, 29) used just such a variation of stimulus frequency, and thus were likely to produce fibrillation if the threshold was as low as the current strengths (1 ma for 750 μ sec) that they were using; Goodford (30) and Kärki (31), however, using fixed frequencies of 10 and 11 $\frac{2}{3}$ pulses/sec respectively, could only get fibrillation if the vulnerable period was near 100 msec for the former, or 86 msec for the latter.

Measuring the susceptibility to fibrillation. The results obtained in this investigation, and summarised in table 6, show that ventricular fibrillation can certainly be induced in isolated rabbit hearts perfused with McEwen's solution, provided that a strong enough stimulus is used,

but that only some 60% of these hearts will continue to fibrillate indefinitely, the other 40% reverting spontaneously to a normal rhythm within a short time. Such a proportion is reasonable in view of the results of MacWilliam (4), Ferris et al. (10), Wiggers (5) and others with hearts in situ: cat hearts being somewhat larger than rabbit, would be expected to give a still greater proportion of persistent fibrillations, while, as Wiggers (47) showed, dogs give almost 100% persistence.

If a fixed arbitrary current strength is used, as in series 1 of this investigation, or in the experiments of Armitage et al., and the timing of the stimuli is such that some should fall within the vulnerable period, then a proportion of the hearts tested should fibrillate, this proportion rising as the current strength is increased. Of those fibrillating, some will show non-persistent fibrillation, and some persistent. It can be seen, therefore, that the hearts recorded as 'fibrillating' [persistently] by Armitage et al. were those which had a fibrillation threshold as low as the current strength they were using, and which gave persistent rather than non-persistent fibrillation.

Thresholds to faradic stimulation. The only reported ventricular fibrillation thresholds for isolated perfused hearts are those given by Szekeres and Lénárd (57) for cats. DiPalma et al. (58) had determined only atrial fibrillation thresholds for faradic current on cat hearts in situ; Dawes,

in his review (64), was mistaken when he stated that DiPalma et al. had measured fibrillation thresholds in both atria and ventricles. Using 30 pulses/sec faradic stimulation, these investigators found fibrillation thresholds ranging from 2 to 8 ma, which agreed fairly well with the currents needed by Wiggers and Wégria (12) for single shock thresholds on dog ventricle in situ.

Szekeres and Lénárd, also using 30 pulses/sec stimulation, obtained a mean fibrillation threshold for 14 isolated cat atria at 38 C of 1.1 ma, with a standard deviation of 0.3 ma: this is considerably lower than the values of DiPalma et al. Their ventricular fibrillation thresholds under the same conditions had a mean value of 0.96 ma, with a standard deviation of 0.5 ma. No mention is made of defibrillating the ventricles: this is surprising, as the fibrillation would have been expected to persist in some 80% of the cases, judging by the results of Dirken et al. (25) [100% persistent ventricular fibrillation in isolated rabbit hearts at 39 C], and those of the present investigation [60% persistent at 37 C, again with the smaller rabbit heart].

Shortly afterwards, Szekeres, Méhes and Papp (65) described their measurements of ventricular fibrillation thresholds to 30 pulses/sec stimulation with cat hearts in situ, and in heart-lung preparations. There are a number of intriguing features in this report. On this occasion, they stated that "Fibrillation stopped as soon as electrical

stimulation was interrupted or immediately after it, as was expected in a heart as small as that of the cat." Most other investigators would expect about 80% of the fibrillations to persist indefinitely. These 'fibrillations' were diagnosed "(a) by direct observation of the cardiac activity, (b) by the electrocardiogram, (c) by the record of the blood pressure." It was surely not easy to see a fibrillatory pattern on the electrocardiogram in the presence of stimulation artefacts occurring every 33 msec. The expected initial responses of the ventricles to stimulation of the frequency used, the gradual adoption of driven rates of 5, 6, or 7 1/2 beats/sec (the submultiples of the stimulation frequency), were not described; these responses would have produced rapid ventricular beats of small amplitude which would probably have led to a reduced cardiac output and a falling, pulseless blood pressure, similar to the effects of a genuine fibrillation. Examination of the heart by eye would also have been unlikely to have distinguished between a genuine fibrillation and the pattern of 6 or 7 1/2 contractions per second, with, in all likelihood, partial ventricular conduction blocks appearing from time to time.

The 'fibrillation' thresholds shown in this report were in the range of 300 - 600 μ a, with 1 msec pulses. These are of the order of magnitude found for extrasystolic thresholds by Orias et al. (63), and in the present investigation, and very much lower than other reported fibrillation thresholds (Wiggers and Wégria, 12; Hoffman et al., 14; vanTyn and

MacLean, 19). Similarly, the thresholds measured by Szekeres and Lénárd (57) were both lower, and showed less variation, than other reported fibrillation thresholds, and again correspond better with extrasystolic threshold values. The most convincing evidence in favour of the presumption that Szekeres, Méhes and Papp were merely measuring the threshold current required to establish a rapid driven rate was the complete absence of any persisting arrhythmias; this is in complete disagreement with the results of every other investigation of electrically induced ventricular fibrillation.

The induction of fibrillation. Wiggers (5) describes the effects of a properly timed pulse of suitable strength as producing an extrasystolic response, followed by a series of reentrant beats of the same character before fibrillation proper became established. This happened with stimulation of a localised region on the dog's ventricle: a different picture was seen in the present investigation when stimuli were applied between base and apex of the ventricle of the isolated rabbit heart; as shown in fig. 6B, an oscillatory response was immediately apparent, and the fibrillatory state was presumably established at once. However, there is some evidence of a normal response from part of the heart at the time the next sinus-controlled beat was due. The two pictures can be reconciled if we consider the fibrillatory state as being initially established between the stimulating electrodes, and then spreading to

cover the whole heart. In Wiggers' experiments, the area initially affected was so small a proportion of the heart that the responses from the remainder of the tissue were the most apparent: in the present investigation, not only was the proportion of the total ventricles initially affected much greater, but also the activity was recorded from electrodes within the affected area: thus the beginnings of the fibrillatory state were more obvious in this case than in that of Wiggers.

Defibrillation

Ferris et al. (10), amongst others, showed that very strong electric currents could arrest fibrillation. Dirken et al. (16) used cooling, while Wiggers (47) had obtained good results with intracardiac administration of potassium. All these methods probably work in the same way, by producing a complete depolarisation of the ventricular muscle, thus interrupting the local circuits involved in maintaining fibrillation. Following the complete depolarisation, gradual repolarisation then leaves the muscle ready for the next normal impulse arriving from the atria.

Electrical defibrillation, although simplest in principle, requires a controlled source capable of supplying large currents, and, particularly with an isolated heart suspended in a metal apparatus, can present a shock hazard to the operator. The use of the large plate electrodes

considered desirable would also have proven awkward, as they would have further obstructed access to the heart.

Dirken et al. obtained their best results with cooling, by pouring cold saline over the fibrillating ventricles. This was easy for them: their hearts were set so as to project upwards from the cannula. It is also easy with a perfusion apparatus of the Palmer type, for the very reason that its use was avoided - the presence of a long slim cannula, unobstructed by a heat exchanger jacket. Once the advice of Gunn (42) is followed, however, and the heart suspended as closely as possible below the heat exchanger system, it is no longer so accessible for the pouring of solutions over it.

Thus potassium injections were used. By injecting slowly until the electrogram was completely silent, and then allowing the potassium to be washed out by the continuing flow of the perfusion fluid, good results were obtained.

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Control Fibrillation Thresholds

The initial ventricular fibrillation thresholds listed in table 6 are claimed to represent the first reliable fibrillation thresholds described for isolated hearts, and also the largest population (59 hearts) from which such a description has been made, whether isolated or in situ. Shumway et al. (18) give means from 355 determinations, in 3 groups, differing in the anatomical location of application of the testing stimuli, but these were determined (on hearts in situ) in a total of only 10 dogs, and no indication is given about the relative contribution of variations between animals and within individuals to the overall variance of the results. Further, Shumway et al. determined thresholds by applying stimuli at the time of the T wave of the standard electrocardiogram. Vulnerable times in the present investigation were never as late as the 'T' wave of the ventricular electrogram. This difference may be a species difference, or may arise from the difference in responses of isolated and in situ hearts.

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vanTyn and MacLean (19) used various electrode spacings, mostly between 10 and 35 mm, and in somewhat fewer than 40 dogs, mention fibrillation thresholds ranging from 10 to 33 ma. They claimed to obtain results consistent to within ± 3 ma in any given dog for a fixed location and spacing of the electrodes, and frequency of interpolation of the test stimulus. All these factors would significantly affect the value of the threshold, lower values being obtained with frequent testing, or at the apex rather than the base; electrode spacings less than 5 mm always gave very high thresholds. Again, groups of 6 to 11 animals were used, and results from within these groups were given with little indication of which animals were used in more than one group.

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The present results, from 59 different hearts, show a wider variation than was apparent with the results obtained in vivo. This may be due to the less physiological

conditions of the isolated perfused heart; a species difference is also possible: most of the data from hearts in situ are from dogs, while the present study was made on rabbit hearts. In favour of a species difference is the fact that both persistent and non-persistent fibrillations were obtained with the rabbit hearts, and the thresholds for the non-persistent were significantly greater (table 6).

The drifts in threshold seen with some of the isolated hearts contrast with the constancy of those obtained in situ by vanTyn and MacLean; gradual changes had been found by Wiggers and Wégria, and they stated that only sharp changes in threshold were significant. This attitude, aided by interpolation of the drifts, was adopted in evaluating the results of the present study.

As already mentioned, the fact that some two-fifths of the hearts tested gave non-persistent fibrillations originally was no surprise. That many of these later gave persistent fibrillations indicated that some of the changes which occur on continued perfusion with McEwen's solution predispose to persistence of fibrillation; whether this is due to potassium movement, leaching of soluble protein or lipid, or just the edema that develops is difficult to say. No claim is made that McEwen's solution is the most efficient serum substitute, but rather that it is the simplest practicable solution which will maintain activity for a reasonable time.

The distribution of the durations of non-persistent fibrillations into two groups is interesting, but of doubtful significance, since a given heart could fibrillate for durations in either group indifferently in response to the same challenging stimulus.

Potassium Concentrations

The normal potassium concentration of a perfusion fluid for mammalian tissues is 5.6 mM (Locke, 38; Krebs and Henseleit, 66). This concentration of potassium certainly gives good results, and Greiner and Garb (67) have shown that the range of potassium concentrations where normal excitability exists is from 4 to 8 mM. Outside these limits, the threshold for excitation of isolated cat papillary muscles rises markedly. McLean, Bay and Hastings (68) found an optimum potassium concentration of 6 mM for perfused rabbit hearts, with marked changes arising if the concentration was less than 3 mM or greater than 9 mM. Antoni, Herkel and Fleckenstein (69) observed disturbances in pacemaker function at potassium levels above 10 mM. All this information agrees with the observations of the present investigation that potassium levels less than 4 mM or greater than 8 mM produced marked changes in the activity of the isolated perfused heart.

The liability of low potassium concentrations to precipitate spontaneous ventricular fibrillation is well documented. Grumbach et al. (26) observed that fibrillation

appeared after some minutes perfusion with potassium-free fluid, and that this fibrillation was arrested by restoring potassium to the fluid. Melville and Mazurkiewicz (27) observed that the fibrillation produced by such perfusion with potassium-free fluids could be temporarily arrested by injections of potassium-containing solutions. Armitage et al. (28) obtained 3 spontaneous fibrillations in 4 hearts perfused with McEwen's solution containing only 1.4 mM potassium.

Wide variations of potassium concentration are only possible with isolated preparations, for in vivo both homeostatic and toxic effects limit the variations possible. The results from the present study thus appear to be the first measurements of the effect of varying potassium concentrations on ventricular fibrillation thresholds, whether in situ or in isolated preparations. As shown in table 7, variation of the potassium concentration between the normal 5.6 mM and the well tolerated limits of 4 and 8 mM produced well marked and consistent changes, with the threshold at 8 mM being some 12 times that at 4 mM. Thus there is no doubt that the ventricular fibrillation threshold is quite sensitive to the potassium concentration of the perfusion fluid within the normally tolerated range.

Outside this range, most of the thresholds determined did not have the necessary control observations made to permit accurate estimation of the changes produced.

Secondary effects may also occur: McLean et al. (68) found

ventricular fibrillation to appear frequently if solutions of potassium concentration greater than 12 mM were perfused through isolated rabbit hearts. This may have been due to the direct depolarisation produced by these concentrations.

The failure in the present investigation to find changes in rate or force of contraction in the equilibrated state with changes in the potassium concentration from the normal 5.6 mM to 4 or 8 mM suggests that there is some homeostatic mechanism present at the level of the cardiac cells themselves, at least over this range of potassium concentration: this idea is supported by the fact that changes did appear, but were transient. Thus the changes in fibrillation threshold found were not related to changes in rate or force of contraction of the ventricles, nor to any variations in excitability (measured by the diastolic threshold), which were also absent.

The highest potassium concentration tested was 11.2 mM; at this level, only non-persistent fibrillations were found, but fibrillation could always be induced. The failure of Armitage et al. (28) to produce any fibrillation in the presence of 11.2 mM potassium is almost certainly due to the weak stimulation used by them.

The reversible interconversion of persistent and non-persistent fibrillations with changes in potassium concentration was much less frequent than had been expected from the results of Armitage et al. and Kärki (31), but this is probably due to their confounding of

non-fibrillating and non-persistently fibrillating hearts. Such conversions did occur, as stated, and in the expected directions, lowering of the potassium concentration promoting persistence of fibrillation. Once again, the effect was all-or-none, and there was no correlation between the duration of the non-persistent fibrillation and the sensitivity to induction of fibrillation. The experiments with varied potassium concentrations were the only ones where consistent interconversions of persistent and non-persistent fibrillations were observed, but these were also the only ones where such an effect was particularly sought, and the treatments chosen in such a way as to expose possible conversions.

Adrenaline

Although the effects of adrenaline on the heart have been widely studied, it is almost impossible to correlate the results obtained with different preparations because of the differing methods of administration used. For example, Trendelenburg (70), investigating the effects on isolated atria, suspended the atria in a fluid containing a known concentration of the drug. Melville and Mazurkiewicz (27), using isolated perfused hearts, injected 1 ml of an adrenaline-containing solution into the fluid flowing to the heart, and had no means of knowing in what volume this adrenaline was finally distributed (this being irrelevant in their study). Dresel and Nickerson (71) infused a fixed volume in a fixed time (1 ml in 15 sec) intravenously in vivo, and proportioned the amount of the drug used to the weight of the animal; such proportioning is not normally used for injections into the perfusion stream of the isolated heart. How effective doses compare in the three systems is impossible to determine, so that during this investigation, the useful dose of adrenaline was determined *de nouveau*.

The dose was normally administered in terms of quantity (moles) per unit time; as the infusion was made in a volume of less than 10% of the coronary flow, it could be assumed to be distributed in this, and knowledge of the total coronary flow permitted calculation of the concentration of adrenaline in the perfusion fluid. A wide range of

concentrations was infused, and the effects observed as described on page 85.

The effect on rate was, as expected, a marked acceleration: this tended to reach a fixed maximum rate, rather than be related to the control value. Trendelenburg (70) had already found the absolute change in rate of isolated atria to show less variation than the relative change (percentage of control), and Nickerson and Chan (72) had suspected an absolute ceiling to the stimulant effects of adrenaline, unrelated to the control value, such that an increase in the control decreased the change that could be observed.

Although Dirken et al. (25) remark on the induction of ventricular fibrillation in isolated hearts by the administration of large quantities of adrenaline, there is little recorded description of the arrhythmias with smaller doses. These followed the pattern of increasing severity described for dog hearts in situ (Dresel and Sutter, 21), except that ventricular tachycardias were always monofocal, never multifocal; this was probably a species difference, due to the smaller size of the rabbit heart. Ventricular fibrillation was, as mentioned, occasionally seen with large doses of adrenaline.

Although adrenaline doses were expressed both as quantity infused per minute and as concentration in the perfusing fluid, no greater consistency of results was gained by using the latter mode, so the former was adopted,

the results being more easily analysed as many infusions were made at the same dose levels.

Hoffman et al. (16) had shown a transient effect of adrenaline on the ventricular fibrillation threshold of dog hearts in situ, with a lowering of the threshold to about half its control value, the effect disappearing within 5 minutes, even with continuing infusion of the adrenaline. Wégria and Nickerson (13), on the other hand, found a rise in threshold following adrenaline injection in dogs.

An attempt was made in the present investigation to choose a dose of adrenaline which would produce the minimum arrhythmic effect of itself, and yet cause alterations in the fibrillation threshold. When this proved impossible, a higher dose was tried, and still no pronounced fall in threshold could be seen.

Goodford's experiments (30) showing facilitation of ventricular fibrillation induction in the presence of adrenaline alone could not be duplicated. It is quite possible that the shifts in the vulnerable time and maximal follow rate produced by the adrenaline could have made his fixed frequency of stimulation more effective.

The failure to obtain a marked fall in the ventricular fibrillation threshold due to adrenaline alone is not really so surprising: Riker et al. (22) have stressed the fact that their dose of adrenaline would produce many arrhythmias by itself, but not fibrillation; some sensitising agent such as petroleum ether or chloroform (Levy, 6) was necessary to

give the latter response. The results obtained with adrenaline infusions when chloroform was present in the perfusion fluid confirm this, in that a marked decrease in the fibrillation threshold was indeed obtained: this was a true synergism, as neither the adrenaline nor the chloroform had any marked effect on their own.

The synergism of carbachol with adrenaline to produce a marked fall in the fibrillation threshold is also important. Recent evidence has implicated vagal action in the production of idioventricular rhythms by adrenaline (Dresel, 49). The mechanism proposed is that the vagal action will produce A-V nodal blocks, thus removing sinus dominance, and allowing the emergence of ventricular pacemakers; Mendez, Han and Moe (73) have shown that vagal action will indeed tend to produce such blocks at high heart rates, and these blocks were observed in the present investigation (p. 91). (Most of the studies in vivo have been concerned with the induction of arrhythmias rather than fibrillation).

The lack of effect of carbachol alone on the ventricular fibrillation threshold confirms the results of Armitage et al. (29), who found its only effect to be on the rate of stimulation needed to produce fibrillation; this may be indirectly related to alterations of the vulnerable time.

Perfusion Pressure: Coronary Flow: Procaine

An increase in the perfusion pressure of the isolated perfused heart is not equivalent to the rise in systemic blood pressure produced by the administration of adrenaline, for it only acts within the ventricular wall, and not from the interior of the ventricle as a transmural pressure. Both Locke and Rosenheim (41), and Lu (74) have shown that the maximum perfusion pressure well tolerated by the isolated perfused rabbit heart is about 45 mm Hg: higher pressures produce rapid edema (Locke and Rosenheim) and a fall in contractile force (Lu). The main effect of a moderate increase for a short period of time will be an increase in the coronary flow, and this will tend, if anything, to raise the fibrillation threshold; such a rise was indeed found. Shumway et al. (18) have shown a reduction in ventricular fibrillation thresholds in areas affected by partial coronary occlusion in vivo; small falls were also seen with transient decreases in coronary flow in some of the experiments of the present investigation, these changes in flow being considered due to temporary local obstructions in the vessels.

Panisset et al. (51) claim to have found increased susceptibility to fibrillation with raised perfusion pressures. The susceptibility was measured by counting the number of successive 2 minute periods of stimulation with 10 volt [!] pulses at 20 pulses/sec required to produce persistent fibrillation. One heart was tested at each of

8 pressures ranging from 18 to 67 mm Hg, one test being carried out on each heart. The hearts tested at the lower perfusion pressures required more periods of stimulation before persistent fibrillation was produced. What this method was measuring is not clear; hardly fibrillation thresholds, but maybe variations in vulnerable time.

In another series of experiments, they induced fibrillation after perfusing the hearts for 20 minutes with a pressure head of 67 mm Hg, and then reduced the pressure: reduction to about 30 mm Hg within half an hour resulted in a reversion of the fibrillation to a normal rhythm; then a return to the original pressure brought about a return of the fibrillation. If the pressure reduction was spread over more than an hour, the fibrillation did not revert to normal rhythm. Attempts made to duplicate this, by suddenly reducing the perfusion pressure of a fibrillating heart to as low as 5 mm Hg failed to arrest the fibrillation. No decreases in fibrillation threshold, or consistent conversions of non-persistent to persistent fibrillations, were seen when the perfusion pressure was raised from 40 mm Hg to 56 - 80 mm Hg.

One possible explanation of the results of Panisset et al. may lie in the effects of the edema produced by their high perfusion pressures. Only one test was carried out on each heart, and the total duration of any of their experiments was never much more than two hours, in contrast to the 6-7 hour experiments carried out in the present

investigation.

The results with procaine were much as expected: the increase in fibrillation threshold found with moderate concentrations agreed with the results of Wiggers and Wégria (12) in vivo. Although the one test with the higher concentration failed to produce fibrillation, this concentration was at a toxic level, as shown by the effect on the rate. The reduction of the coronary flow produced with procaine is of interest, and shows that this agent has more than a simple anti-arrhythmic action on the heart.

Validity of Threshold Measurements

Dawes (64) claims that the electrical fibrillation threshold is not a true measure of the susceptibility of the ventricles to fibrillation. He bases his claim on the fact that the fibrillation thresholds in normal animals are so much higher than the extrasystolic thresholds that triggering of fibrillation by reentrant activity would be unlikely. This is true: but conditions which merely raise ventricular automaticity (e.g., administration of sympathomimetic amines, particularly isoprenaline) do not normally produce ventricular fibrillation of themselves; it is only under such conditions as decreased potassium concentrations, or the combined presence of chloroform and adrenaline, that ventricular fibrillation occurs readily, and, as the present investigation has shown, it is precisely in these conditions that the fibrillation threshold is

reduced, thus making induction of fibrillation more likely. In other cases, the absence of decreases in the fibrillation threshold testifies to the unlikeliness of fibrillation occurring spontaneously.

CLAIMS TO ORIGINAL RESEARCH

- 1) The first measurements of electrical ventricular fibrillation thresholds in isolated hearts were made.
- 2) The conditions required for the induction and persistence of ventricular fibrillation in isolated rabbit hearts were clarified, and these two effects shown to be separable.
- 3) The first measurements of the effects of varying concentrations of potassium on ventricular fibrillation thresholds were made.
- 4) The arrhythmic effects of adrenaline infusions on isolated hearts were described in detail.
- 5) The lack of effect of adrenaline alone on the ventricular fibrillation threshold of isolated hearts was demonstrated.
- 6) The synergism of chloroform and adrenaline in lowering ventricular fibrillation thresholds was proven by measurement.
- 7) The synergism of carbachol and adrenaline in lowering ventricular fibrillation thresholds was demonstrated and measured.
- 8) The failure of raised perfusion pressures to decrease the ventricular fibrillation threshold was shown.

NOTE

Some of the results obtained in this investigation were presented to the Halifax meeting of the Canadian Federation of Biological Societies in June 1964 (75).

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