ON FUNCTIONAL ANALYSIS BY THE ACTION OF ELECTROLYTES.

By GEORGE RALPH MINES,
Fellow of Sidney Sussex College, Cambridge.

(*From the Physiological Laboratory, Cambridge.*)

CONTENTS.

In the majority of experiments in which the environment of the heart has been accurately controlled, attention has been paid only to the mechanical expression of the heart's activity. Until recent years the study of the electrical phenomena of the heart could be carried out only by the use of the capillary electrometer. Those who acquired the skill necessary to make good experiments with this instrument did not often turn their attention particularly to the exact control of chemical environment. And indeed it is only quite recently that so important a factor as hydrogen ion concentration has received recognition by physiologists.

Since the introduction of Einthoven's string galvanometer great numbers of researches have been published dealing with the interpretation of the electrocardiogram. The majority of these have been concerned with the mammalian heart *in situ*, while in those concerned with the excised heart, with a few exceptions which will receive attention later, the control of chemical environment has been inadequate. Probably one reason why so many observers have been content to record only the mechanical changes in the excised heart is that so much stress has been laid in the past on the parallelism between electrical and mechanical changes in living tissues.

Now although such parallelism can be clearly traced in many instances it is not inevitable. And it is precisely through certain alterations in the chemical environment of the heart muscle that divergence between the mechanical and electrical responses may be brought into prominence. From the standpoint of an investigator of tissue mechanisms the electrical response is certainly not less important than the mechanical response. And as a guide towards functional analysis their occasional divergence is even more significant than their usual parallelism.

In the following pages I shall describe an experimental enquiry as to what features of the electrogram of the isolated heart yield definite information about the condition of the heart muscle, and I shall then give some examples of the way in which the relations between different characteristics of cardiac activity may be explored through alteration in the concentrations of electrolytes in solutions bathing the muscle.

METHODS.

Animals. Medium-sized or large male specimens of *Rana temporaria* were used, all of them being vigorous and apparently healthy. The experiments, on which the chief conclusions of this paper are founded, were made in the winter months from November to March. A large number of experiments carried out in the spring and early summer months showed in a general way the same results, but since their technique was much less perfect and in particular the records were taken at lower speed, they do not admit of deductions as to any seasonal variations.

Every observer who has attempted quantitative work on any tissue has been aware of great individual variations. Until we have some system of standardising the history of our material, so that the age, the diet, the surrounding temperature and other conditions of life of the animal before the experiment are subject to control, such differences will remain large. Probably they will never be wholly eliminated. An individual heart, examined by quantitative methods, generally shows clean and definite relations between its behaviour and the variations in concentration of some substance in its environment. It is certainly a mistake to blur the lines of such relations by merging them in an average. The cause of individual variations must be sought. This can be done only by the elaborate study of individuals. As many factors as possible must be studied in each individual taken. The "individual factor" cannot be ruled out by taking averages without our losing essential information. We should rather aim at keeping the "individual factor" constant throughout a whole series of experiments by making them all on a single specimen! If we understood the physico-chemical nature of the heart-beat in some one particular frog, it is certain that the mechanism of the hearts of other frogs would be found to differ from it only in a quantitative sense.

Perfusion. The chief danger in the use of perfusion methods for the control of the chemical environment lies in possible variations in the perfusion pressure. The sinus and auricles are very sensitive. In perfusion with an ordinary cannula there is especial risk of causing a sudden brief rise of pressure at the instant when the perfusion fluid is changed. All sorts of effects may be produced in this way; inhibition through stimulation of the intra-cardiac vagus, extra contractions, modification of the rate of rhythm, and so on: such changes of course alter the state of equilibrium of the tissue and complicate to an unknowable extent the results due to the change in composition of the perfusion fluid.

The desiderata for perfusion in experiments of the type considered in this paper are chiefly the following:—

The sinus venosus and the great veins close to it should not be touched.

The flow through the heart should be abundant.

The perfusion pressure should be sufficient to distend moderately the auricle during its diastole, but should not prevent its emptying in systole.

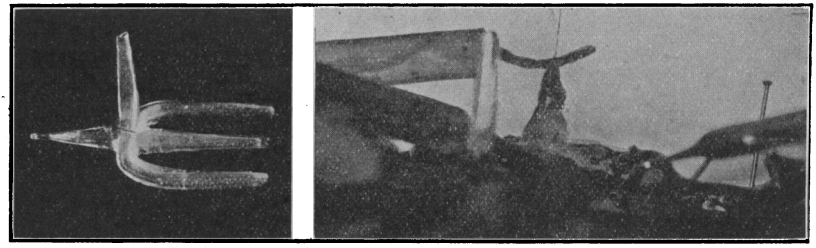

Fig. 1. Fig. 2.

Fig. 1. Cannula.
Fig. 2. Method of applying electrodes to heart. See text.

The heart must be safeguarded absolutely from accidental changes in pressure.

The dead space of the cannula must be known and ought to be as small as possible, so that there is no doubt as to the exact time at which some particular solution enters the heart.

The method which I have found to fulfil these conditions best is the use of a special cannula inserted into the inferior vena cava taken below the liver. The form of cannula which I have used is shown in Fig. 1. It consists of a five way piece made of glass tubing of 2 or

3 mm. bore. One tube is vertical (the chimney): it is about 20 mm. in height. Of the four horizontal tubes, one is drawn out with a neck for insertion into the vein, the other three are for connexion with rubber tubes coming from the perfusion bottles. The perfusion bottles are placed at such a level that the solution rises to the top of the chimney. The least rise of pressure causes overflow; this ordinarily occurs when the solution is changed. The dead space in this cannula amounts to less than 0·05 c.c. The chimney also allows air bubbles to escape should any have been left in the rubber tubing. This is very useful when it is required to perfuse a fourth solution. Without in any way disturbing the preparation, one of the perfusion bottles is removed from the rubber tube, a small bubble of air is allowed to enter the tube and the new perfusion bottle is attached. If now the new bottle is slightly raised, the solution runs out of the chimney, and when the air bubble arrives one knows that the new solution has arrived at the cannula. The chimney also offers a ready means of introducing a dose of some drug directly to the heart. The needle of a hypodermic syringe is put down the chimney and the orifice directed at the tube in the vein.

I have employed Mariotte's bottles of 250 c.c. capacity, made of ordinary glass. The use of rubber tubes and of ordinary glass in perfusion apparatus is open to criticism. With regard to rubber tubing, the danger lies chiefly in the absorption of small amounts of toxic substances from the fluids which may be given out later to another fluid passing along the same tube. In my experience, if the tubes are well washed out with glass distilled water immediately after use, and before any particular experiment with the solutions which they are going to conduct, there is no objection to their employment in experiments with saline solutions. As regards the use of ordinary glass, Herlitzka (1912) has made the very interesting observation that it may so far alter the composition of a solution that a fluid which kept in Jena glass will not allow the mammalian heart to beat, will start the beat at once if used in a perfusion apparatus of ordinary glass. This effect is due to the alkali of the glass lowering the hydrogen ion concentration of the fluid. In the present series of experiments I have eliminated such alterations by the use of 'buffers' (Sörensen, 1909) in sufficient amount to prevent measurable change in hydrogen ion concentration through solution of the glass or absorption of carbon dioxide from the air during the course of an experiment. Naturally the vessels were washed thoroughly with glass distilled water, and all solutions kept for any length of time were stored in Jena flasks or in bottles of 'resistance' glass.

The choice of a vein for perfusion is of importance. The anterior abdominal vein is not well suited for this work because the vein breaks up to some extent in the liver. Liquids perfused by it do not run direct to the heart but are exposed to an unknown but probably large surface of tissue on the way. This increases the dead space and possibly alters the composition of the solution reaching the heart. To insert the cannula in the inferior vena cava, I have found the following procedure most convenient. The abdomen of the frog is opened by cutting to one side of the middle line: the sternum is then

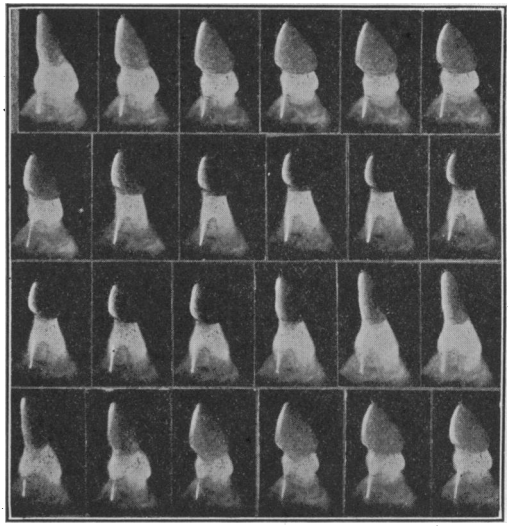

Fig. 3. Serial photographs of the perfused heart of the frog, from a cinematograph film. 15 images per second.

divided in the middle line. The anterior abdominal vein is pinched with the forceps and divided. The frog is pinned out by the upper extremities. The liver is then turned forwards so as to lie over the heart. The stomach is seized with the forceps, drawn downwards, and the œsophagus cut through at its lower end. The whole alimentary canal is then removed by cutting through the mesentery keeping close to the gut. The genitalia are next removed. The inferior vena cava is seen running up from between the kidneys into the liver. It is further exposed by cutting away the remains of the common intestinal artery and the peritoneum which binds it to the back wall of the abdominal cavity. A silk thread is looped round the vein. At this stage it is

convenient to remove the lower parts of the frog by cutting through the whole of the tissues at the level of the lower borders of the kidneys. This facilitates placing the cannula in position. The cannula, which has been connected with the perfusion bottles, is arranged on a bed of plasticine so as to bring the nozzle in the right position. A gentle stream of fluid coming from the cannula enables it to be inserted into a slit made in the vein, without difficulty. After the cannula has been tied in, the liver is turned back and the heart exposed by opening the pericardium. When it is desired to record the beats of auricles and ventricle separately, the fraenum ventriculi should not be cut. If it is required to collect the outflow from the heart a cannula is placed in one of the aortæ and the other aorta is tied. In other cases escape of fluid is permitted by cutting a slit in the bulbus aortæ. Burridge (1912) recommends making a slit in the ventricle itself, in order to allow the escaping fluid to bathe the exterior of the heart. But when studying the electrical changes as well as the mechanical, the advantage gained by slitting the ventricle is more than counterbalanced by the complications in the electrogram introduced by the injury. The appearance of the heart when properly perfused is shown in the serial photographs reproduced in Fig. 3[1].

Chemical materials. The chemicals used were in nearly all cases Kahlbaum's purest. The materials employed in making the hydrogen ion concentration determinations were obtained from the sources indicated by Sörensen in his papers (1909, 1912). Pure hydrochloric acid was obtained from the chemical laboratory. Strontium chloride, of which no sample of specially controlled purity was available, I obtained by repeated crystallisation from Merck's product. Solutions of sodium and potassium chloride were prepared by weighing: solutions of the deliquescent chlorides of magnesium and calcium were prepared by titration with standard silver nitrate. The cane sugar solution used in certain experiments was prepared from thoroughly washed 'coffee sugar' crystals. The water used was boiled in a tinned vessel and condensed in glass.

Temperature. The control of temperature was one of the weakest points in this research. The experiments were made in a cellar. The bottles containing the perfusion fluids were allowed to stand side by side in the cellar for some time before the experiment began. The temperature was taken from time to time by a standard thermometer either

[1] I wish to thank M. Lucien Bull for his kindness in giving me the opportunity of taking this film at the Institut Marey, Boulogne-sur-Seine.

placed in one of the bottles or near the heart. Sometimes the temperature changed during the course of an experiment by as much as 1° C. though usually there was much less change than this. This defect in the technique I shall attempt to remedy in future work. I do not think the variation in temperature in these experiments upsets any of the conclusions to be drawn from them; at the same time the effect on the rhythm of the heart of a change in temperature of 0·2° C. is very readily appreciable, by the methods adopted.

Mechanical records. The mechanical movements are recorded by the suspension method. Very fine silk threads are tied, one to the extreme tip of the ventricle, the other to one of the auricles. These threads should be attached without injuring the muscle. A special pair of forceps should be reserved for this operation: the finest iris forceps may be used, or, better, forceps with broad hollow shafts tapering to sharp points in perfect alignment. An exceedingly small bit of tissue is picked up and tied with a single knot. The threads pass over light pulleys to be attached to levers of special construction which move in front of the slit of the photographic recorder. To make the moving parts as light as possible, and to avoid friction, I have constructed a very simple form of lever (Fig. 4), adapted only for taking shadow records. It consists of a thin slip of straw supported by a stretched loop of silk. The loop of silk which forms the axis is held between two pins, one of which is attached to a brass support directly, the other mounted so that its distance from the first can be adjusted over a certain range by a screw. The straw is passed through the loop and twisted round several times. The tension of the loop is then altered by means of the screw until the lever gives the desired amount of pull on the thread from the heart which is attached to it. I have found this arrangement more sensitive and more convenient than a lever pivoted in metal bearings.

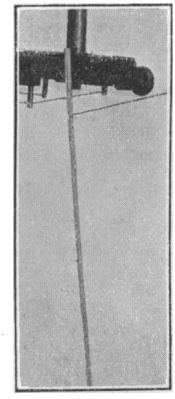

Fig. 4. Mode of suspension of lever. See text.

Electrodes. For obtaining records of the electrical changes in the heart I have used a simple form of zinc electrode, in which the Ringer's solution is prevented from mixing with the zinc sulphate by a 10 % solution of gelatine, as suggested by Noyons. The gelatine is made up with Ringer's solution and a little thymol: a glass tube of about 6 mm. bore and about 8 cms. in length is half filled with the gelatine,

and in it is partly embedded a piece of worsted. When the gelatine is set, the other end of the tube is filled with zinc sulphate solution, and a thin zinc rod, soldered to a brass terminal (the junction being well coated with Prout's glue), is introduced into the tube and fixed there with the aid of a short piece of rubber tubing. The electrodes are kept when not in use with the worsted dipping into Ringer's solution. They will last for weeks, and if properly treated they seldom need compensation.

In most of the experiments to be described, one electrode was placed on the apex of the ventricle. The worsted was tied on by the thread already attached to the tip of ventricle. This arrangement is shown in Fig. 2. The other electrode was usually placed on the sinus venosus or in contact with the liver or the back of the abdomen. In some cases a third electrode was employed, this being fixed in contact with an auricle, with the base of the ventricle or with some other part. In such instances a telegraph key was arranged so that the electrodes could be coupled to the galvanometer leads as required.

Galvanometer. The instrument used was the Cambridge model of Einthoven's string galvanometer. A silvered quartz or glass 'string' of about 4 or 5 μ was employed. For illumination a 20-ampère Zeiss arc lamp was used, but in the later experiments this was satisfactorily replaced by a small Leitz hand-feed arc, taking about 5 or 6 ampères. The intensity of the luminous crater and not its size is the factor of importance for this work. The condensing system was composed of a double convex lens attached to the lamp by a sliding tube, and a $\frac{2}{3}''$ objective in the galvanometer tube. The beam passed through a heat filter of distilled water. For projection a $\frac{2}{3}''$ objective and a Zeiss projection ocular were used. The recording surface was placed about $1\frac{1}{2}$ metres from the galvanometer: the linear magnification of the string was about 350.

Time marking. An aluminium disc 7·5 cms. in diameter carrying 10 projecting teeth of which two, placed opposite to each other, are wider than the rest, is so arranged that the teeth pass through the focus of the projection ocular. The disc is mounted on the spindle of a small phonic wheel kept in rotation by the intermittent current from a tuning fork. The arrangement is a slight modification of that used by Bull. If the tuning fork gives 50 vibrations per second, the wheel revolves 5 times per second, and the light is cut off by the teeth 50 times. But as every fifth tooth is wider than the rest, tenths of a second are marked more strongly, and thus it becomes very easy to measure intervals of time on

the tracing. In these experiments the frequency of the fork was 50·25 d.v. per sec.

A correction of + 0·5 % should be applied to all the time values given.

Photographic recorder. The records were taken on rapid bromide paper about 8 cms. wide. Since it was often necessary to use 10 or 15 metres of paper in a single experiment a special apparatus was made, the chief point of which is that the exposed paper is wound on a roller, as in a cinematograph, instead of being left loose in a box, as is the case with several types of recorder on the market. The arrangement is of the utmost simplicity (Fig. 5).

On a teak base 28 × 20 × 1·5 cms. is erected a teak box 39 × 23 × 15 cms. The box is subdivided by an incomplete partition half way up. Each compartment has a door at the back and will accommodate a roll 9 cms. wide by 14·5 cms. in diameter.

A brass rod 4 mm. in diameter in each compartment pierces the sides of the box and to it is fixed by an arrangement of nuts and washers a wooden roller 9 cms. long and 5 cms. in diameter. The roller in the lower compartment carries about 60 metres of bromide paper. The unexposed paper passes from the lower reel over a small roller fixed inside the box, out through a wide slot and round a Sandström drum placed horizontally so that the point of contact of a vertical tangent to the drum comes at the focus of a cylindrical lens which covers the slit transmitting light from the apparatus. Above the drum is a rubber-covered roller mounted loosely as shown in Fig. 5, with springs, which holds the bromide paper firmly in contact with the drum and absolutely prevents slip. The paper re-enters the box, passes under a roller, reaches the upper compartment and is wound on the roller there. The paper is kept in tension all the time by a string which is wound on the axis of each roller. This string passes over pulleys and carries a clock weight placed out of the way against the wall. The motor is allowed to run throughout an experiment, the movement of the drum is started and stopped by a clutch arrangement worked from the experimental room. To avoid waste of paper the upper roll has wound upon it a few metres of non-sensitive paper. Before using the apparatus this is joined to the end of the bromide paper with seccotine, and the junction brought opposite the slit. If the experiment is a long one a pencil mark is made on the paper occasionally to indicate where it may be cut without interfering with a record. If several experiments are done in succession, each involving a number of exposures, a double line is drawn across the paper at the end of each experiment. When the paper is to be developed, it is cut across

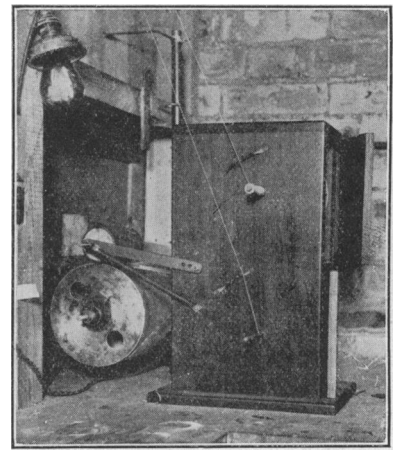

Fig. 5. Photographic recorder for long rolls of bromide paper. See text.

at the level of the slit, and the exposed paper is rolled by hand from the upper roller. The new roll thus formed has the part first exposed on the outside. This is at once inscribed with pencil, with the date, the number of the experiment and the symbols '§ 1.' Starting with this end a roll is made until about 6 or 8 metres have been wound off. The paper is then cut at the nearest pencil mark, the remaining roll marked '§ 2' and so until the whole paper has been divided into a convenient number of sections. With a little practice a roll of 6 or 8 metres of paper may be developed by hand without special apparatus. The paper should not be too thin. A whole-plate dish is used and plenty of developer. The roll of paper is passed from one hand to the other several times. A satisfactory and cheap developer is made by taking a saturated solution of sodium sulphite, to which immediately before use is added a little amidol, and an equal bulk of water. For fixing, a nearly saturated solution of sodium hyposulphite, with no additions, is best.

On the interpretation of the electrocardiogram.

The variation in form of the electrocardiograms obtained from different frogs demands some discussion before it can be decided what features of the curves are of importance for our investigations. The derivations I have used are similar to those employed by Samojloff (1910) and I confirm absolutely the general statement to be found in his admirable paper, viz. that with this mode of derivation there are two features of the greatest constancy, a small deflection, indicating 'negativity' of the basal electrode, to the apical electrode preceding the auricular systole, and a large deflection in the same direction preceding the ventricular systole. The further course of the curve is variable: I have encountered all the types mentioned by Samojloff as well as others. The final variation due to the ventricle may be in the same direction, or in the opposite direction to the initial variation: occasionally it is scarcely perceptible.

The ventricular complex. The physiological significance of the sharp deflection which the ventricular complex begins has never been called in question. It indicates, as Burdon-Sanderson showed, that the base of the ventricle becomes excited before the apex. The excitation spreading to the apex, if the latter is uninjured, the whole ventricle again becomes isoelectric; since the second phase follows the first with great rapidity owing to the rapid conduction of the excited state through the muscle, the result is the characteristic 'spike' of Burdon-Sanderson, 'first ventricular wave' of Waller or 'R-wave' of Einthoven.

Regarding the remainder of the ventricular complex opinions are much divided.

We may, I think, simplify the discussion by disposing of certain suggestions which have been made recently as to the nature of

the final ventricular wave. Straub (1910), Hoffmann (1910) and Samojloff (1910) consider that the final wave is the expression of a different process in the muscle from that indicated by the initial wave. More recently Seemann (1912), as the result of extensive researches on the heart of the frog, concludes that while the initial wave is the electrical expression of excitation, the final wave is the electrical expression of contraction. Seemann's hypothesis is, it appears to me, completely upset by the experimental fact that the contraction of the heart muscle may be abolished under certain conditions without abolishing or even reducing the size of the final wave. The evidence on which this assertion rests will be discussed and illustrated in a later section of this paper.

But others have suggested that the final wave is dependent on some process in the muscle of a different kind from that responsible for the initial variation, without making the assumption that this other process is the mechanical shortening. It must always be borne in mind that a deflection of the galvanometer shows only that one region of the heart is at a higher potential than another region at the time.

If the final variation depends on some process in the muscle other than that responsible for the initial variation (and yet like it in being accompanied by a disturbance of electric potential) it must be a process which takes place in one region of the musculature and not in another, or at different times in different parts, or to a greater degree in one region than in another. Apart from its orientation no 'process' taking place in the muscle generally can be thought of as yielding a difference of potential between two distant regions of the muscle.

There is much positive evidence that the final variation of the ventricular complex is the electrical expression of the passing off of that process, the beginning of which is signalised by the initial variation.

It is universally admitted that when one region of the muscle assumes the excited state while another region remains unexcited, an electrode on the excited region is at a lower potential than that on the unexcited region. This, of course, is the state of affairs at the beginning of the ventricular complex. The base is 'negative' to the apex. The excitation spreads with great rapidity over the muscle and when the whole muscle is excited the electrodes are at the same potential. Now it is obvious that unless the excited state passes off simultaneously in all regions of the muscle, or unless it passes off symmetrically with regard to the two electrodes, there must be a difference of potential between the electrodes towards the termination of the excited state.

A priori it is improbable that the excited state will pass off symmetrically with regard to the two electrodes, except in rare instances, for it started asymmetrically with regard to these fixed points. It follows that as a rule the passing away of the excited state must be signalised by the appearance of a deflection of the galvanometer. In the typical electrogram, the 'T-wave' is the only feature of the complex which occurs anywhere near the finish of the excitation process in the muscle. As shown by the comparison of an electrogram from an uninjured ventricle with an electrogram from the same ventricle after local injury under one electrode, the time relations of the final variation show the closest agreement with the passing off of excitation as indicated by the fall of the monophasic variation of the injured heart.

A deflection of some kind, due to the same process as the initial variation, is bound to appear in the great majority of cases at just that time when the final variation actually makes its appearance. It is therefore at present superfluous to make the assumption of some other process to account for the appearance of the final variation.

The initial variation is the electrical expression of the asymmetric orientation of the excited state in the muscle with regard to the two electrodes, at the beginning of ventricular excitation. The final variation is the electrical expression of the asymmetric orientation of the excited state in the muscle when the excitation is passing away. Such a view was the outcome of the classical researches of Burdon-Sanderson and Page (1880, 1883) on the ventricles of the tortoise and frog. They showed that by local warming of the muscle in the region of one of the electrodes the capillary electrometer record which resulted from stimulation of the muscle was modified in just that sense which would be predicted as the result of hastening the termination of the excited state under the warmed electrode. Bayliss and Starling (1892) made somewhat similar observations on the naturally beating heart of the mammal, and concluded that the form of the final ventricular variation was determined by the unequal duration of the excitation process at the base and at the apex of the ventricle.

The effects of local warming may be demonstrated with the greatest ease and certainty on the naturally beating frog's heart. If the final wave is positive (*i.e.* in the same direction as the initial wave) the effect of warming the apical lead is to render the final wave more strongly positive. If the final variation is negative, it is easily rendered positive by warming, even very slightly, the apex. Conversely by warming the

base of the ventricle a positive final variation can be rendered negative, or a negative variation rendered more strongly negative.

In these experiments the local warming was carried out by placing a little loop of platinum foil round the required region, but at some distance from it. With a suitable resistance in series a current was

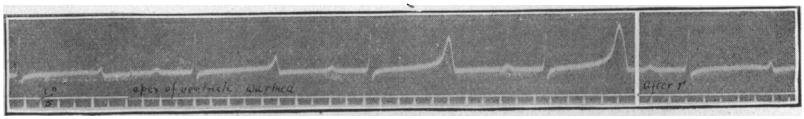

Fig. 6. Warming of apex begins at signal and ends with the first portion of tracing. Second portion one minute later. Time marked in ·2″. ×½.

sent through the foil, so as to warm the heart by radiation. A thermometer, the small bulb of which was placed in the position occupied by the heart in the following experiments, rose from 14° to 26° in ½′ and to 33° in 1′. Fig. 6 shows that the effect of warming appears within 2″ of the commencement of warming. Figs. 7 and 8 illustrate the various points mentioned above: each of them shows the perfect reversibility of the effect produced. When the local warming is stopped, the final wave regains its original form whatever this may have been.

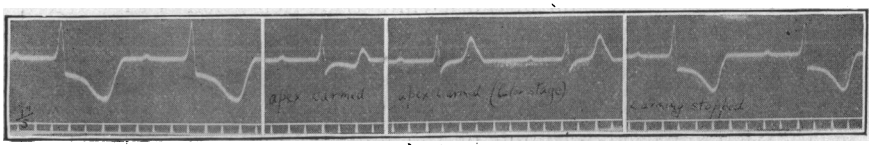

Fig. 7. Stages in warming apex of frog's heart are shown in the second and third parts of this tracing. The first and last pieces taken before and after the warming. See text. Time in ·2″. ×¼.

It remains to consider the sign of the final variation when the different regions of the ventricle are kept so far as possible under similar conditions. The chief point requiring explanation is that with the electrodes arranged as usual, one in contact with the base and the other with the apex, the final wave is very frequently positive. According to Gotch (1907) this is invariably the case with the normal heart *in situ* properly supplied with blood, and in my experience it is very common in the perfused heart under the experimental conditions which I have described.

As stated most clearly by Bayliss and Starling (1892) the positive final variation means that the excited state persists longer at the base

of the ventricle than at the apex. In the light of the evidence which I have reviewed there appears to be no escape from this conclusion.

Why should the base of the ventricle which becomes excited first remain excited last?

Gotch's hypothesis. In order to account for the fact that in the frog's heart left *in situ*, the basal region of the ventricle is 'negative' to the apical region both at the beginning and at the end of the ventricular complex, Gotch in 1907 advanced the very attractive hypothesis that the form of the electrocardiogram is reminiscent of the ontogenetic

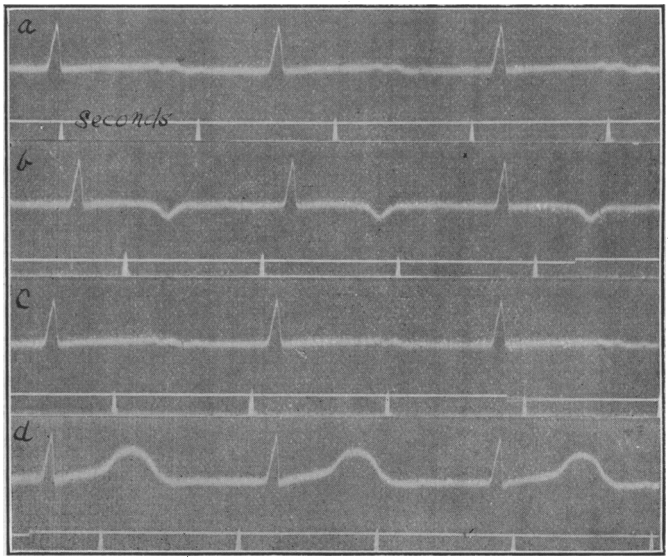

Fig. 8. Frog's heart. *a* and *c* normal. In *b* the base of ventricle was warmed. In *d* the apex was warmed. ×⅔.

history of the heart. The ventricle is developed as a tube, which becomes folded on itself. The aortic region of the heart represents the most distal part of the tube in the primitive heart, the last part to become excited. Gotch supposes then that in the frog's ventricle the wave of 'negativity' starts in the base in the region remote from the aorta, is propagated to the apex and thence to the aortic region of the base.

This conclusion is based chiefly on the following observations:

(*a*) With one electrode on the apex of the ventricle and the other going across the auriculo-ventricular groove, the form of the ventricular

complex is such that the final deflection is of the same sign as the initial deflection.

(*b*) With one electrode on the aortic region of the base, and the other on the left margin of the base, the latter region is found to become excited before the former.

Gotch lays stress on the fact that in order to demonstrate these effects it is necessary to leave the heart *in situ* and well supplied with blood. More recently Gotch (1910) has extended his observations to the hearts of the tortoise and the rabbit.

Meek and Eyster (1912) have lately objected to Gotch's hypothesis on the ground that every region of the base of the tortoise heart becomes excited before the apex. What they have shown is that every region of the base becomes 'negative' to the apex at the beginning of the ventricular complex. It is equally true that with the frog's heart *in situ*, an electrode placed at the region of the aortic exit shows that this region becomes negative to the apical region at the beginning of the ventricular complex. But this does not upset Gotch's position, as Meek and Eyster assume. For the 'negativity' of the aortic region at this stage may possibly be a passive affair, an expression of the fact that the aortic region of the heart is much closer and connected by tissue presenting less electrical resistance to that region of the base in which excitation, and therefore 'active negativity,' starts than is the apex. If we suppose that the muscle tissue which is involved in the return path of excitation in the ventricle postulated by Gotch is small in amount, it is possible that the electrical changes involved in the assumption by it of the excited state might be swamped in other electrical changes involving wider tracts of musculature. It yet remains possible that the 'negativity' beginning later and therefore presumably leaving off later in the aortic region should render the basal region of the heart negative to the apex at the end of the ventricular complex. Yet the uncertainty in the form of the final variation under experimental conditions makes it difficult to accept any hypothesis involving structural considerations in explanation of one particular form of wave which is only sometimes encountered.

But before deciding whether Gotch's hypothesis is in any way available for the explanation of these results, I thought it best to attempt to satisfy myself as to its validity under the conditions laid down by Gotch. I therefore made a series of experiments in which the hearts of healthy, freshly caught frogs were exposed and while beating normally were led off to the galvanometer without interference

with blood supply and without displacement from the normal position. Gotch found that opening the pericardium in no way upset his results: I therefore did this since there are obvious advantages for our purpose in confining the contacts with the heart to small areas.

Referring to the diagram in Fig. 9, we may recapitulate the chief experiments of Gotch on the frog's heart thus: placing one electrode across from B_1 to B_2 and the other at A, he obtained the triphasic variation whose origin we are discussing. Placing one electrode at B_1 and the other at B_2 he found that B_1 becomes excited before B_2.

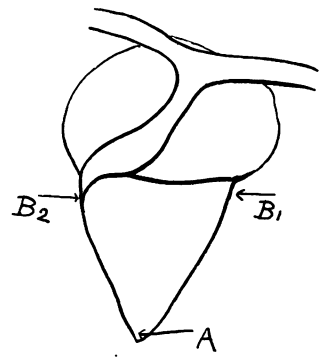

Fig. 9.

The method by which I endeavoured to control these observations was as follows:

I used three similar electrodes which made contact at A, B_1 and B_2. A was connected to one pole of the galvanometer while B_1 and B_2 were connected successively, by means of a two-way key, to the other pole. The electrocardiograms obtained from these pairs of leads were then compared. The connexions were always arranged so that negativity of the base caused an 'upward' movement in the record. If the character of the final variation of the ventricular complex depends on the passage of excitation by the route B_1, A, B_2 through certain of the fibres, the relative negativity of B_2 to A at the end of the complex should be much more pronounced than the relative negativity of B to A at this time. On the other hand the converse relation should obtain at the beginning of ventricular excitation.

I found that the results obtained by derivation from the heart *in situ* showed individual differences of a much more disturbing character than those associated with the mode of derivation I have adopted in my perfusion experiments. The differences of potential set up in the heart are short-circuited to a greater or less extent by the tissues on which the heart lies—the movements of the heart affect the extent of this short-circuiting: differences of potential are transmitted from one region to another through the tissue, and thus when the electrodes are both on the ventricle, the auricular variations may show in the electrogram. This is uncommon when the heart is raised from its bed.

To economise space and to facilitate discussion I give in Fig. 10 tracings made with pen and ink from the photographic records of 14

consecutive experiments. The first column shows the curves obtained from the derivation apex-left base, the second column those from the derivation apex-aortic base. These experiments yield some evidence in support of Gotch's hypothesis as to the course of excitation. For with

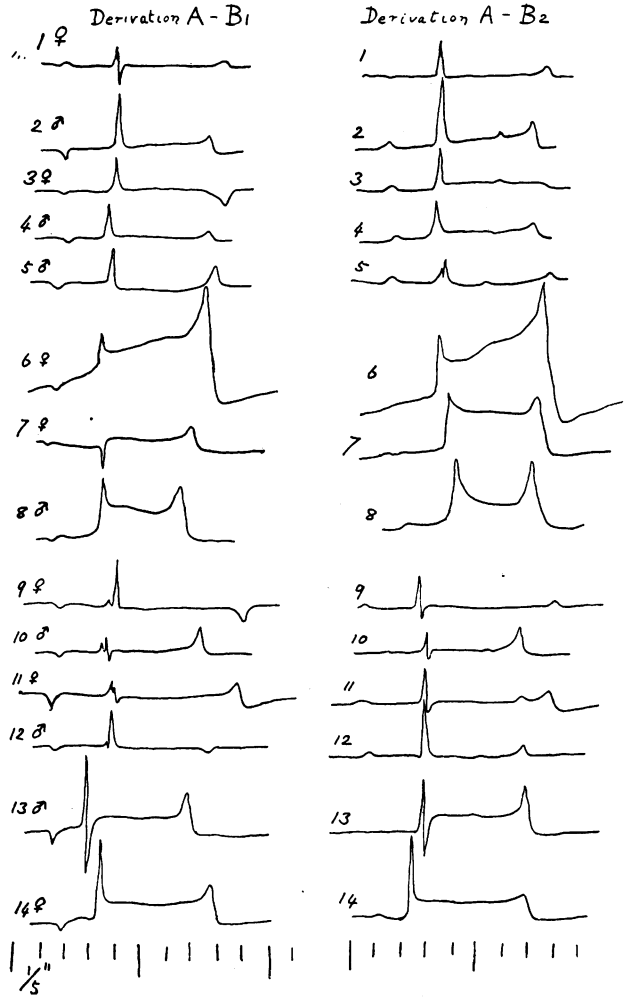

Fig. 10. ×⅜.

the first derivation in Exps. 3, 9 and 12, the final deflection was downwards, while in all cases (except 3, where it was practically absent) with the second derivation the final deflection was upwards. But against this must be set Exps. 5, 11 and 14, where the final wave is actually more

positive with the first derivation than with the second. And in Exps. 1, 6, 10 and 13, the difference is scarcely of the magnitude to be deduced from the hypothesis. Moreover the deduction as to the relations between the initial parts of the ventricular complexes as found in the two different derivations receives no support. This deflection is sometimes greater in one derivation and sometimes in the other. It is curious that in Exp. 7 this wave was inverted with the first derivation.

Certain other points of interest may be noted. With the first derivation, the auricular excitation is indicated by a downward deflection in all experiments except the first—with the second derivation the converse is true. In more than half the experiments, the second derivation gave clear indication of the excitation of the bulbus aortæ. This is most striking in Exps. 2 and 11, but is also unmistakable in 3, 4, 5, 10, 12 and 13.

The fact that the aortic bulb contracts some time after the ventricle is not proof that the excitation reaches it by a devious route; it is strictly comparable with the pause at the auriculo-ventricular junction. It seems to me that these experiments lead to the conclusion that in the frog's heart the 'circular' conduction occurs only to a very limited extent.

Even with the heart *in situ*, in seems then that the hypothesis of Gotch cannot be regarded as a sufficient explanation of the form of the final ventricular variation. Meek and Eyster have justly pointed out that the duration of the final ventricular variation is often too great to be accounted for merely by the later arrival of excitation at one particular region of the base, calculated on the basis of the rate of propagation of excitation in ventricular muscle given by Gotch.

Gotch insists that his observations applied only to the heart left *in situ*. And indeed the difficulties in the way of the application of his hypothesis to the heart under experimental conditions such as I have described at the beginning of this Paper are very great.

As Samojloff (1910) found, the sign of the final variation may change when no external condition is intentionally altered. Such a progressive change always takes place very gradually. There exist certain observations which apparently correlate changes in the final variation with known changes in the conditions under which the heart is placed. Thus Samojloff found that stimulation of the vagus caused a marked change in the character of the final variation. If it was positive in sign, it tended to become negative when the vagus was

stimulated; if negative, it became more strongly so. I was able to confirm this observation (1912), both for stimulation of the vago-sympathetic trunk and for stimulation of the sinus venosus in a considerable number of cases. But in some instances, both on stimulation of vago-sympathetic trunk and on stimulation of the sinus venosus, although good inhibition was obtained, the final variation was practically unaffected when the beats returned. In other cases, in which also the inhibition was well marked, the effect on the final variation was to render it much more strongly positive. How far this result was due to the effect of sympathetic stimulation I shall consider in a subsequent paper where I propose to describe the effects of stimulation of the sympathetic. For our present purpose we need only note that the amplitude and the sign of the final variation are liable to be affected by nervous influences.

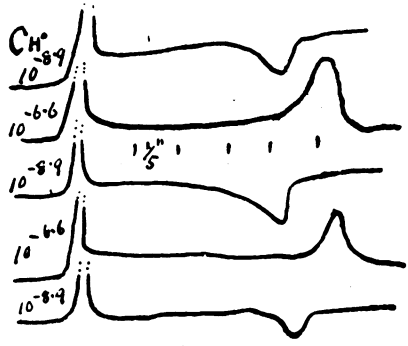

Fig. 11.

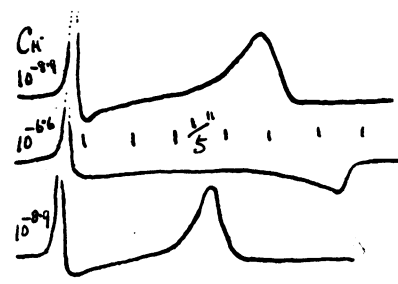

Fig. 12.

In several cases I found an apparent relation between the hydrogen ion concentration of the perfusion fluid and the sign of the final variation. Thus for example Fig. 11 shows tracings taken from an experiment in which on the perfusion of a solution of $C_H \cdot 10^{-8\cdot9}$ the final variation in the course of some minutes became negative. Change to a solution of $C_H \cdot 10^{-6\cdot6}$ was followed by a gradual change to a positive final variation. This was repeated a number of times. The figure shows that the total duration of the ventricular complex is greater in the 'more acid' than in the 'more alkaline' solution. There is no question that in this particular experiment there was a definite relation between the sign of the final variation and the C_H of the perfusion fluid. But Fig. 12 shows an experiment on another frog with the same perfusion fluids. Here again we see a relation but it is exactly opposed to the result of the preceding experiment. Here too the 'more acid' solution increases the length of the ventricular complex; but here the final

variation is positive in the alkaline solution and negative in the acid. The rate of development of the change was similar in these two experiments. It occurred always some minutes after the new fluid had started running through the heart: when the reversal started, it took place much more quickly than in the spontaneous reversals to which allusion has been made. In one case I recorded the act of reversal on a rapidly moving paper. To economise space, I have traced a series of eight consecutive ventricular complexes from this record. They are shown in Fig. 13.

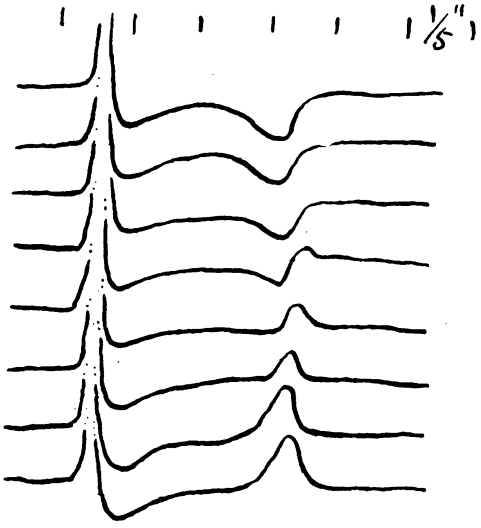

Fig. 13.

A change in the C_{H^+} of the perfusion fluid, then, may cause alteration in the sign of the final variation. But the reversal may be in opposite senses in hearts of different frogs. In other cases no clear relation at all was discoverable.

Now these circumstances specially liable to alter the form of the final variation, namely changes in the hydrogen ion concentration and the stimulation of the cardiac nerves, are changes which affect the duration of the excitation process in the muscle. In the case of nerve stimulation, it is very likely indeed that one region of the muscle should be more affected than another. But it is less obvious why the perfusion fluid should alter one region more than another. Probably the whole problem arises out of the remarkably great length of the excitation wave in heart muscle. The excited state—the condition of

'negativity'—lasts so long in the heart muscle that those little differences in the condition of the muscle in one region or another, which are to be expected in a mass of tissue of the magnitude of the ventricle, may decide the state of affairs at the end of excitation. In other words, the length of time during which the excited state persists in any region of the heart muscle is very long, while the time taken for the excited state to travel from one region to another is very short. A small percentage difference in the time of persistence of excitation in one portion of the muscle as compared with another—a difference, that is to say, such as will be produced by quite trivial differences in environment or in relations to environment—will result in differences in the time of ending of excitation of one part of the muscle as compared with another which will entirely override the very small differences in time which separated the beginning of excitation in the one region and in the other.

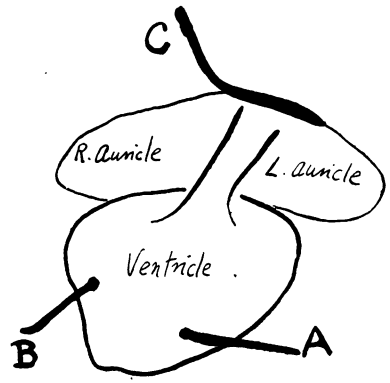

Fig. 14.

This idea is strongly supported by the highly important observation of Samojloff (1910), that in an extrasystole, provoked by stimulation of the apex of the ventricle, the initial variation in the ventricle is inverted, but the final variation has the same form as in the response of the ventricle to a normal auricular excitation—whether this form is positive or whether it is negative.

Finally I will describe an observation, which struck me as being very interesting in this connexion, on a case in which there was alternation in the ventricle of a tortoise. The heart was that of a large 'Spanish terrapin.' It was excised and placed on a paraffin block. Electrodes were fixed so as to touch the heart in the regions indicated in the sketch (Fig. 14). Region *A* was treated with a few drops of a modified Ringer's solution of $C_H \cdot 10^{-4.5}$. I cannot say whether the effect to be described was due to this treatment: alternation is very frequently developed in the excised tortoise ventricle in the absence of any special treatment. Fig. 15 shows the records obtained by the three modes of derivation indicated. In all three there is alternation in the form of the ventricular complex. With the derivation *BC* the final ventricular wave is alternately positive and negative.

This state of affairs lasted for many minutes; a short piece of the tracing taken on a slower paper is reproduced in Fig. 16.

In previous papers (1912), dealing with the condition of alternation in the electrograms from the frog's heart, I have shown how Gaskell's (1882) original explanation of mechanical alternation may be applied to the interpretation of the complex phenomena presented both in clinical and experimental studies of the condition. I need not recapitulate the evidence here. It may suffice to state that the condition of alternation depends essentially on local differences in the condition of

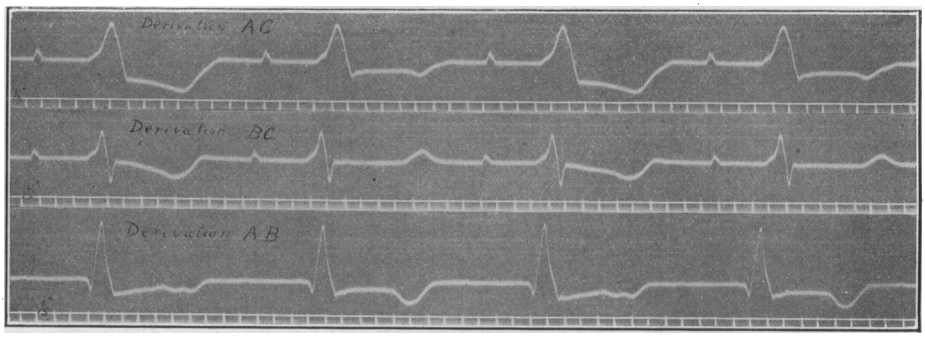

Fig. 15. Alternation in form and in sign of the final ventricular variation, heart of tortoise. See text. Time in ·2″. $\times \frac{1}{3}$.

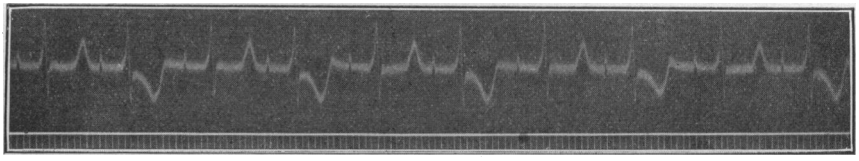

Fig. 16. From same experiment as Fig. 16. Time in ·2″. $\times \frac{1}{2}$.

the ventricular musculature. There is no more difficulty in explaining on these lines a case where there is alternation in sign of the final ventricular variation than where there is merely a slight difference in the form of the ventricular complex in successive beats. But as the condition of alternation in sign of the final variation is rare, I have thought it worth while to describe this instance. It shows most clearly that the sign of the final ventricular variation gives information only about relative differences between different parts of the muscle and tells one nothing as to the condition of the musculature as a whole.

The electrical variation due to the auricles.

In the electrogram obtained by derivation from the apex of the ventricle and the base of the heart, the only expression of the activity of the auricles which is ordinarily apparent is a small deflection in the same sense as the first ventricular wave, returning quickly to the base line. The rise and fall are as a rule symmetrical. A little consideration is needed to understand why the auricular excitation should yield this simple form of curve—the P-wave. If electrodes are applied directly to a piece of excised auricular muscle, as Noyons has shown in the tortoise (1910), its excitation results in an electrogram quite as complex as that obtained from the ventricle. But the electromotive force is smaller. The auricles are very thin-walled and therefore the short-circuiting fluid-bathed surface is large in proportion to the internal membrane system where the potential differences arise. The production of a difference of potential between the electrodes depends on an asymmetric orientation of the excited state with respect to the two electrodes. This occurs in the highest degree, with the ordinary modes of derivation, both in auricle and in ventricle at the beginning of excitation. It is then that the greatest potential difference in the whole complex appears. If the sensitivity of the galvanometer is reduced to a suitable extent

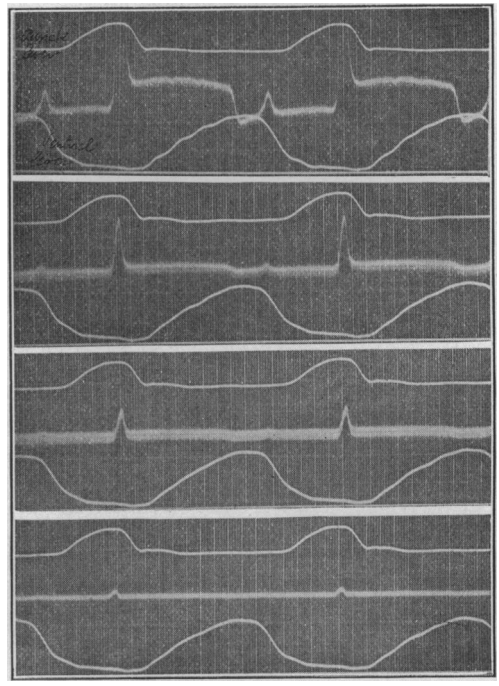

Fig. 17. Records of frog's heart with different sensitivities of the galvanometer. The tension of the string was in every case great enough to obviate errors due to deflection time. See text for further description. Time marked in ·1″ and ·02″ with phonic wheel apparatus. $\times \frac{2}{3}$.

the initial 'spike' may be the only part of the complex recorded. And if auricles and ventricle are included in the same derivation, since the ventricular 'spike' is much higher than the auricular, the latter will disappear while the former remains. This is illustrated in Fig. 17. In the first tracing the electrogram is taken with a moderate tension of string. In the succeeding portions, *B*, *C* and *D*, the tension was increased. It will be noticed that in *D* nothing remains obvious except a little notch corresponding to the first ventricular deflection. The final ventricular variation is not to be seen. And this little notch, the identity of which with the first ventricular waves of the preceding tracings, is established at once by comparison with the simultaneous mechanical tracings (lever shadows), resembling closely in form the auricular wave of the ordinary electrogram. In this particular curve, very close inspection reveals the first auricular variation.

But there are other reasons why the electrical sign of the passing away of auricular excitation is likely to escape notice in the electrocardiogram. It must usually be small in comparison with the initial variation. Leading off from the base and the apex of the heart the electrical connexions with the auricles are by two very extensive leads. The auricles are connected with the 'base' electrode by the whole sinu-auricular junction and by the aortæ which touch the auricles in front and run to the general mass of the frog's body. The connexion with the 'apex' electrode is formed by the very large area of the junction between auricles and ventricle as well as by the aortæ which spring from the ventricle and are in contact with the auricles.

The appearance of a 'final variation' depends upon the excited state persisting longer in one region than in another, these regions being placed asymmetrically with respect to the two electrodes.

The instant at which any particular region ceases to be excited depends not only upon the moment at which it became excited but also on the condition of the muscle. For since the rate of spread of excitation is high, very small local differences in the condition of the muscle may determine that a region which became excited early shall remain excited late, or *vice versâ*. With such factors at work it is easy to see that the orientation of the excited state in the muscle when the excitation is passing away need not be the mirror image of the orientation of excitation when it is spreading over the muscle. And thus while at the beginning of the excitation one may say that the muscle near one electrode is excited while that nearer the other electrode is not yet excited—the time at which excitation begins in any particular

part of the syncytium being independent of the time during which that excited state will last— the passing away of excitation will be far less definite. The more extensive the electrodes, the greater the chance that the E.M.F. set up in one direction by one part of the muscle will be counterbalanced by an opposite E.M.F. due to another region.

Lastly, the time at which the final electric variation in the auricle occurs is most unfavourable for its recognition in the electrogram of the complete heart. It takes place as a rule at the same time as the initial ventricular variation. This is seen in comparing records taken with the electrodes alternately on sinus and auricle, and sinus and ventricle. With the former leads the tracing shows indication of the ventricular complex, but it is easy to distinguish the final variation due to the auricle. Fig. 18 illustrates these points. In this instance, as often with the electrodes on sinus and ventricle, the final variation is entirely swamped in the much greater ventricular variation.

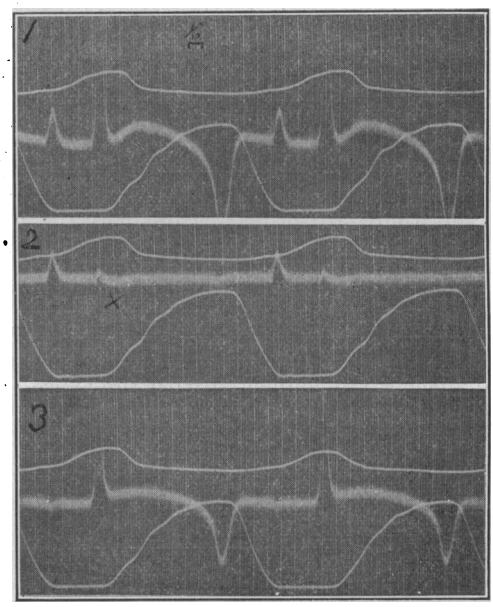

Fig. 18. Frog's heart. Not perfused. Records of contractions of auricle and ventricle in each tracing. Electrograms taken with the electrodes (1) on sinus and tip of ventricle, (2) on sinus and auricle, (3) on auricle and ventricle. The final auricular variation visible in (2) is marked with a cross. $\times \frac{2}{3}$.

Occasionally one may detect the final auricular variation in the electrogram of an uninjured heart with the electrodes on sinus and ventricle. I have already published an example of this (1912). It may be made apparent with these leads by making a section through the greater part of the auriculo-ventricular junction. In this way the area of contact of the ventricle with the auricle is reduced and the ventricular beat is, for a time, stopped, if the section is performed successfully. In such a preparation the whole electrogram of the auricle

becomes obvious (see Fig. 19). It is similar to that obtained by direct derivation from the auricle and it presents the same uncertainty regarding the form of its termination as does the ventricular electrogram.

If the ventricle starts beating with an independent rhythm, the interference of the auricular and ventricular electrograms meeting in

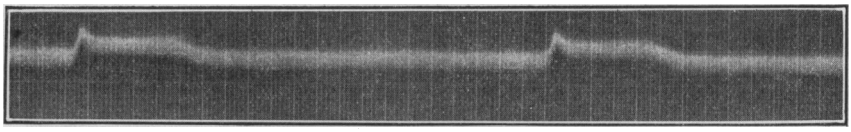

Fig. 19. Frog's heart. Auricle electrogram by derivation from sinus and ventricle, after partial section through auriculo-ventricular junction. The ventricle was quiescent. See text. $\times \frac{3}{5}$.

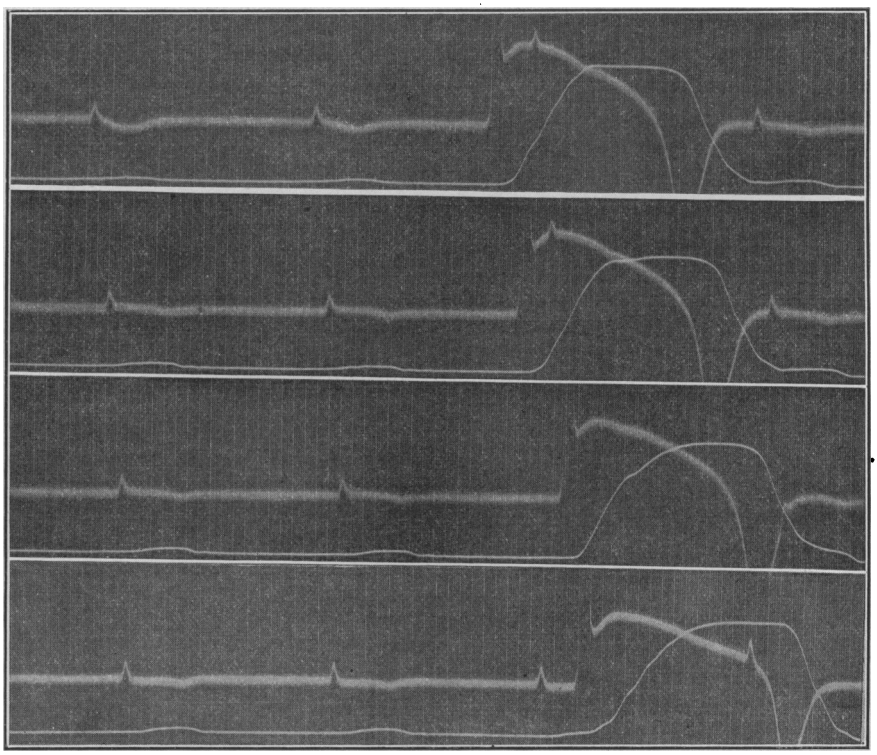

Fig. 20. Dissociation of auricle and ventricle, to show influence of phase of interference on form of electrogram. The mechanical record indicates both auricular and ventricular movements. $\times \frac{1}{3}$.

different phases illustrates how the former may sometimes be swamped in the latter. Fig. 20 gives an illustration of this, the cycle of the ventricular rhythm was here very slightly more than four times as long as that of the auricular rhythm.

The swamping of the initial auricular variation by the initial ventricular is familiar to every student of the electrocardiogram. It is seen in almost any case of dissociation of auricles and ventricles.

In the normally beating heart of the frog, with derivation from sinus and ventricle, the final auricular variation, even if it is big enough to affect the galvanometer, will often escape recognition because it is so timed as to fall within the great electric disturbance which occurs at the beginning of ventricular excitation.

Information to be derived from the experimental electrocardiogram.

We may now summarise the chief points of interest in our experimental electrocardiograms and decide which of their features may be studied with least risk of disturbance by experimental errors.

(1) *Duration of the cardiac cycle.* The first ventricular variation gives a delightfully sharp point in the cardiac cycle. The interval elapsing between the beginning of one ventricular excitation and the next may be measured with accuracy from this deflection.

(2) *Duration of the excited state in the ventricle.* Although its form is variable, the final variation of the ventricular complex is seldom absent. It is generally possible therefore to determine the total duration of the ventricular excitation by measuring the interval between the beginning of the initial and the end of the final ventricular variation.

(3) *The auriculo-ventricular interval.* As has been remarked, the initial variation of the auricle and that of the ventricle form constant features of the experimental electrocardiogram. The interval between the rise of the first and of the second of these waves indicates the interval between the beginning of auricular and of ventricular excitation. Even with considerable alterations in the position of the electrodes the value determined for this interval remains nearly constant. Thus in the curves given in Fig. 21, in (*a*) the electrodes were on the sinus and the tip of the ventricle. The electrogram gives ·48″ as the value of the auriculo-ventricular interval. In (*b*) the electrode previously on the apex was placed in contact with the auricle, close to the auriculo-ventricular junction. The first ventricular variation still affects the galvanometer and the interval shown is ·49″. In (*c*) the electrodes

remained as in (*b*) but the sensitivity of the galvanometer was increased. The interval still has the value ·49″.

(4) *The rate of propagation of the wave of excitation from base to apex.* Burdon-Sanderson pointed out that the time occupied in the passage of the excited state from base to apex is represented by the duration of the rising phase of the first ventricular variation. The

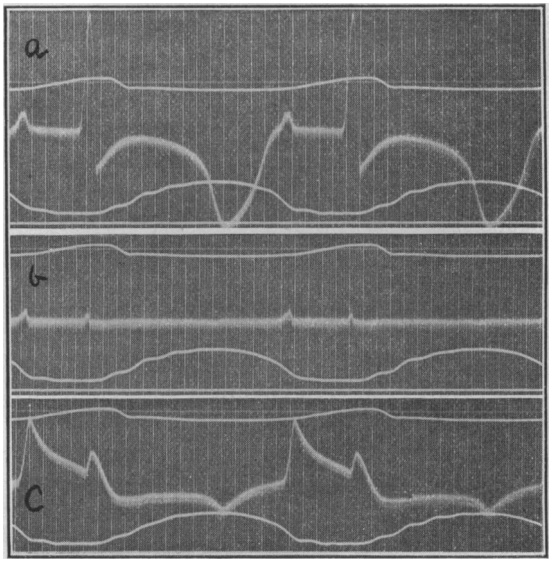

Fig. 21. $\times \frac{2}{3}$.

speed of recording surface which I have used (about 50 mm. per second) was too low for the satisfactory study of small changes in this value. I shall therefore refer only incidentally to measurements of this factor.

I have attached no particular importance to the absolute values of the potential differences recorded. The electrodes derive only a fraction of the total E.M.F. This fraction will be altered in amount according to the degree of tone of the heart, and therefore the amount of short-circuiting by fluid within the cavity. The potential differences available have been sufficient in every case to allow of a well stretched and therefore quickly responsive string. The deflection time has been as a rule between ·01″ and ·02″. My usual practice has been to adjust the tension of the string so as to give an easily read auricular variation.

The following data show that within wide limits of variation in sensitiveness of the galvanometer the time relations of the factors to be dealt with remain sensibly constant.

Deflection for ·01 volt.	Interval between initial aur. and ventr. variations	Duration of ventricular complex	Rising phase of initial ventricular variation
18 mm.	·45″	·92″	Off paper
4 mm.	·45″	·92″	·07″
2 mm.	·45″	·90″	·07″
Less than 0·5 mm.	·45″	Final variation illegible	·07″

From the mechanical records of auricles and ventricle, and from comparison of these with the simultaneous electrograms, information may be obtained on the following points:

(5) the relative amplitudes of the mechanical responses under various conditions,

(6) their durations under various conditions,

(7) the intervals between the beginning of excitation and of contraction.

It will be shown that these factors undergo striking and characteristic changes as a result of altered chemical environment.

THE INFLUENCE OF HYDROGEN ION CONCENTRATION.

If the heart is treated with a strongly acid (*e.g.* $C_H \cdot 10^{-2}$) or a strongly alkaline solution (*e.g.* $C_H \cdot 10^{-12}$) it stops in a firmly contracted state. But in both cases the stoppage and the going into contraction are two distinct affairs. For, as has been known from the time of Gaskell's experiments on the effects of lactic acid on the frog's heart (1880), a very moderate degree of acidity stops the heart in diastole: this is in fact the generally recognised effect of acid on the heart. Tonic contraction is produced by acidity only when far greater than that required to arrest the heart. With alkaline solutions on the other hand, the concentration needed to arrest the heart is very near that needed to send the muscle into systole. Consequently the effect of alkaline solutions on the heart is generally stated to be stoppage in systole. Yet it was pointed out by Gaskell, that occasionally the perfusion of an alkaline solution stopped the frog's heart in diastole and only some minutes later did it go into systole.

As I have shown elsewhere (1912) these effects of gross alteration in hydrogen ion concentration on the heart may be further analysed by comparison with the effects of certain polyvalent ions. Here I shall

consider the results of some experiments in which the reaction of the perfusion fluid was changed less extensively so that it remained always within those limits which permit the heart to continue beating for some minutes at all events. Acting on a suggestion made to me by Prof. Sörensen I used salts of weak acids to 'stabilise' the reaction of my solutions. The whole question of the mode of action of such substances in regulating hydrogen ion concentration has been most lucidly explained by Sörensen (1909) and by Henderson (1908). The 'buffers' naturally present in the blood, phosphates and carbonates, I avoided as a rule for special reasons which need not be discussed at this stage. In general I used borates and acetates. Mixtures were prepared containing each the proper concentration of calcium, potassium and sodium chlorides, and equal amounts of boric acid and sodium acetate. One of these mixtures was rendered strongly alkaline with soda, the other strongly acid with hydrochloric acid.

For example:—

Stock solution 1 HCl M/10 400 c.c.	Stock solution 2 NaOH M/10 400 c.c.
Boric acid M/10	100 c.c.
Sod. acetate M/10	100 c.c.
$CaCl_2$ M/10	40 c.c.
KCl M/10	60 c.c.
NaCl M/8	to 2000 c.c.

From such a pair of solutions it is easy to prepare a mixture of the desired hydrogen ion concentration by the following device. One of the Sörensen standard mixtures of the required C_{H^+} is taken, and a definite amount of an indicator added, the indicator selected being of course in the proper range. 10 c.c. of solution 1 are taken in a test tube of the same bore as the tube containing the standard: the same concentration of the indicator is added. 10 c.c. of solution 2 are taken, the same concentration of the same indicator added and the solution placed in a small burette. Solution 2 is then run into the test tube containing solution 1 until the mixture of 1 and 2 matches the Sörensen standard. It is thus determined in what proportions the stock solutions must be mixed. With the above mixtures the proportions were as follows:

Amount of 1		Amount of 2	C_{H^+}
6·2	+	10	10^{-10}
8·4	+	10	10^{-9}
9·85	+	10	10^{-8}
10·1	+	10	10^{-7}
10	+	9·7	10^{-6}
10	+	8·6	10^{-5}
10	+	6·8	10^{-4}

These values were used only as rough indications of the proportions in which the solutions should be mixed. In each experiment determinations were made on the actual mixtures used, by Sörensen's method.

Control experiments showed that the hydrogen ion concentration of mixtures of this type was not appreciably changed by one passage through the frog's heart. It is quite otherwise when approximately neutral solutions containing no 'buffers' are perfused. It is true that such mixtures differ in the concentration of sodium ions and of chlorine ions. But these constituents are present in very large amount in every case, in comparison with which the differences are trivial. It is theoretically impossible to have two solutions at the same temperature differing in hydrogen (and hydroxyl) ion concentration only; this would mean an alteration in the dissociation constant of water. But by the use of different solutions we can change the equilibria immediately dependent on the hydrogen ion concentration in different cases, and thus distinguish between effects due to the influence on hydrogen ion concentration on the composition of the fluid in other respects, and those due directly to the altered hydrogen ion concentration of the solution brought in contact with the living tissue. Indirect effects cannot be eliminated except by elaborate series of experiments; yet there are some serious instances which can be avoided. For example if we are going to study the effects on the heart by diminishing the hydrogen ion concentration of solutions containing calcium, it will be unwise to use solutions containing carbonates. For as the alkalinity of the solution increases calcium carbonate will be thrown out, and thus the composition of the fluid with respect to a most significant constituent for the activity of the heart will be modified. Experiment confirms the operation of this factor. It does not enter when using the borate-acetate Ringer solutions within the limits observed in this paper. In some experiments, acting on a suggestion given by Clark (1912), I replaced a part of the sodium chloride in the solution by a 7 % solution of cane sugar.

When the C_H of the perfusion fluid is changed to a relatively small extent, the resulting changes in the behaviour of the heart are manifested gradually. It takes a long while for equilibrium to be attained. In order to appreciate the course of such changes, the records taken at intervals of a minute or two must be measured and the results plotted against the time. As a rule, three or four beats were recorded on the rapidly moving paper on each occasion. The measurements made on

successive beats in the same tracing showed, with few exceptions, very close concordance.

Fig. 22 shows an instance where perfusion for over 20 minutes with a neutral solution $C_H \cdot 10^{-7}$ produced no change in the duration of the electrical response. There was a slight slowing of the heart during this time and a distinct lengthening of the electrical A–V interval. Change to an alkaline solution 10^{-9}, caused a temporary slight acceleration and then marked slowing. The duration of the ventricle electrogram diminished, and the electrical A–V interval diminished slightly. After

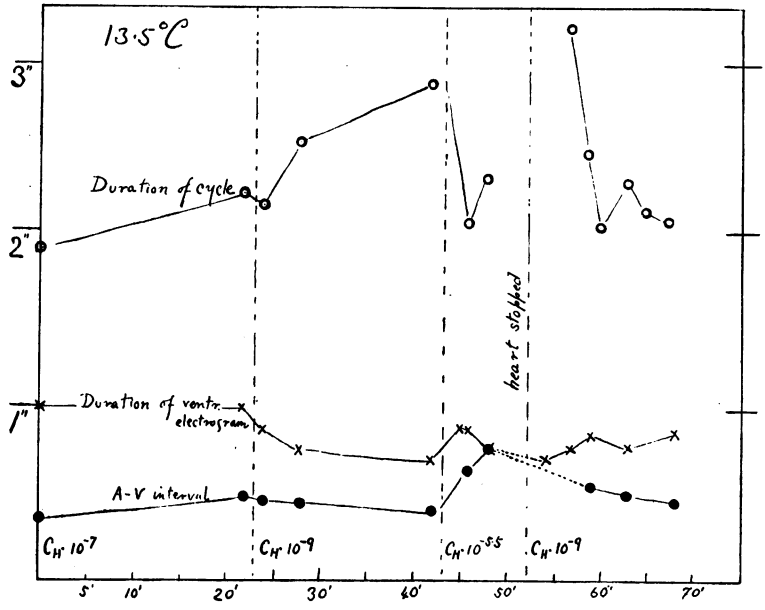

Fig. 22.

20 mins. perfusion with the alkaline solution, a change to a solution on the acid side $C_H \cdot 10^{-5.5}$ caused acceleration of the rhythm at first, slight alteration in the duration of the ventricular electrogram and very pronounced lengthening of the A–V interval. After about 9 mins. the heart stopped. An alkaline solution restored the beat, which at first was slow, but soon got quicker. The duration of the A–V interval was steadily reduced but at the end of the experiment it had not quite returned to the value it had when the heart was perfused with the solution of $C_H \cdot 10^{-9}$ for the first time.

The very large effect on the A–V interval has been noticed in numerous experiments. In every case in which two solutions have

been used of hydrogen ion concentration within the limits where function is possible, the heart in equilibrium with the more alkaline solution has shown an A–V interval distinctly shorter than when it is in equilibrium with the more acid solution.

The effect of a change in C_{H^+} is often well marked within a few minutes. For instance:

Frog's heart. Borate-acetate Ringer mixtures.

After 6 mins in C_{H^+}	$10^{-6.45}$	A–V interval ·35 sec.
2	$10^{-9.1}$	·29
1·5	$10^{-6.45}$	·34
2	$10^{-9.1}$	·29

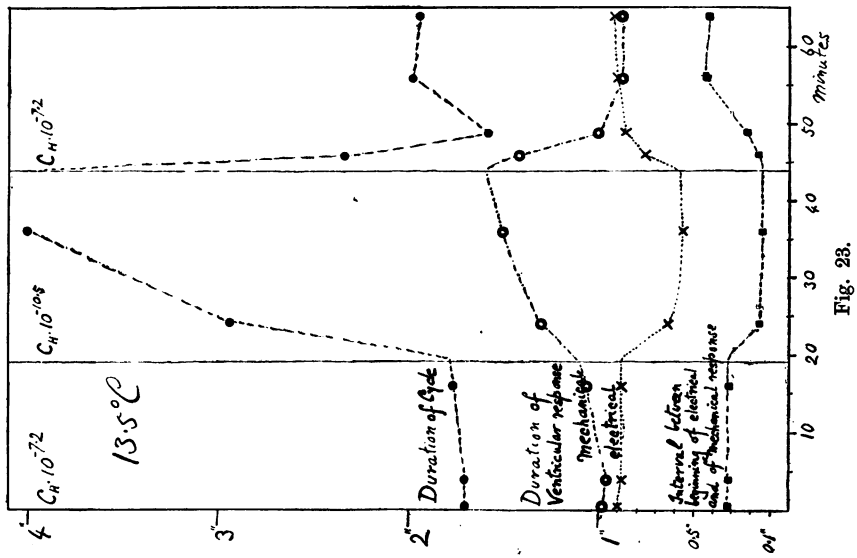

Fig. 23.

When the heart has been perfused with a solution near the acid limit for a long time, the A–V interval may become very long, *e.g.*, 1·5 secs. or even more. If the rhythm of the sinus is not arrested, and if beats continue to get through to the auricle it is observed that further prolongation of the A–V interval leads to heart block, at first partial and then complete. Perfusion with an alkaline solution quickly removes the block and then steadily reduces the A–V interval.

The interval between the beginning of the electrical response and the beginning of the mechanical response in the ventricle appears to follow the same course as the A–V interval in all experiments where

both these factors have been measured. This point is illustrated in Fig. 24. It will be discussed later.

Fig. 23 shows a case in which the same change of environment caused very marked lengthening of the mechanical response and shortening of the electrical response in the ventricle. It is seen from the figure that 19 mins. perfusion with a solution of $C_H \cdot 10^{-7.2}$ caused practically no change in the duration of the cycle, in the duration of the electrical response of the ventricle or in the interval between this and the beginning of the mechanical response. There was a slight increase in the duration of the mechanical response. It would seem that this solution was not very far removed in its properties as a perfusion fluid from the frog's blood. Changing to a markedly alkaline mixture $C_H \cdot 10^{-10.5}$ caused slowing of the heart and increase in the duration of the mechanical response of the ventricle. But the duration of the electrical response was reduced. The interval between the beginning of the electrical and that of the mechanical response was reduced to less than half its original value. Return to the solution of $C_H \cdot 10^{-7.2}$ was followed by the gradual return of these four factors to something near their original values. This eliminates progressive changes due to uncontrolled factors in the tissue in a satisfactory way.

Fig. 24 shows the results of a more prolonged experiment. Here perfusion was started with an acid solution. Change to the alkaline solution caused, as usual, diminution of the A–V interval and of the interval between the beginning of the electrical and mechanical responses. As in the last experiment it caused lengthening of the mechanical response and shortening of the electrical response of the ventricle. It also produced increase in the height of the ventricular contraction.

It must be noted that the lines connecting the points marked in this and other figures are put in for convenience in following the points. They are not to be taken as necessarily representing the condition of affairs at periods between the points. The effect of prolonged treatment with a solution near the acid limit is well seen in the next part of this experiment. The A–V and 'elect.-mech.' intervals become greatly lengthened, the duration of the mech. and elect. responses of the ventricle, at first reduced, become longer, the mechanical response of the ventricle is reduced in height, and the duration of the complete cycle is markedly increased. Prolonged treatment with the alkaline solution reverses completely the effects of the acid solution on the A–V interval and the 'elect.-mech.' interval; it increases the height of the contraction, reduces the duration of the electric response, but after a primary

reduction, it increases the duration of the mechanical response. It is interesting to compare the effects of the acid solution on the heart after prolonged treatment with the alkaline solution with the effects produced after the short treatment. A marked discrepancy appears only in the effect on the rhythm.

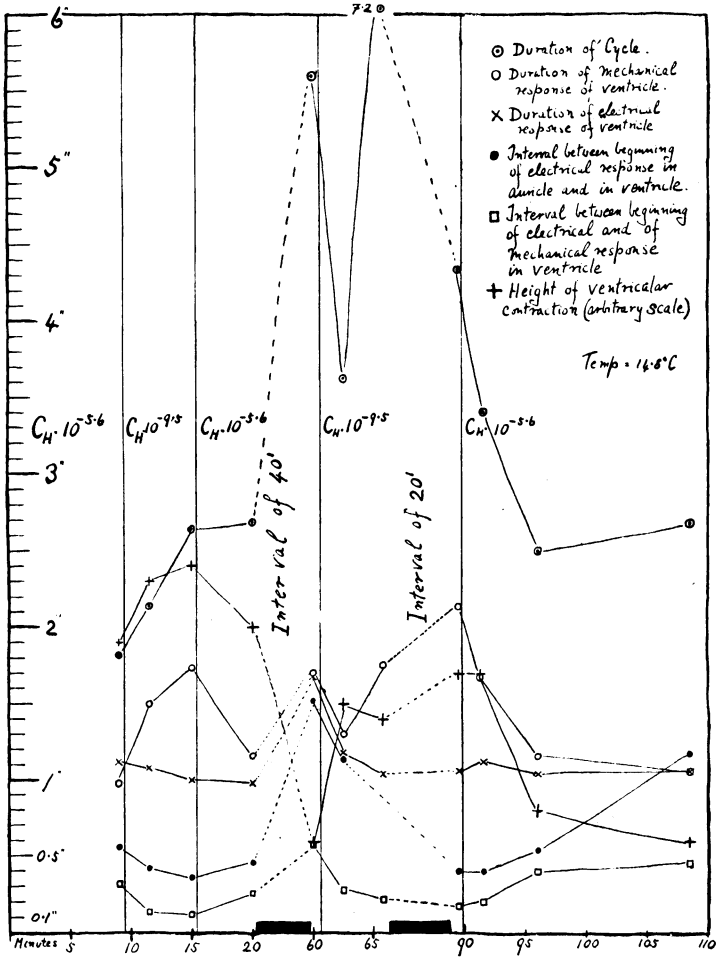

Fig. 24.

The comparison of the height of contraction in two different solutions, one of which is markedly alkaline, is not satisfactory, because in the alkaline solution the increase of tone means that the degree of relaxation is influenced largely by the tension placed on the heart. It

would of course be preferable to have records of the tension set up in the muscle: but I am unable at present to see any way of registering this without sacrificing some of the great advantages of the free perfusion method. Fig. 25 shows the progressive change in the rhythm, height of contraction, duration of electric response and A–V interval of a heart perfused with a solution on the acid side of the optimum but well

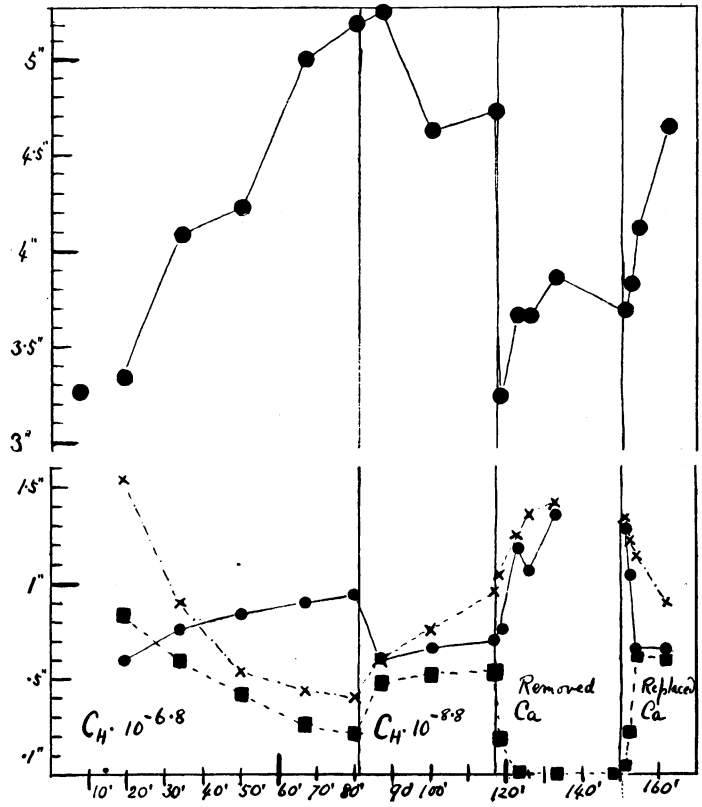

Fig. 25. Large black circles = duration of cycle. Small black circles = A–V interval, elect. Crosses = duration of ventricle electrogram. Black squares = height of ventricular contraction (arbitrary scale). Further description in text.

within the limits which permit of long continued activity. The beats become less frequent, they become weaker, the duration of the electric response is diminished while the A–V interval is increased. All these changes except that of the rhythm are immediately reversed on changing to the more alkaline solution. It is clear then that the change in rhythm is not the determining cause, in this instance, of the other

changes. I shall discuss the influence of the frequency of beat on the character of the individual beats in another place. The figures presented in this and in the following section show that in the heart beating spontaneously no simple statement of a relation between frequency and other factors in cardiac activity under the various conditions here described is possible.

Influence of the concentration of calcium ions.

Since Ringer's discovery of the peculiar importance of calcium salts for the activity of the heart, perhaps the most interesting observation in connexion with this subject is that of Locke and Rosenheim (1907). In the course of their work on the consumption of dextrose by the excised mammalian heart they found that a heart brought to rest by perfusion with a solution devoid of calcium, consumed sugar at a rate scarcely inferior to that of a normally beating heart. Locke made the further very significant discovery that in the mammalian heart without calcium, the spontaneous action current (observed with the capillary electrometer) remained strong, long after the mechanical beat had become minimal. Locke concluded that "calcium is necessary for the conversion of the heart's chemical energy into the mechanical energy of its beat."

My experiments entirely confirm Locke's observation regarding the dissociation of the electrical and mechanical responses of heart muscle.

Noyons (1908) has shown the possibility of dissociating the mechanical from the electrical response by various poisons.

In numerous experiments I have seen the heart-beat reduced to much less than 1 % of its original strength while the electrical response remains vigorous—very often not reduced in extent at all.

Occasionally I have seen the heart, perfused with a calcium-free solution, brought into a condition in which no movement whatever was perceptible on the most careful examination, while the galvanometer showed strong regular deflections of the same general form as the ordinary electrogram and no smaller. This I noted in two or three early experiments, but in many subsequent experiments I observed that though there was an enormous reduction in the mechanical response as compared with the electrical response, yet some mechanical movement remained, especially in the auricle—movement too weak to affect the delicate levers used, yet perceptible if the light was suitably arranged so that reflections from its surface could be examined. These observations made me doubt whether my examination of the heart in the

earlier experiments, in which I had considered the abolition of movement to be complete, had been sufficiently thorough: moreover, certain theoretical considerations led me to think it probable that a certain mechanical movement always persisted. Under these circumstances, I attach peculiar importance to one of the last experiments on which this paper is based. The experiment was satisfactory from the point of view of technique. Removal of calcium reduced the mechanical response, but not the electrical; for some time the movements of the auricle and ventricle remained distinct though slight, then the movements of the auricle only could be seen; but later, the most careful examination I could make, lighting the heart in different ways and observing it from all points of view, failed to show me when the 'beats' occurred: yet the galvanometer showed large and perfectly rhythmical deflections[1].

It may be objected, of course, that some internal portions of the musculature were even in this case still contracting. Such movements, if they existed, must have been extremely feeble to communicate no perceptible displacement to the surface. Moreover the suggestion does not seem altogether probable for another reason. The weakening of the beat is the result of the removal of calcium. The exterior of the heart, the region which we can best observe, is, in the method employed, the region least in contact with the perfusion fluid. The internal parts are much better placed to secure complete washing out. It is to be expected, then, that the movements of the internal portions of the muscle will be even feebler than the movements of the superficial parts.

Although the electric response of the heart usually remains strong after removal of calcium from the perfusion fluid, provided the other conditions in the perfusion fluid are favourable (and in particular that an alkaline reaction is maintained), the electrocardiogram is not unaffected. Leontowitsch (1912) states that the form of the electrogram of the frog's heart is affected in various ways by alteration in the calcium concentration. Unfortunately the description of his methods is insufficient to allow of comparison between his results and mine. In spite of a sensitivity of the galvanometer over 100 times greater than that which I found it necessary to employ, Leontowitsch found that sometimes records of the normal frog's heart gave him no P (auricular) or R (first ventricular) waves.

Removal of Ca from the perfusion fluid, resulting in abolition of the

[1] The electrical variation due to the auricles is nearly always reduced on removal of Ca. This is certainly largely, if not entirely, due to their much increased distension and the consequent short-circuiting of current through the fluid.

mechanical response, does not necessarily alter the form of the final ventricular variation. In some instances, during perfusion with these solutions there has been change in the character of this very variable part of the electrogram; but I have seen no definite correlation, even in individual experiments, such as appeared in some instances where the factor varied was the hydrogen ion concentration.

An interesting and constant change produced in the electrogram by removal of calcium is a marked increase in the interval between the initial auricular and ventricular variations. My friend Dr A. J. Clark

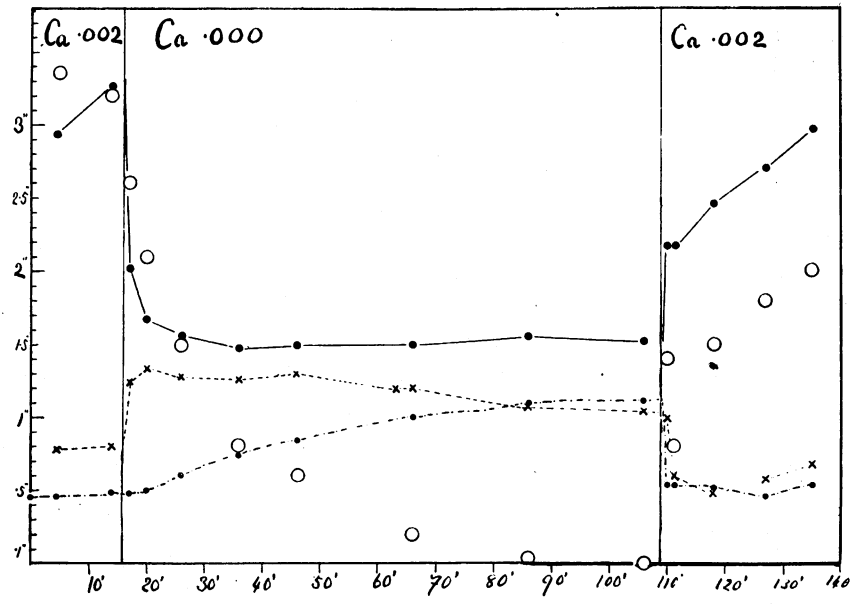

Fig. 26. Large black circles = ½ duration of cycle. Small black circles = A–V interval. Crosses = duration of ventricle electrogram. Large clear circles = height of ventricular contraction (arbitrary scale). Temp. = 13·2°. $C_H \cdot 10^{-7.7}$.

drew my attention to the fact that in a heart perfused with a calcium-free solution, mechanical records of auricle and ventricle showed a marked increase of the interval between them. My experiments confirm this observation and show further that the increased mechanical A–V interval is compounded of (1) an increase in the interval between the excitation of the auricle and that of the ventricle, (2) an increase in the time lost between the excitation of the ventricle and the appearance of its mechanical response.

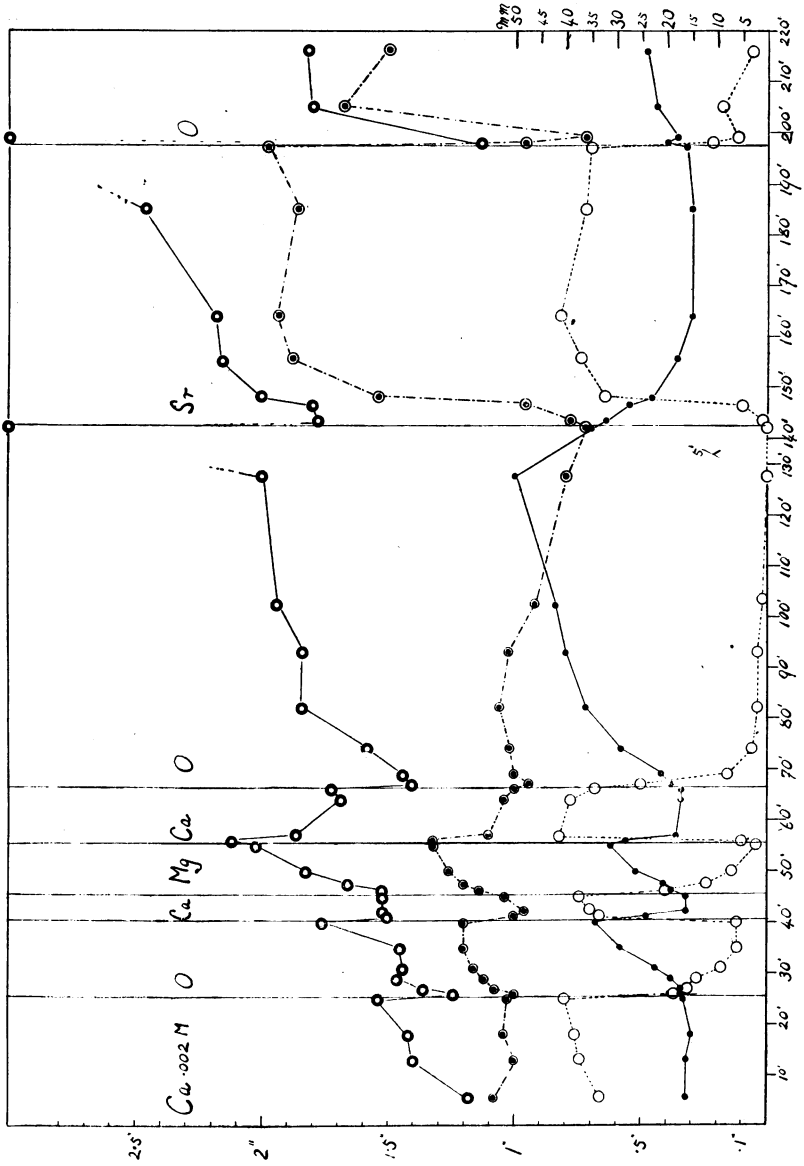

Fig. 27. The points represent, reading from above downwards at the start, $\frac{1}{2}$ duration of cycle, duration of electric response of ventricle, height of ventricular contraction recorded (mm. scale on right) and A–V interval (electr.). At 0, solution containing no calcium, at Mg and Sr solutions containing ·002 M Mg or Sr instead of Ca. All solutions of $C_H \cdot 10^{-8.8}$. Temp. = 14° C.

I find also that with the heart in this condition where the beats, though enfeebled, still remain, that the latent period of the mechanical response to electrical excitation of the ventricle is notably increased. The relation of the A–V interval to the calcium concentration appears only when the latter is very much reduced. The Ca concentration may be varied from, *e.g.*, ·008 M to ·001 M without producing any but trivial effects on the (electrical) A–V interval. But on complete removal of Ca from the perfusion fluid the interval begins to increase almost at once. After some time (*e.g.*, in 15 or 30 mins.) the interval may be doubled. I have sometimes seen partial block follow (cf. p. 233). The restoration of Ca to the perfusion fluid results in the very prompt reduction of the A–V interval to its original value.

Removal of calcium tends to lengthen the duration of the ventricular complex of the electrogram. As may be seen from Figs. 26 and 27 this change usually reaches its maximum earlier than the change in the A–V interval. Prolonged perfusion with the Ca-free solution may result in a secondary diminution of the duration of the ventricular complex, which, however, generally remains greater than in the heart supplied with calcium, and drops abruptly when the calcium is restored. I have observed similar changes in the duration of the ventricular complex on changing the calcium concentration from ·002 M to ·001 M or from ·008 M to ·002 M and *vice versâ*: the ventricular complex being in general rather longer in the solution poorer in calcium. The solutions always contained ·0025 M KCl.

It was noticed by Ringer, and I have confirmed the observation many times, that removal of calcium from the solution perfusing the heart causes at first an increase in the frequency of the beats. In the later stages of the washing out of calcium from the heart there is often a marked slowing of the rhythm. But provided a certain small concentration of calcium is present, other factors remaining the same, increase in the concentration of calcium causes slowing of the beat, decrease in concentration of calcium causes quickening.

Reference to Figs. 25 to 28 will show the order of magnitude of this effect. Now it may be recalled that in certain cases which have been investigated there is a very clear relation between the concentration of magnesium and the frequency of the rhythm of the heart. I have plotted such relations in certain elasmobranch hearts (1912). The hearts of the frog and toad are affected in the same sort of way by magnesium. The frequency of the rhythmic impulses arising in the sinus venosus is definitely reduced by the addition of a small concentration of magnesium

ions to the perfusion fluid. The effect is perfectly reversible. When the concentration of Mg is raised to ·01 M or rather more with a perfusion fluid of C_H· about 10^{-6}, the frog's heart very often stops: the stoppage is due to the cessation of the rhythmic impulses, the ventricle remains fully excitable and capable of contracting well, and very often after a pause strong beats begin again, originating in the bulbus aortæ.

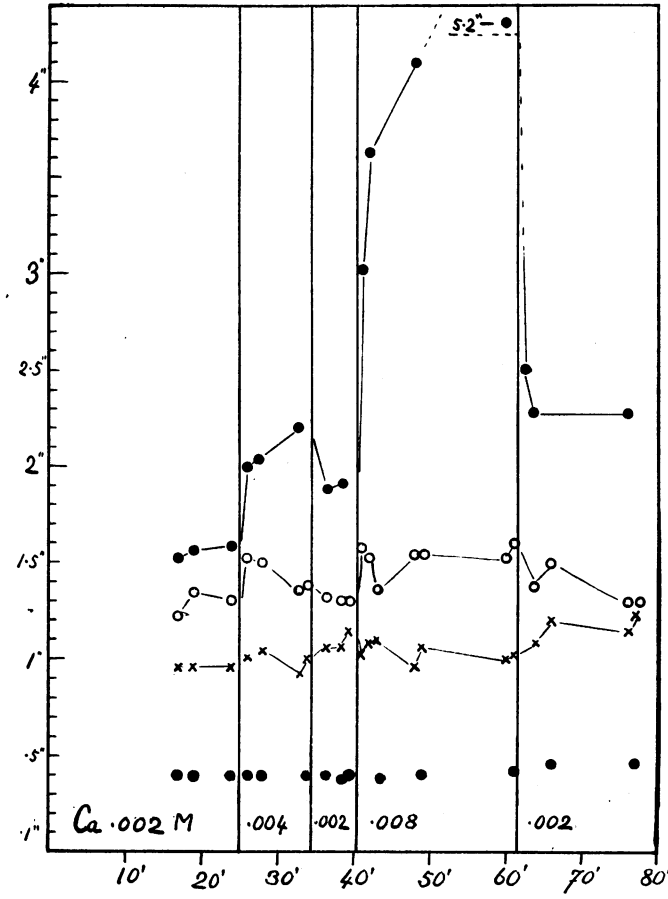

Fig. 28. Points represent, reading from above downwards, duration of cycle, duration of mechanical response of ventricle, duration of electr. response of ventricle, A–V interval.

I have drawn attention in a previous paper (1911) to the fact that a distinction must be drawn between the mode of action of calcium in those cases where it can be replaced by magnesium (*e.g.* skeletal muscle as regards its electrical excitability) and those cases where no such

replacement is possible (*e.g.* neuro-muscular junctions). It now appears that in a single tissue we may trace by this method of analysis two different modes of action of calcium. For while it is very well known that so far as the contractile function of cardiac muscle is concerned calcium cannot be replaced by magnesium, it would follow from the above considerations that in respect of the primary effect of the change of concentration of calcium on the frequency of the automatic rhythm

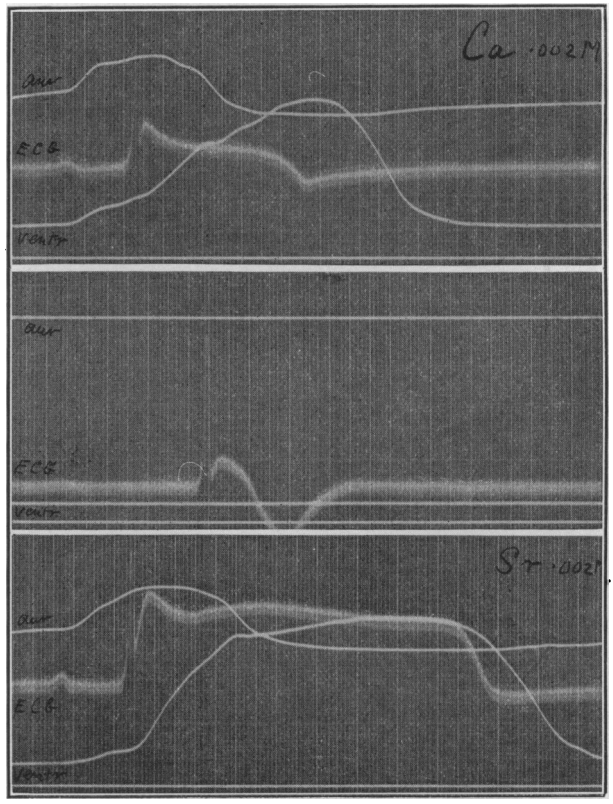

Fig. 29. $\times \frac{1}{2}$.

in the sinus such replacement should be possible. This is in fact the case. If instead of changing the calcium containing solution for a solution the same in all respects except that it contains no calcium, we change to a solution in which the calcium is replaced by an equivalent amount of magnesium, the effects of calcium removal on the size of the contractions and on the A–V interval make their appearance as usual,

but the primary quickening of the rhythm is not observed. So far as this particular effect on the rhythm is concerned, calcium is replaceable by magnesium. Fig. 27 demonstrates this point very clearly. It also shows that the effects of removal of calcium on the A–V interval and on the duration of the electric response of the ventricle are not prevented by the introduction of magnesium. An equivalent concentration of strontium added to the calcium-free fluid, as Ringer showed (1883), restores the mechanical beat to its full strength. Fig. 27 shows that it also restores the A–V interval to its normal value. The continued action of this small concentration of strontium leads to enormous prolongation both of the electrical and mechanical responses of the ventricle[1].

In Fig. 29 three beats are reproduced from the 140 records obtained in the experiment plotted in Fig. 27. They show the behaviour of the heart in the calcium containing solution, in the calcium-free solution and in the strontium solution. The relations between the mechanical activity of the heart and the concentration of calcium ions in the perfusion fluid suggest that the effect of removal of calcium is the dissociation of a calcium compound forming an essential part of the mechanism. Since the electrical response remains and the power of consuming dextrose (Locke and Rosenheim) it is to be supposed that there is still a rhythmic production of lactic acid and probably still a rhythmic evolution of heat. The latter point will be tested.

On the general hypothesis of the muscle contraction grounded on the work of Fletcher and Hopkins, which has been outlined by A. V. Hill (1913) and by myself (1913), one would say that in the case of removal of calcium the contractions fail because the actual contractile mechanism which lactic acid in the ordinary course of events plays upon is thrown out of gear. The simplest hypothesis embracing the observed facts is this. As has been pointed out by Strietmann and Fischer (1912) a 'contractile mechanism' which will shorten on the application of dilute acid need be no more complex (in a macroscopic sense) than a series of strands of some colloidal material. If the contractile mechanism in the muscle is of this type, it is not at all improbable that the contractile material is a calcium salt. If the

[1] The effect of the strontium salt is to prolong the excitation wave without in any way reducing its rate of propagation. It will be shown in a subsequent paper that a slow short wave of excitation is characteristic of the heart muscle when in a condition favourable for fibrillation or a circulating rhythm which recalls tachycardia. It is theoretically probable that the pathological conditions of fibrillation and paroxysmal tachycardia would be benefited greatly by injections of strontium salts.

tension of calcium in the surrounding fluid is reduced below a certain level the calcium compound dissociates, and the residue, which forms part of the structure of the muscle no longer, has the property of shortening when small concentrations of lactic acid are liberated in contact with it. The addition of calcium or of strontium to the fluid causes the re-formation of a substance with the requisite property.

We have seen that the initial effect on the rhythm of altering the concentration of calcium is due to a different and less specific property of the calcium ion. This is shown by the facts mentioned above regarding the replacement by magnesium, and still more clearly by the forms of the curves expressing these relations. These are represented in a purely diagrammatic fashion in Fig. 30. In a future paper I shall give data for the more exact working out of these relations.

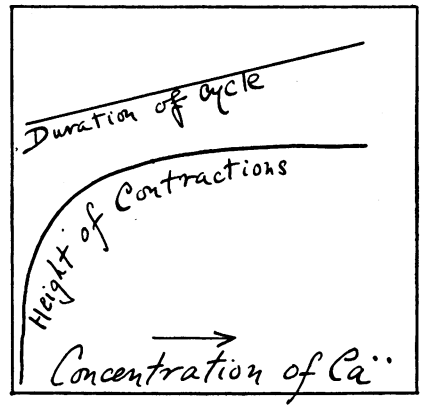

Fig. 30.

How are the effects of removal of calcium on the A–V interval and on the duration of the electric response of the ventricle to be accounted for? It must be borne in mind that when the mechanical response of the muscle is greatly reduced, still more when it is abolished, the relations between perfusion fluid and muscle are altered. Not only is the rate of flow through the heart cavity reduced, but also the fluid in the cavities is no longer mixed with that in the sponge-work of the muscular walls by mechanical squeezing. And as regards the finer structure of which we know so little for certain, there can at least be no doubt that the presence or absence of movement will alter the distribution of the metabolites, if these are produced, and there is good reason to believe, in one region or phase rather than another.

In the absence of movement a new type of equilibrium must be attained in which diffusion is no longer assisted by stirring. At present we are not in a position to decide how far this factor is responsible for the alterations in the electrocardiogram which result from the removal of calcium. I hope to return to the discussion of this point after the completion of series of experiments now in hand

on the dynamic equilibrium in heart muscle excited artificially with different rhythms.

That the intrinsic activity of the muscle may have a very large effect on its subsquent condition when the beats have been cut down by the removal of calcium, is shown in an unmistakable way by the observation recorded in Fig. 31. Here the duration of the A–V interval (elect.) in a series of beats is shown. The auricle was responding electrically to the spontaneous sinus excitations at regular intervals of 3·66 seconds. The first point plotted shows an A–V interval of 1·16 secs.; in the next cycle the interval was 1·18 secs. while in the

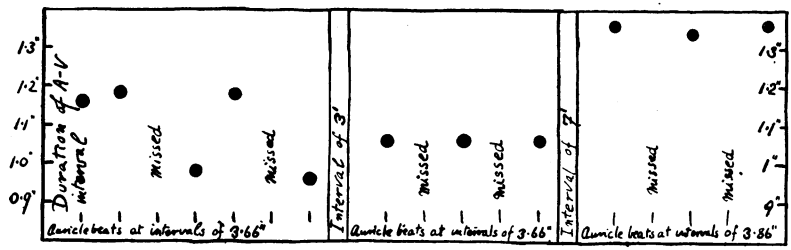

Fig. 31.

next there was no response on the part of the ventricle. After the double pause the next A–V interval was reduced to less than a second, the next, after a single pause, was longer, then again there was a miss, and so forth. After three minutes a new equilibrium was attained in which every alternate beat got over, and the A–V intervals were of equal length, shorter than the long, but longer than the short intervals in the preceding example. This type of rhythm was maintained for over half an hour and never gave place to complete block.

SUMMARY.

A method of taking simultaneous records of the contractions of auricles and ventricles and of the electrical changes in the perfused heart of the frog is described. The form of the electrocardiograms obtained under experimental conditions is discussed. It is shown that the form and sign of the final variation of the ventricular complex is dependent on differences in the duration of the excited state in different regions, such differences very commonly masking effects such as Gotch has described due to differences in the time at which the propagated disturbance reaches different regions.

The experimental electrocardiogram is chiefly of value in (1) the accurate determination of the duration of the cardiac cycle, (2) the

measurement of the duration of the excited state in the ventricle, (3) measurement of the interval between beginning of excitation in auricle and in ventricle, (4) measurement of the rate of propagation of the wave of excitation from base to apex (Burdon-Sanderson). In conjunction with the mechanical records, information is also obtained regarding (5) intervals between beginning of excitation and of contraction in auricles and ventricles.

Experiments on the effects of alteration in concentration of hydrogen ions and of calcium ions in the perfusion fluid demonstrate the following among other points in the functional analysis of the heart. Mechanical movement of the heart may be abolished while regular spontaneous electrical variations of normal form and extent continue. The duration of the A–V interval may be increased to three times its normal value. Such a change is not necessarily associated with any slowing of the rate of conduction in the ventricle; in these experiments it has been accompanied by an increase in the interval between the beginning of the electrical and of the mechanical response of the muscle and by weakening of the mechanical response. The frequency of the spontaneous rhythm may be varied over a considerable range by certain alterations in the perfusion fluids without changing the A–V interval.

The influence of $Ca^{\cdot\cdot}$ on the heart rhythm is shown to depend on a different property of $Ca^{\cdot\cdot}$ from that which determines its relations to the mechanical response and to the propagated disturbance.

If the muscle is in a condition in which the mechanical response is possible, the mechanical response in each cycle will not end before the electrical. The substitution of strontium for calcium in the perfusion fluid for example prolongs greatly the electrical and the mechanical responses. But prolongation of the mechanical response may be produced by a change (decrease of C_H) which at the same time causes shortening of the electrical response.

I wish to express my sincere thanks to Prof. Langley for the special facilities he has granted me in the laboratory and to Miss Dale for her valuable assistance in many experiments.

Part of the expenses of this research have been defrayed by grants from the Government Grant Committee of the Royal Society, for which I would record my gratitude.

REFERENCES.

1880. Gaskell. This Journal, III. p. 57.
1880. Burdon-Sanderson and Page. Ibid. II. p. 384.
1882. Gaskell. Phil. Trans. p. 1017.
1883. Burdon-Sanderson and Page. This Journal, IV. p. 327.
1883. Ringer. Practitioner, XXXI. p. 81.
1892. Bayliss and Starling. Internat. Monatschr. f. Anat. u. Physiol. IX. p. 256.
1907. Gotch. Proc. Roy. Soc. LXXIX. B, p. 323.
1907. Locke and Rosenheim. This Journal, XXXVI. p. 213.
1908. Henderson. Ergebn. d. Physiol. VIII.
1908. Noyons. Koninkl. Akad. Amsterdam, p. 273.
1909. Sörensen. C. R. Lab. Carlsberg, VIII. p. 1.
1910. Gotch. Heart, I. p. 235.
1910. Hoffmann. Pflüger's Archiv, CXXXIII. p. 522.
1910. Noyons. Koninkl. Akad. Amsterdam, p. 680.
1910. Samojloff. Pflüger's Archiv, CXXXV. p. 417.
1910. Straub. Z. f. Biol. LIII. p. 499.
1911. Mines. This Journal, XLII. p. 251.
1912. Burridge. Q. Journ. Exp. Physiol. V. p. 347.
1912. Clark. Proc. Roy. Soc. of Medicine, V. p. 181.
1912. Fischer and Strietmann. Koll. Zeitschr. X.
1912. Herlitzka. Arch. di Fisiol. X. p. 261.
1912. Leontowitsch. Pflüger's Archiv, CXLVII. p. 473.
1912. Meek and Eyster. Amer. Journ. Physiol. XXXI. p. 31.
1912. Mines. This Journal, XLIII. p. 467; Ibid. XLIV. p. xxi. Proc. Camb. Philosoph. Soc. XVI. p. 615.
1912. Seemann. Z. f. Biol. LIX. p. 53.
1912. Sörensen. Ergebn. d. Physiol.
1913. Hill. This Journal, XLVI. p. 28.
1913. Mines. Ibid. XLVI. p. 1. Proc. Camb. Philos. Soc. XVII. p. 34.