A STUDY OF THE ELECTRICAL FIELD SURROUNDING SKELETAL MUSCLE[1].

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PART I. THE PROBLEM OF ACTION CURRENT.

A STRIP of muscle is taken from the body, suspended in air and stimulated at one end; two contacts are placed in line upon the muscle, at a distance from the stimulating electrodes, and are connected to a sensitive galvanometer. As is well known from the experience of many workers, the electrical effect recorded is a diphasic curve of which the first phase indicates a potential at the proximal contact lower than that at the distal contact, the proximal contact being that first reached by the excitation wave.

In a previous investigation (1) of cardiac muscle, the tissue strip was examined not suspended in air but deeply immersed in a quantity of saline. The electrical currents developed about excitable tissue so immersed seem never to have been investigated; the need for so proceeding has arisen out of the method of electrocardiography in which the muscle from which records are taken always lies deeply buried in a conducting medium. Using immersed heart muscle strips and stimulating and leading off as above, a simple diphasic effect was never obtained. The curves were always polyphasic, and the initial deflection invariably indicated relative positivity of the proximal contact. On the basis of these and related observations views were expressed in regard to the manner in which currents arise and flow in the medium surrounding immersed cardiac muscle. It was considered that the field of potential due to such currents could best be explained on the basis of doublets; and this hypothesis was fully discussed.

From further consideration it seemed that the records obtained from immersed cardiac muscle might conceivably be peculiar to that type of muscle, composed as it is of a syncytium of extremely short individual fibres. It seemed important therefore to investigate the action currents

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[1] Work undertaken on behalf of the Medical Research Council.

of some simple long parallel-fibred skeletal muscle, similarly immersed, in order to ascertain whether there is any essential difference in the manner in which action currents arise from the two tissues in question.

Galvanometric responses have accordingly been obtained from two leads applied to skeletal muscle under the following conditions: (*a*) when the saline surrounding the tissue is limited, as for example when a muscle is kept moist and is suspended in air; (*b*) when the saline surrounding the tissue is in considerable amount, *e.g.* when the muscle is either immersed in, or lies in contact with the surface of a large quantity of saline; (*c*) when two contacts are on injured and uninjured regions respectively of a partly injured muscle, and the latter is suspended (i) in air, (ii) in a considerable body of saline; and (*d*) when one contact is at the surface of the tissue, and the other at some distant point in a considerable quantity of saline surrounding the tissue. These variations in leading off were chosen for reasons that developed as the investigation proceeded.

The responses recorded are a continuous expression of the differences of the potentials of the regions in the saline to which the leads are applied; these differences of potential are in turn an expression of the lines of current flow that result from muscle activity. Any hypothesis put forward to express these responses in terms of specific levels of potential at specific regions of the active tissue must, clearly, explain all recorded curves with equal facility provided the conditions of their inscription are understood.

Method.

The five experiments that follow have certain features in common.

Preparations used. The sartorius of the frog was selected as it is composed of long parallel fibres. Nerve conduction was eliminated by preliminary curarisation. This was obtained by injecting 0·1 to 0·2 c.c. of a 1 p.c. solution of curare into the dorsal lymph sac of the decerebrate animal some 20 minutes before exposing the muscle. Curarisation was considered complete when powerful electrical stimuli applied direct to the vertebral column produced no response in the muscles of the lower limbs.

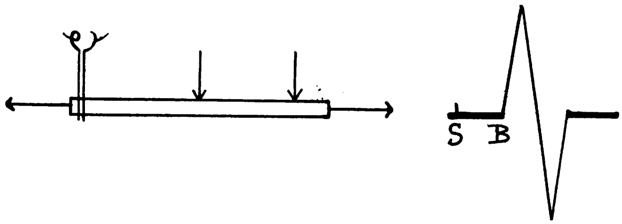
Electrodes. The stimulating electrodes consisted of two parallel silver wire needles connected to an adjustable induction coil. They were placed in contact with one end of the tissue in such a way that the plane passing through them was perpendicular to the long axis of the muscle. The recording contacts were composed of glass tubing of 3 mm. bore drawn to a point of 1 mm. in diameter or less. Polarisation was diminished by filling the contact end with 12 mm. of gelatin made up with Ringer's solution. Above this was placed 5 cm. of saturated copper sulphate solution into which dipped pure copper foil.

Interpretation of curves. The electrical responses were recorded by means of a string galvanometer. The string used had a diameter of 1·5 μ and a length of 6 cm. Its period of vibration was of the order of 0·002 sec. with a resistance in the circuit of 14,000 ohms, and a tension such that the shadow, distant 1 metre, was deflected through 1 cm. by a potential difference of 30–50 millivolts. The recorded curves were interpreted with due regard to the limitations of the string. The tension of the latter was, whenever possible, so adjusted that its speed was sufficient to record accurately the changing potential differences between the leading off contacts. Certain of the experiments were conducted at room temperature; in such circumstances the degree of tension required to effect accuracy of the complete curve is not obtainable without reducing the dimensions of the graphic record beyond convenient limits; in consequence, such records obtained at room temperature are used only to indicate the direction of the initial deflection. In curves obtained at temperatures below 12° C. the rate at which the deflections are inscribed is much slower than in uncooled preparations. In these curves the measured properties of the string, maintained at a tension yielding conspicuous deflections, prove the graphic record to be qualitatively exact, and to be without sufficient quantitative error to produce any material distortion.

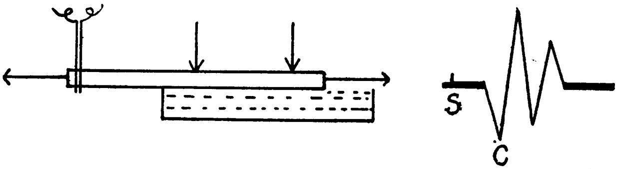
To test recorded responses for escape of stimulating current. Security that the deflections of any given curves are uncomplicated by escape of stimulating current was obtained as follows. (1) If reversal of the direction of the stimulating current had no effect on the form of the recorded response, the latter was regarded as containing no elements of the stimulus. (2) In certain experiments the stimulus and the muscle response were recorded on the same plate by means of a second galvanometer. If the initial movement of the string recording the stimulus was unaccompanied by a simultaneous movement of the string connected to the contacts on the muscle, the latter were regarded as unaffected by the stimulating current; see *e.g.* curves *a* and *b*, Pl. I. All the curves used for analysis were examined in one or other of the above ways, and complication by stimulating current definitely excluded.

Exp. I. To study the string galvanometer record obtained by placing two contacts on a curarised sartorius stimulated directly at one end, (A) when the muscle is suspended in air, (B) when both leads are on a portion of muscle in contact with, or surrounded by, a considerable quantity of conducting medium.

The sartorius is removed from a curarised frog by carefully dividing the connective tissue adhesions between it and underlying muscles. The tendinous and muscular insertions into tibia and pelvic girdle respectively are divided close to the bone. It is suspended in air with its normally subcutaneous surface uppermost by means of two threads tied to its ends. These are attached to two upright supports and the tension of the strip adjusted so that direct stimulation results in an approximately isometric contraction. Stimulating electrodes are placed in contact with the pelvic end. Two recording contacts are fixed resting lightly on the uppermost surface of the muscle, one near the middle and the other towards the tibial end. Fig. 1, method *A*, shows this arrangement of muscle and electrodes. The stimulus from the coil is so adjusted that conspicuous contractions occur at break, and feeble or no responses at make. The tissue is kept just moist by occasional washing with Ringer's solution at room temperature. Curve *a*, Pl. I, shows one example of the typical diphasic action current responses obtained from a series of sartorii thus examined.



Method *A*.



Method *B*.

Fig. 1. Showing the arrangement of the muscle, electrodes and dish of saline in methods *A* and *B*; and also the typical resultant curves when the muscle responds to single shocks.

A flat dish filled with saline is next brought up under the muscle so that, without in any other way disturbing the preparation, about two-thirds of the tissue are brought into contact with the fluid in the dish, while one-third of the tissue from the point of stimulation remains in air. Fig. 1, method *B*, shows this arrangement of muscle and electrodes. The proximal contact is at least 6 mm. from the edge of the dish. Responses are recorded from single stimuli precisely similar to those previously used.

Curve *b*, Pl. I, is typical of the responses obtained in several such experiments. Curve *a* shows a diphasic variation in the potential difference of the two contacts. The first phase represents relative negativity of the proximal contact, the second phase relative negativity of the distal contact. Curve *b*, on the other hand, shows an initial deflection below the base line, revealing a relative positivity of the proximal contact[1]. This is followed by a deflection above the base line indicating relative negativity of the near contact. The remaining deflections vary in form in different experiments and will be discussed later.

We may conclude from these curves that whenever the muscle is suspended in air and surrounded by an extremely limited layer of conducting medium, the electrical record of the response to a single shock is typically diphasic. When, however, the surrounding conducting medium is increased to considerable dimensions, an initial relative positivity of the proximal lead is invariably displayed.

In further support of this conclusion may be described briefly the original experiment of which method *B* is a simple modification. An isolated sartorius is suspended vertically in a vessel containing saline at 10° C., the pelvic third remaining in air. It is stimulated at the upper end and led off by two contacts applied to the surface of the completely immersed section. The galvanometric responses are always of the general type shown in curves *c* and *d*, Pl. I. The response is always polyphasic and an initial relative positivity of the proximal contact is invariably recorded. The number of phases present depends on the distance separating the leads. Curve *c* was obtained when the leads were 6 mm. apart, and curve *d* when 17 mm. apart.

We may next similarly examine curves obtained from muscle injured beneath one contact, a method well known to yield *monophasic curves* from muscle suspended in air.

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[1] For the initial positive deflection to be well marked it is essential that the proximal contact should be an appreciable distance from the edge of the trough.

Exp. II. To examine the string galvanometer record obtained from a partly injured curarised sartorius by means of one contact on an uninjured and another on an injured region of the tissue (A) when the muscle is suspended in air, (B) when the regions led off are associated with an extensive conducting medium.

An isolated sartorius, curarised as before, is partly injured by dipping the distal fourth into saline at 55° C. It is suspended in air by threads attached to the ends at a tension sufficient to render contraction approximately isometric. Two leading off electrodes are placed on injured and uninjured regions respectively. The tissue is kept moist by occasional washing with Ringer's solution. It is stimulated at the uninjured pelvic end. Curve *e*, Pl. I, shows a record of the action current following a single stimulus obtained in such an experiment. The response is approximately monophasic in character, there being some overshoot at the termination of the deflection.

A flat dish containing saline is now brought up beneath the muscle so that both regions led off are in contact with the surface of the saline. Special care is taken to ensure that the electrodes and tissue remain otherwise undisturbed. About 1 cm. of the tissue from the region of stimulation is allowed to remain in air, as in Fig. 1, method *B*. Curve *f* is one example of the action current response now obtained. Such curves invariably show an initial positive phase.

From these observations we may conclude that a monophasic curve is obtained when the partly injured tissue is suspended in a very limited conducting envelope. The presence of a considerable body of saline in the region of both contacts, however, produces an initial relative positivity of the proximal lead.

It has thus been shown that immersed skeletal and cardiac muscle behave similarly in an important respect, namely, that when a wave of excitation passes beneath two contacts in turn, the graphic record displays an initial deflection representing a relative positivity of the lead first encountered. It remains therefore to determine whether the current flow round skeletal tissue is to be explained on a basis similar to that put forward for cardiac muscle in the previous investigation.

In any investigation of the source of action currents from excited tissues there is one difficulty that must constantly arise in connection with the interpretation of graphic records. So long as both leading off contacts are in close relation with the active tissue, their potentials will be simultaneously influenced; and it will be a practical impossibility to analyse the graphic record of their potential difference in terms of their individual levels. Injury beneath one contact has been advocated as meeting this difficulty; there is, however, no obvious reason why such a procedure should ensure fixation of the level of potential at the injured surface. As will be shown later, there is indeed every reason to suppose that activity of uninjured tissue in the neighbourhood of the injury does materially affect the level of potential at the injured surface.

The ideal method clearly is one in which changes in potential at the surface of active tissue can be measured relative to some unvarying standard. Such a method would disclose those regions at which the level of potential is at a maximum, indicating the presence of sources or anodes, and other regions simultaneously at a minimum, disclosing the situation of sinks or kathodes.

The method that suggests itself as nearly meeting the ideal requirements is to immerse the active tissue in a large body of saline and place one electrode near the tissue and the other at a distance. This distance should be sufficient to eliminate any material change in the potential at the distant electrode in consequence of tissue activity.

Exp. III. To study the changes in potential occurring at points close to the surface of an uninjured curarised sartorius immersed in a considerable saline medium, and directly stimulated at one end.

Method C. The sartorius of a curarised frog is carefully removed by dividing the connective tissue binding it to the neighbouring structures of the thigh. The tibial tendon and the muscular pelvic ends of the muscle are cut close to their attachments to bone. It is suspended in air between two upright supports by threads attached to the ends, and its tension adjusted until contraction following direct stimulation is isometric. A glass dish filled with saline is brought up beneath the muscle so that the tibial half is in contact with the surface of the saline, while the pelvic half is suspended in air. The naturally subcutaneous surface is arranged to remain uppermost. One contact is placed at any distant point in the saline; the other is fixed resting lightly on the surface of the immersed half. Stimulation is applied at the pelvic end, and responses following single stimuli recorded. Curve *g*, Pl. II, is typical of responses obtained by this method.

To eliminate so far as possible the effects of dissection injuries on the excitation response, it was thought desirable to repeat the experiment in a modified form.

Method D. The thigh of a curarised frog is skinned and placed on a large pad of cotton wool saturated with Ringer's solution. The distal half of the sartorius is carefully dissected away by dividing its connective tissue attachments to the limb. It is gently stretched and suspended in air by means of a thread attached to its tibial tendon. Additional pads of saturated wool are laid round the preparation, until the attached half of the muscle is freely bathed in saline, while the distal freed portion remains exposed in air. Stimulating electrodes are applied to the muscle close to the tibial end. Leading off contacts are arranged, one anywhere on the pad at a distance, the other resting lightly on the surface of the sartorius in its uninjured proximal half. Single break stimuli of sufficient strength to evoke a good contraction are imparted to the tissue. The tension of the muscle is adjusted to render contraction practically isometric. In this method there is a minimum of disturbance in the region of the muscle led off. Curve *h*, Pl. I, was obtained in such an experiment. It is typical of responses recorded by this method.

The curves obtained by these two methods resemble each other closely. They consistently show first a positive effect, corresponding to an elevation of the potential at the near contact. This is followed by an equally definite negative effect, *i.e.*, a fall in potential below the level present previous to excitation. The remaining deflections, as previously stated, are difficult to analyse in view of the tendency of the string to oscillate when returning from the wide negative excursion. It was consequently decided to cool the muscle in the hope that a slowing down of the electromotive mechanisms would facilitate accurate analysis of the final deflections of the recorded response.

Preparations were set up in precisely the same way as described under methods *C* and *D* above, except that all saline employed was previously cooled down to the region of 6° C. The tissue was allowed to rest in the cooled saline for some time previous to stimulation. Curves *i* and *j*, Pl. II, are examples of the responses now seen. They are triphasic in form, with a definite positive end phase.

Discussion and a possible explanation of the recorded electrical responses of active skeletal muscle.

We are faced with the necessity of having to explain on a common basis three characteristic responses from uninjured tissue. They are: (i) a diphasic curve from two contacts on tissue suspended in air; (ii) a polyphasic curve with an initial positive deflection from two contacts on tissue suspended in a considerable body of saline; and (iii) a triphasic effect including two positive phases from immersed tissue with only one direct contact.

In attempting to explain these recorded responses, it must be borne in mind that the current flow in the saline external to the tissue is alone under consideration. This qualification is to be emphasised since all our observations refer only to the difference of potential between two regions in the external medium.

On theoretical grounds it may be assumed that when current flows in a limited homogeneous medium, such as the body of saline surrounding the muscle strips in the foregoing experiments, there must be at least one region at the boundary at which it enters, and at least one other at which it leaves. For brevity we will refer to two such regions simultaneously present as a doublet. The region at which current enters the medium will be referred to as an anode, and that at which it leaves as a kathode.

If now we can explain on a common basis involving doublets all responses recorded under known conditions we shall have postulated an hypothesis experimentally supported and theoretically acceptable.

It was previously shown (1) that the field of potential in the saline surrounding immersed cardiac muscle can be explained on the basis of doublets in the following way. As a wave of excitation passes down the tissue it is supposed to be accompanied by a wave or succession of doublets, each of which is so arranged that the positive pole is a short distance ahead of the negative pole; that is, the region in which the current enters the external medium from the tissue is in front of the region at which it re-enters the tissue, the term "front" indicating the direction in which the wave is travelling. Doublets thus oriented are referred to as primary doublets. Subsequently there is supposed to follow a second succession of simultaneous regions of current entry and exit at the tissue surface, but oriented reversely, that is, the region of exit is now ahead of that of entry. The latter doublets are referred to as secondary doublets.

We will consider first the galvanometric response which is to be expected from two contacts on an air surrounded strip of moist muscle; it is the moisture on the tissue that constitutes the external medium the current flow in which is under consideration. Let us consider the conditions obtaining at the instant at which the excitation wave has reached the middle of the strip, and confine ourselves for the moment to the theoretical effect we should expect were the wave of excitation to give rise to a single primary doublet as postulated above, and situated near the middle of the strip. Current will flow through the saline envelope from the anode to the kathode, through all regions of the envelope in which flow can take place. If the layer of moisture be excessively thin, it is clear that current arising at the anode cannot flow towards the corresponding end of the strip, for if it does so it has to return by the same path eventually to reach the kathode; and current cannot flow in the same path in opposite directions at once without being reduced to zero.

This is equivalent to saying that provided the saline envelope is sufficiently limited, all regions between either pole of the doublet and the corresponding end of the strip are isopotential, for if in a conductor there is no flow of current between two points the potentials at those points are equal. That is, the potential in the saline between the anode and the corresponding end of the strip is at a constant level; and the potential between the kathode and the end of the strip corresponding to it is also at a constant level, the latter being lower than the former.

Under these circumstances two contacts leading to a galvanometer and placed either between the anode and the corresponding end of the muscle, or between the kathode and the end corresponding to it, will yield no deflection. If, however, the two contacts be arranged one on either side of the doublet, the galvanometer will immediately record a current flowing from the contact nearer the anode towards that nearer the kathode. Hence, if the saline be sufficiently limited, current will flow through the galvanometer from two contacts on a strip only when one or both poles of the doublet are situated between them; and it will flow in only one direction, namely from the contact on the same side as the anode towards the other.

If therefore we postulate a sufficiently limited layer of saline, and a succession of similarly oriented doublets to pass down the strip, two contacts will yield a monophasic curve indicating a relative negativity of the contact first reached. Similarly, a succession of doublets of reversed polarity passing down a strip similarly led off will yield a monophasic response in the reverse direction, *i.e.* the contact first encountered will be relatively positive.

In practice a strip of skeletal muscle under these conditions yields a diphasic curve, the phases of which are qualitatively similar to those just described. We may thus regard the diphasic response obtained from a strip of moist tissue suspended in air to be readily explained on the basis of two sets of primary and secondary doublets as postulated above.

We will now consider the field of potential in a large volume of saline due to a doublet at the surface of immersed tissue. It is easy to show experimentally, and is readily understood theoretically, that any region nearer the anode than the kathode is at a higher potential than any other region nearer the kathode than the anode; also that as we approach the anode from any region on the anode side of a plane surface perpendicularly bisecting the line joining anode and kathode, the potential steadily rises. This is equivalent to saying that the potential in the surrounding medium is at a maximum at the anode and at a minimum at the kathode. As the primary doublets approach the proximal of two contacts on a strip of immersed muscle, the potential at this contact therefore steadily rises above that at the far lead. We would thus expect, on the basis of the doublet view, just such an initial positivity as has been seen in all curves recorded from immersed strips.

We come next to the curve we would expect from an immersed strip with one direct and one distant lead. It follows from the above description of the field of potential round a doublet at the surface of immersed tissue, that as the primary doublets approach the direct contact, its potential is gradually raised to a maximum; it falls to a minimum immediately after the wave of primary doublets has passed. The primary doublets alone would thus produce a diphasic curve with an initial positive phase. Similarly, a wave of secondary doublets alone would produce a diphasic curve with an initial negative phase.

If the effects of the first and second waves are sufficiently separated in time, we would expect at most four deflections in the order + −, − +. The positive sign here represents positivity of the near electrode relative to the potential of the distant lead, and *vice versa*. It is manifest that if the secondary doublet follows the primary doublet sufficiently quickly, its field of relatively low potential may fuse with that of the primary, and so yield a triphasic curve as the whole effect. The same result would happen if, as is conceivably the case, our tight string of $1{\cdot}5\,\mu$ diameter, quick and sensitive as it is, fails to record one deflection completely before the next begins. The fact that triphasic and not tetraphasic curves are actually recorded is not surprising therefore, and is not opposed to the hypothesis put forward.

Further tests of the doublet view may be carried out as follows.

Test I. Referring to Exp. I it may be recalled that two leading off contacts were maintained in a fixed relation to a curarised sartorius. Responses were recorded from the tissue (*A*) when suspended in air, and (*B*) when in contact with a considerable body of saline. Fig. 1 shows the two arrangements along with tracings of typical responses. According to the doublet postulate there is in method *A* no differential effect on the potentials at the two electrodes until the wave of excitation has passed the proximal contact. This then becomes relatively negative. In method *B*, on the contrary, the potential at the proximal contact becomes increasingly positive as the excitatory process approaches it. Its potential changes abruptly to relative negativity when the wave of excitation has passed beyond it. Hence, apart from the lag of the recording instrument, we would theoretically expect the interval between the stimulus and the beginning of the deflection in method *A* to agree closely with the interval between the stimulus and the peak of the initial positive deflection in method *B*[1]. Table I shows the measurements of these intervals in a series of six experiments. Four different muscles were employed, the contacts being shifted and the experiment repeated in the case of two.

TABLE I.

Preparation	Muscle in air Time in secs. between stimulus and beginning of relative negativity of proximal electrode *S B*	Muscle on conducting medium Time in secs. between stimulus and peak of initial relative positivity of proximal electrode *S C*
1	0·0074	0·0080
2	0·0054	0·0055
3	0·0059	0·0060
4	0·0043	0·0046
5	0·0057	0·0058
6	0·0061	0·0063

There is a slight excess in favour of the response derived from the partly immersed tissue. This excess is well within the error of measurement. The agreement is, indeed, sufficiently close to conclude that the experimental findings bear out the theoretical expectations referred to above.

Test II. As shown in Exp. III, one near and one distant contact in relation with an immersed strip yield a graphic record triphasic in form. It follows, on the basis of previous reasoning, that two contacts on the same strip should yield a response which, when analysed, should be composed of two superimposed triphasic effects. The second of these should be an approximate mirror image of the first. In the event of the two contacts being sufficiently far apart, we might expect all six phases to be seen in the record in the order + − +, − + −. This distance will depend on the velocity of the wave of excitation, the time relations of

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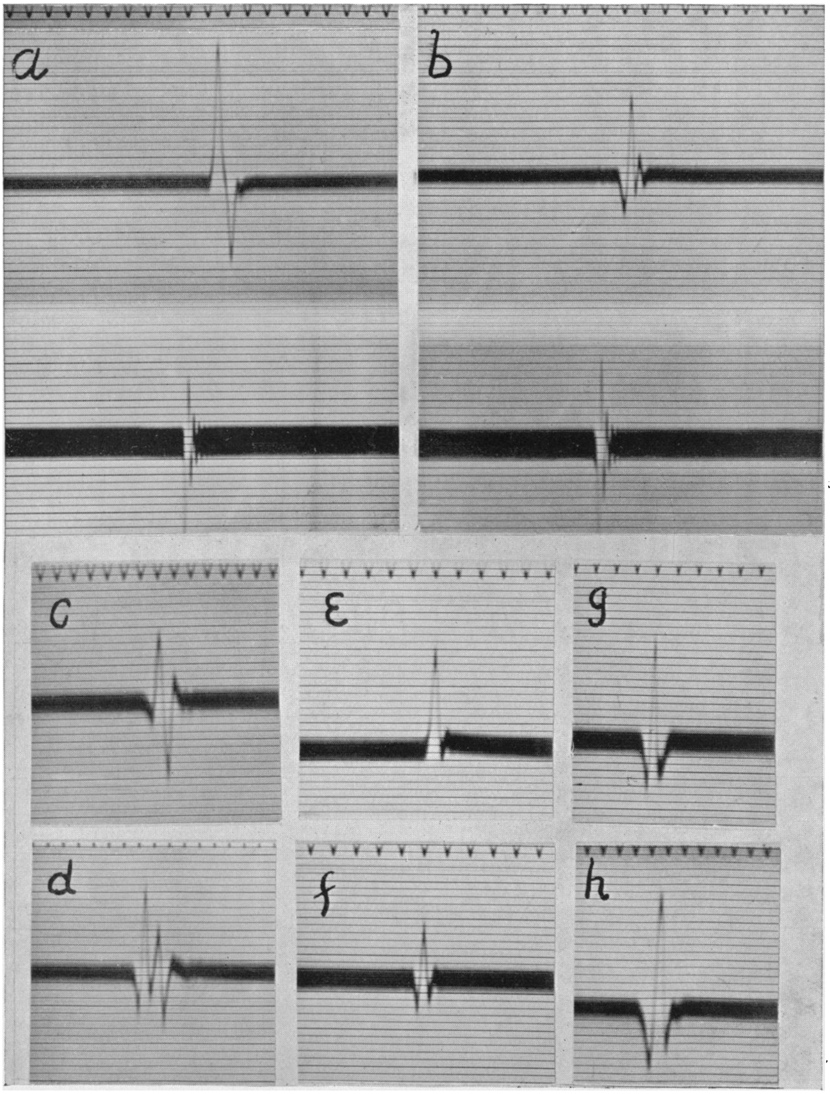
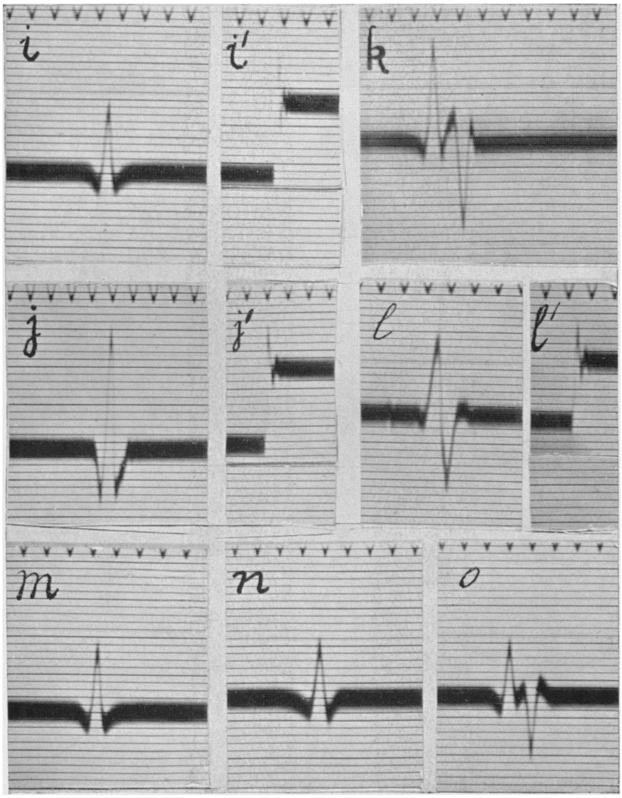
[1] In practice, however, since the string is moving from rest in method *A* and is reversing its direction of movement in describing the positive peak in method *B*, we would expect a measurement of the actual curve to show a slight excess in favour of those derived in method *B*.



Records at room temperature (about 17° C.). In records *a* and *b* curves of electrical response of muscle (above) and signal of stimulation (below) are shown. The diphasic curve (*a*) obtained from the unimmersed muscle is altered by immersion of the muscle, an initial positive deflection appearing in record *b*. Records *c* and *d*, two polyphasic records from immersed muscle, the pair of leading-off contacts being 6 mm. apart in *c* and 17 mm. apart in *d*. Records *e* and *f*, records from a muscle injured under one contact, immersed and unimmersed, respectively.

Records *g* and *h*, triphasic curves taken from immersed muscles with one contact on the muscle and the other at a distant point in the surrounding saline (methods *C* and *D*).



Records from muscle immersed in saline at about 6° C. Records *i* and *j* were taken in the same way as were records *g* and *h*; records *i'* and *j'* show the corresponding deflection times of the string. Records *k* and *l*, showing the effects of bringing the two leading-off contacts close together (*l*); *l'* exhibits the corresponding deflection time of the string. Records *m*, *n* and *o* were obtained from the three leads represented by *AD*, *BD* and *AB*, respectively, in Fig. 2.

In this and the preceding plate, the time is marked by 1/100 sec. tuning fork; the ordinates are 20 millivolts = 1 cm.; resistance of electrode-galvanometer circuit 10,000 ohms.

the doublets involved, and the strength, or potential difference, of the latter. The conditions must be such that the entire galvanometric consequences of excitation are over in the region of the first contact before they materially affect the potential at the other. The following experiment was accordingly carried out.

Exp. IV. To study the string galvanometer record of the response obtained from a curarised sartorius immersed in chilled saline and led off by two contacts separated as far as possible.

The sartorius of a bull frog is set up precisely as in Exp. III, method *C*. Two leading-off contacts are placed on the uninjured surface of the muscle, one 3 mm. from the edge of the dish, and the other about 17 mm. nearer the distal end. The electrical responses from isometric contractions are recorded, and curve *k*, Pl. II, shows one example of the type of response seen. The distal electrode is then moved to within 6 mm. of the proximal contact. Curve *l* shows the type of response now obtained. Curve *k* distinctly shows six phases in the order named above. In view of the measured properties of the string, these phases probably represent with fair accuracy the relative potentials of the two contacts during the course of excitation. Curve *l*, inscribed when the contacts were moved closer together, shows a curve of only four phases, indicating the fusion of six phases into four that has occurred as the result of bringing the leads closer together. The presence of six phases in the response from immersed skeletal muscle led off by two contacts is a striking phenomenon, when we consider that it was theoretically anticipated on the basis of what appeared to be a rational explanation of the typical diphasic curve from a strip suspended in air. Test II thus offers further evidence in support of the doublet view.

That the response from two contacts on an immersed curarised sartorius is composed of the interference of two opposite triphasic effects originating at the near and far contacts respectively may be further demonstrated as follows.

An isolated sartorius from a curarised bull frog is again set up as in Exp. III, method *C*. The arrangement of saline dish and suspended tissue is represented in Fig. 2. *A* and *B* are two leading-off contacts resting on the muscle about 25 mm. apart, and *D* is a third contact fixed at any distant region of the saline. The responses obtained from single stimuli when leads *AD*, *BD* and *AB* are employed are shown in Pl. II, curves *m*, *n* and *o* respectively. Clearly the six-phased response *o* is an algebraic summation of two curves, namely, the triphasic response *m* and a mirror image of the triphasic response *n*. If leads *A* and *B* are placed sufficiently close together, the interference of their potentials will result in a four-phased response, as was seen in curves *c* and *l*.

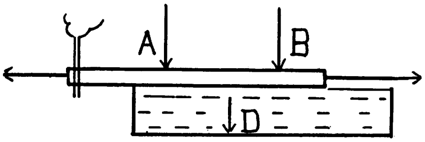


Fig. 2. The arrangement of muscle, saline bath and electrodes to illustrate leads *AD*, *BD* and *AB*, described in the text.

It has thus been shown that two contacts on a strip of muscle may yield responses which are two-phased (curve *a*), four-phased (curves *c* and *l*), or six-phased (curves *d*, *k* and *o*), depending upon the volume of the saline surrounding the tissue, and upon the distance separating the leading-off contacts. It has also been shown that these very different responses are in strict accord with the doublet postulate.

PART II. THE PROBLEM OF INJURY CURRENT.

It is known that the potential at the surface of the injured region of a partly injured muscle is lower than that at the uninjured surface of the same muscle. Whether the potential at the latter changes at all, and how the change, if any, is related to the external surroundings appear at present to be unknown. This will now be examined.

The problem of the origin of injury current has to be approached from two distinct points of view: (i) we must ascertain the lines of current flow in the conducting medium outside the tissue boundary, *i.e.* in the external circuit; and (ii) we must relate these lines of flow to specific electromotive mechanisms localised within or at the surface boundary of the tissue itself. If the latter mechanisms are known the lines of flow in the external circuit may be deduced, provided the nature and configuration of the external medium be known; the converse is, however, not necessarily true. It might be possible to deduce the seat of the E.M.F.'s of injured tissue from a knowledge of the lines of flow of injury current, if the tissue were sufficiently simple in structure to render its phase interfaces few in number and obvious in situation. For in any system of fluid conductors electromotive forces may be said to occur only across the interfaces or phase boundaries separating two different homogeneous media.

The problem thus naturally falls under two headings: (*a*) the field of potential in the medium surrounding the tissue; (*b*) the mechanism and sites of the electromotive forces concerned in its production. Since the present investigation aims solely at the interpretation of galvanometric records in terms of the electrical events in the medium external to the tissue boundary, the problem of injury current will be approached experimentally from the former standpoint only.

In studying the lines of current flow in any homogeneous medium, the problem may be said to be solved when all the regions of current entry and withdrawal at the boundary are known; that is, when all anodes and kathodes have been localised.

The sites of origin of positive and negative ions respectively at the surface of an injured muscle are clearly independent of the quantity of the saline surrounding it. The latter can influence only the course and the amount of current flowing per unit time.

To locate the regions at which current arises and disappears at the surface of a partly injured muscle, we may usefully employ the device adopted in the previous section and seek the maxima and minima of potential at the tissue boundary relative to an approximately unchanged level at a region distant in the fluid surrounding the preparation. Thus, the injured tissue is bathed in a large volume of saline, one lead is fixed at a distance, and an exploring electrode is moved from point to point at the surface of the tissue and the difference of potential noted.

Preliminary note on method. To investigate the field round any given tissue the latter is either totally or partly immersed in a bath of Ringer's saline sufficient in extent to permit one electrode to be fixed some 7 to 10 cm. from the tissue. The other electrode, to be referred to as the exploring electrode, is mounted on an easily adjustable carrier permitting ready movement of the electrode from one region to another, its exact position being read on a scale specially arranged. The electrode-galvanometer circuit is temporarily completed at will by closing a suitably inserted contact key, the contact being maintained sufficiently long for the galvanometer deflection to be noted. In order to eliminate electromotive forces arising within the electrode-galvanometer system, sufficient compensatory current is introduced to ensure a zero reading with both contacts close together at a distance from the tissue, immediately before moving the exploring contact to the neighbourhood of the preparation. The difference in the potentials at the ends of the two electrodes is estimated in the usual way by comparing the deflection with that given by a standard potential difference. For reasons depending on the general coarseness of any injured preparation, there is no point in attempting to record potential differences arising from injured tissues more accurately than can be done in the above simple way. In order to make the galvanometer readings approximately equivalent to the actual potential differences existing in the external medium, it is essential that the resistance of the electrode-galvanometer circuit should be as high as is commensurate with adequate sensitivity. In the experiments to be described the external resistance was of the order of 10,000 ohms.

Exp. V. To explore the electrical field in the neighbourhood of an injured muscle.

Two different arrangements of injured tissue and saline bath will be described in order to demonstrate the constancy of the experimental findings.

Method I. The skin is removed from the hind leg of a frog and the muscle to be used, whether sartorius or gastrocnemius, is left *in situ* on the limb. The exposed limb is placed upon a large pad of saturated cotton wool which is so arranged that the upper surface of the muscle to be examined appears above the level of the surrounding saline, and is therefore separated from air by only a limited conducting envelope. The exploring electrode is allowed to rest in this limited layer barely touching the muscle. Preliminary exploration over the uninjured muscle is first carried out to observe the extremes of potential difference between distant and near contacts before gross injury is inflicted. If the skin is removed with care it is possible to obtain a preparation practically free from injury currents; and provided preliminary measurements reveal potential differences of not more than a millivolt or two between the distant and the near contact, unavoidable dissection injuries may be regarded as too small to interfere with, or mask, the very conspicuous differences observed after gross injury. Transverse injuries are then made in the selected muscle by snipping cleanly with sharp scissors or by a single brief contact with a heated needle. The exploring electrode is now moved a millimetre at a time along the uninjured surface towards the injury, and the deflection noted for each position. The results are shown in Graph (*a*), Fig. 3.

Method II. Single muscles are removed as carefully as possible and totally immersed in a saline bath, or, as in the previous method, are placed upon a cotton wool pad sufficiently saturated to keep the tissue covered by a thin layer of fluid. Removal of the entire muscle involves relatively gross injury at distal and proximal insertions, and along the under surface where the muscle is attached to the limb by nervous, vascular and fibrous structures. In the case of the sartorius, injury at the tibial end may be minimised by careful detachment of the tibial tendon; complete transverse division of the muscle through its pelvic third provides the injury that gives rise to the field. When completely immersed the muscle may be suspended from a suitable frame by threads attached to the ends. Exploration is carried out by keeping one contact fixed at a distant region of the saline and moving the exploring contact 1 mm. at a time along a line running parallel to the long axis of the muscle and in contact with its surface.

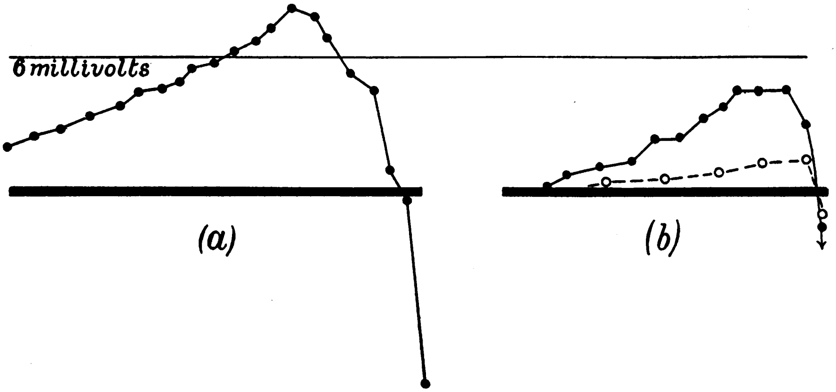


Fig. 3. A muscle injured at its right-hand end is represented by the heavy black line. The graph represents the potentials found at corresponding points of the surface. Potentials positive to a distant point are plotted above, and negative below.

(*a*) a 28 mm. muscle examined *in situ*;
(*b*) a 21 mm. muscle removed from the limb.

Graph (*b*), Fig. 3, pictures the results of an experiment carried out in this way. The line joining the small black circles represents the potential difference at the surface when the muscle was placed upon a moist pad; the line joining the white circles, the potential difference when the same tissue was completely immersed in a large bath and suspended by threads tied to its ends. These two curves from the same tissue are qualitatively similar, and differ only in degree.

Comment on Exp. V. The main feature of interest that emerges from this experiment is that, relative to the level of potential at the distant fixed contact, the potential at the uninjured surface of the injured muscle becomes increasingly positive as we approach the injured region, falls to isopotential at the region approximately 2 mm. from the edge of the visible injury, and then rapidly falls to a conspicuous negativity when the actual injured surface is reached.

There is every reason to believe that the same result would be obtained if a partly injured muscle were immersed in the sea and explored in similar fashion. The uninjured surface would display potential raised above that of the earth, the injured surface a potential below that of the earth, an earth-zero line being found somewhere about 2 mm. from the injured edge.

The method of immersing the tissue in a large field of saline overcomes the difficulty of interpreting a potential difference in terms of the actual changes in potential that occur at the injured and uninjured surfaces respectively.

Actually the precise level of potential at injured and uninjured regions respectively will depend upon the environment of the tissue. For example, suppose we suspend an injured muscle upright in air with its injured surface just dipping into a large bowl of saline. If we explore the surface of the muscle as before by means of one electrode at a distance in the saline, and an exploring contact moved from point to point on the tissue, relatively large positive values will be found on the air-surrounded portion of the uninjured surface, and relatively small negative values, if any, at the injured end. Suppose we reverse the muscle so that the uninjured end is dipping into the saline, and again explore its surface. Large negative values will now be found on the injured region and small positive values, if any, at the immersed uninjured surface.

Now since the saline in the bowl remains at a more or less constant level of potential, we must conclude from the above findings that the actual potentials at the surface of the tissue vary with the position of the tissue relative to the saline; that is to say, the actual potentials at the surface are modified by the environment of the tissue, while the current flows through the external medium between regions which are determined only by the site of the injury.

This point is stressed to emphasise the need of avoiding descriptions of injury and action currents in terms of variable quantities whose values in any given case depend on the conditions of the external medium. When the muscle current is described in terms of the regions at which the current enters and leaves the tissue, the phenomenon is described in terms which specify activities completely independent of the conditions of the external medium.

An approximate but clear impression of the lines of current flow round an injured muscle may be gained from the following simple experiment. It is based on the principle that in any conductor the current flows in a direction which is perpendicular to the equipotential surfaces. Suppose, therefore, that we reduce the conducting medium surrounding the tissue to an approximate plane, and map out experimentally the equipotential lines, it is possible to obtain a fairly good idea of the actual lines of current flow by drawing in continuous lines which will cut the measured equipotential loci at right angles. A saline-saturated circular piece of filter paper forms an excellent "plane" conductor and an isolated sartorius divided near its attachment to the pelvic bone a suitable preparation for the purpose.

Exp. VI. To map out the equipotential surfaces in a plane circular field surrounding a sartorius injured at one end.

Method. A circular filter paper of diameter about 18 cm. is divided into small numbered squares by ruled pencil lines in order to facilitate localisation of any particular point on its surface. It is placed on a plane horizontal wax surface and saturated with Ringer's solution. The tendinous attachment of a frog's sartorius is freed from the tibial bone, and the entire muscle carefully dissected away from its attachments to the underlying structures; a transverse section in its upper third severs it from its proximal end. It is placed upon the filter paper so that its long axis passes over the centre of the paper, with its injured surface about 2 mm. away. Fig. 4 shows the arrangement of tissue and filter paper.

AE and *CJ* represent two perpendicular diameters specially included when squaring the filter paper; the long axis of the tissue is made to lie along *AE*. Two pointed electrodes are placed upon the paper; they are connected to a sensitive galvanometer through a special contrivance permitting compensation of electromotive forces arising within the galvanometer-electrode circuit. One electrode is kept fixed at a selected site, and the other is moved from place to place on the moist field until the deflection in the galvanometer disappears. The two regions are therefore isopotential, and the position of the exploring contact is noted. In this way a number of "spots" on the moist field may be localised which are at the same potential as the point of application of the fixed contact. The line joining these spots is roughly an equipotential surface at the potential of the fixed contact. The fixed contact is then moved to another selected site and its "equipotential locus" similarly obtained.

Fig. 4 shows the result of one such experiment. Sites *A*, *B*, *C*, *D*, *E*, *F*, *G*, *H* and *I* were selected for the fixed contact, the lines passing through the black circles representing their respective equipotential loci. When the moving contact approaches the tissue, small changes in its position produce great variations in the deflection of the galvanometer, and accurate localisation of the equipotential spot is not practicable.

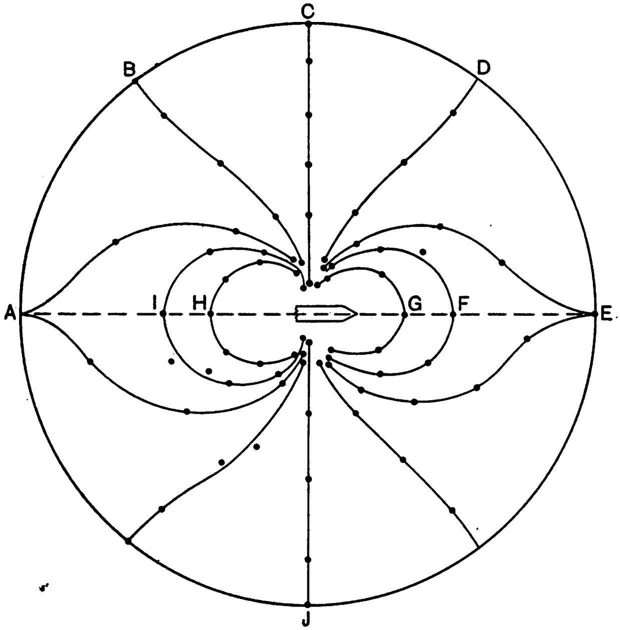


Fig. 4. A chart of the equipotential lines in a plane circular field surrounding an injured sartorius muscle. For comparison with this is Fig. 5, a similar chart of the equipotential lines surrounding two pointed electrodes joined to a battery.

If we now measure the potential difference between the straight equipotential *CJ* passing through the muscle near the injury, and each of the other equipotentials shown, it immediately becomes evident that the field of potential is peculiarly symmetrical. That is, if we regard the straight equipotential line as at an arbitrary level of *O* then all regions to the left (*i.e.* in the direction of the injured surface) are *O* − and all regions to the right are *O* +.

Measurement of the potential difference between equipotential *CJ* and the equipotential passing through *B* = − 13 units; through *D* = + 14 units; through *A* = − 16 units; through *E* = + 17 units; through *I* = − 20 units; through *F* = + 22 units; through *H* = − 25 units; and through $G = +28$ units, revealing the degree of symmetry to which reference has been made.

If the field were exactly symmetrical about the diameter *CJ* it would mean that we could describe the tissue as behaving precisely as though the current flow were arising from a "point" anode and a "point" kathode situated symmetrically near the centre on the diameter perpendicular to *CJ*, the anode being on the side of the uninjured surface and the kathode on the side of the injury. That is, in the language employed in the previous section of this paper, and from the point of view of the external field of current flow, the muscle would behave precisely as though it were replaceable by an equivalent doublet.

The similarity between the current flow round an injured sartorius and the flow round an artificial doublet may be demonstrated in the following way. A saturated circular piece of filter paper is prepared as before. Two pointed electrodes joined to a battery are placed on the diameter *XY*, Fig. 5, equidistant about 3 mm. from the centre on either side of it. A make and break contact key is inserted near the battery so that current flow may be controlled and unnecessary polarisation avoided. The same points are chosen as in Exp. VI for the position of

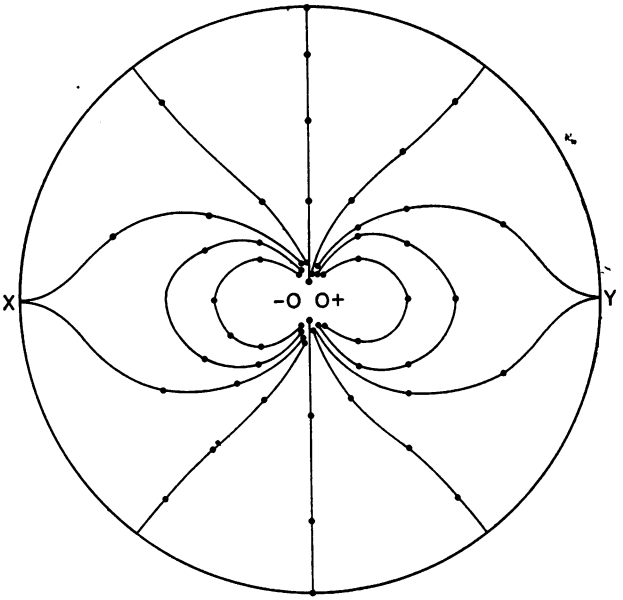


Fig. 5.

the fixed contact, and equipotential loci ascertained as before. Fig. 5 shows the result of one such experiment. Comparison of the equipotential lines in Figs. 4 and 5 reveals how very similar the two fields are.

The question arises whether the results of these experiments on injured sartorii afford any evidence by means of which we may localise the sources of origin of negative and positive ions respectively at the surface of an injured muscle. It is not possible to specify how much of the injured surface is the source of negative ions, and how much of the uninjured surface is the source of positive ions; but the field of potential revealed by these methods suggests strongly that the main source of positive ions is from the uninjured surface over a region situated comparatively near the edge of the visible injury.

Certain useful deductions may be based on the evidence presented by Exp. VI. Histologically the sartorius is composed of a number of separate long parallel fibres, each of which in the above circumstances has an injured and an uninjured surface. The aggregate of injured fibres has been shown to give rise to an external field of flow which can be reproduced very closely by an artificial doublet, *i.e.* by a simultaneous anode and kathode in close proximity, and localised at the region of injury. From this it may be deduced, so far as the flow in the external medium is concerned, that each individual injured fibre may also be regarded as replaceable by an equivalent doublet; or, conversely, that the field of potential due to an injured muscle is the summation of the individual effects of its injured fibres. The reasons leading up to these conclusions are given in an appendix.

SUMMARY AND CONCLUSIONS.

Experiments are described to show that the form of the galvanometric response obtained from two contacts on a strip of curarised uninjured skeletal muscle excited at one end depends upon (1) the quantity of the saline medium surrounding the tissue; and (2) the distance apart of the recording contacts. When the tissue is suspended in air the record is the well-known type of diphasic curve; when suspended in a considerable quantity of saline the response is polyphasic, and is composed of an interference between two triphasic effects occurring successively at each of the leading-off contacts. It is shown that during the passage of a single wave of excitation down the strip the potential at any arbitrary point on the surface first rises above, then falls below, and subsequently again rises above the level of potential obtaining at the surface during the resting state.

When the tissue is injured beneath the distal of the two recording contacts, the record of the action current is monophasic only when the strip is suspended in air; it shows an initial deflection disclosing a relative positivity of the proximal contact when the partly injured strip is immersed in saline.

The conclusion is drawn that electric currents flowing round active skeletal muscle arise in precisely the same manner as was previously found to be the case in cardiac muscle, the difference being merely one of degree.

The manner in which action currents are supposed to arise may be briefly described as follows. When a wave of excitation passes down a strip of long-fibred skeletal muscle, the flow of current in the saline surrounding it may be explained on the assumption that the initial electrical effect of excitation of each small fraction of an excited fibre is to produce a simultaneous anode and kathode at the surface of the tissue, with the anode in the direction in which the wave is travelling. This simultaneous anode and kathode (or doublet) is followed shortly afterwards by a second electrical effect also of the nature of a simultaneous anode and kathode, of which however the anode is now in the direction from which the wave has come. That is to say, in terms of potential theory, the excitatory process is accompanied by a three-fold change in the level of potential at the surface of the muscle, namely, first a rise (positivity), then a fall (negativity), and finally a further rise (positivity), the terms positivity and negativity being employed in relation to the level of potential obtaining at the surface of the quiescent tissue.

On this view, it is clear that the potential at the surface of the excited area at a certain instant of time is indeed negative to that of the saline at all other regions of the tissue surface, and in this sense it is correct to speak of the "negativity of the active region." Such an expression, however, is one readily leading to misconception. Misconception arises when development of the "negativity" is regarded as the sole or chief change that occurs at the surface of active tissue, when actually the change that occurs is three-fold and in the order "positivity," "negativity," "positivity."

Experiments are described in which it is shown that the current round a resting injured sartorius arises somewhere on the uninjured surface and disappears somewhere on the injured surface. When the muscle is immersed in a large volume of saline, the potential of the saline at the injured region is conspicuously negative, and that at the uninjured surface quite definitely, though less conspicuously, positive to the level of potential at distant regions of the surrounding medium. Further, this positivity reaches a maximum at a region a short distance from the edge of the injury.

It is shown further that under certain conditions the current flow round an injured sartorius is very similar to that which occurs when the muscle is replaced by an artificial anode and kathode situated at the region of injury. From this it is deduced that so far as the current in the surrounding saline is concerned, an injured muscle behaves very much as though replaceable by an equivalent simultaneous anode and kathode (or doublet). From this and supplementary arguments it follows (*a*) that a single injured fibre may also be regarded as replaceable by an equivalent doublet, and (*b*) that the field of potential round an injured muscle is the summation of the individual contributions of the injured fibres.

I desire to acknowledge my indebtedness to Sir Thomas Lewis and Professor A. V. Hill for their kindly help and criticism throughout this investigation.

REFERENCE.

1. Craib. Heart, 14. p. 71. 1927.

APPENDIX.

The distribution of potential and the current flow, in a conducting field around a doublet of the type considered in the preceding paper, is a well-known mathematical problem, of which the solution must satisfy Laplace's equation and the boundary conditions characteristic of the system. The problem cannot in general be solved, but whether it can or not we may imagine the potential at any point P due to a doublet at the point A to be represented by some mathematical function $V_{P,A}$, where V depends upon the coordinates of P and of A, and on the strength and direction of the doublet at A.

Imagine for example that we are interested in the potential difference which will arise between two different points P and Q when a number of similar injured sartorii are arbitrarily arranged at the points A, B, C, etc. The potential at P due to the first is $V_{P,A}$, at Q it is $V_{Q,A}$, so that the difference of potential arising from the first sartorius alone is $V_{P,A} - V_{Q,A}$. Potentials, however, are additive, so that if we imagine the injured muscles to be placed simultaneously at the different points A, B, C, ... the total potential difference between P and Q is

$$V_{P,A} + V_{P,B} + V_{P,C} + \dots - V_{Q,A} - V_{Q,B} - V_{Q,C} - \dots.$$

To take a simple application of this result, two identical injured sartorii side by side, facing in the same direction, will produce twice the potential difference between any two distant points in the conducting field surrounding them: three identical injured sartorii, side by side, similarly will produce three times the potential difference of one alone, and so on. Conversely, the field of potential produced by an injured parallel-fibred muscle is twice the field produced by half the muscle, three times the field produced by one-third of the muscle, and so on. Hence, if the muscle be composed of n individual injured fibres the field of the whole muscle is n times the field due to a single injured fibre. Again, two parallel identical injured sartorii placed near to one another and facing in opposite directions will neutralise each other's potential effects at distant points, so that no potential difference will exist between such points. These deductions can be verified experimentally.

Thus we see that the potential at any arbitrary point in a conducting medium surrounding an active tissue is related directly to the potential at all regions of origin of positive and negative ions respectively. Moreover, the potential at P is the sum of the potentials produced at P by anodes and kathodes individually. The problem therefore of the distribution of potential, or current, around an active or injured tissue is one which can be expressed in simple mathematical terms, although its actual solution may in any given case be outside our reach.