THE REVERSIBLE LOSS OF EXCITABILITY IN ISOLATED AMPHIBIAN VOLUNTARY MUSCLE[1].

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A. INTRODUCTION.

IN the spring of 1928 an attempt was made to confirm, by direct chemical estimation, the very low values obtained by Stella(1) by a diffusion method for the inorganic phosphate content of resting frog's muscle. It was hoped to secure a really resting condition by employing very thin muscles and suspending them in moist oxygen for some hours. The slowness of diffusion on the one hand and the consumption of oxygen in the resting metabolism on the other (see (2)) may prevent the inner region of a thick muscle from being adequately oxygenated, so that resting conditions may not really exist there: when, however, a thin sartorius muscle of *Rana temp.* or of *Rana esc.* is suspended at rest in pure oxygen the whole of its interior may be regarded as being adequately supplied with that gas. Experiments, therefore, were made in which frogs' sartorii were suspended in moist oxygen for one or more hours at room temperature. The temperature was then reduced to 0° C. approximately and, after an interval sufficient to allow them to become cold throughout, the muscles were ground up in ice-cold trichloroacetic acid. The extract was analysed, with slight modifications[4], by the method of Eggleton and Eggleton(3), with whom these initial experiments were made.

Values for the inorganic phosphate content varying from 10 to 20 mg. p.c. were obtained, with correspondingly high values for the phosphagen phosphate, instead of the previous values of 20 to 30 mg. p.c. The values obtained by Stella were about 8 mg. p.c. for muscles suspended in oxygenated Ringer's solution.

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[1] A preliminary account of these experiments was read to the Physiological Society at Oxford on July 14th, 1928, and a more complete account at the meeting on Jan. 19th, 1929.

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[4] For an improved method of analysis, incorporating these and other modifications, see Eggleton and Eggleton (in the press).

Fletcher and Hopkins[4] have shown, and many subsequent observers have confirmed, that many extraction processes can give fictitiously high values to the lactic acid content of muscle, owing to the "stimulation" which results from the grinding up process. It appears that with phosphagen and inorganic phosphate determinations also, the utmost care must be exercised to avoid this source of error.

During the course of the preliminary oxygen treatment at room temperature it was often noticed that the muscles became inexcitable. Such muscles at first were discarded for the above work, it being suspected that they were in poor condition, as the frogs had been for some time in the laboratory. It soon became evident, however, that this behaviour was the rule rather than the exception, and analyses were made to determine whether the muscles which had become inexcitable showed the characteristic chemical properties of fatigue or rigor. In all cases the phosphagen content of an inexcitable muscle was found to be comparable with that of a resting excitable one, and since Eggleton and Eggleton have shown that in rigor all, and in fatigue most of, the phosphagen is broken down, it was clear that the phenomenon in question could not be attributed to "fatigue" or "death."

B. THE ACTION OF OXYGEN.

If a freshly excised frog's sartorius (dissected out, unless otherwise stated, without the use of Ringer's solution) be suspended in water-saturated oxygen there is a progressive decrease in the inorganic phosphate and free creatine and an increase in phosphagen (estimated by the method of Eggleton and Eggleton) and in combined creatine (estimated by the method of Dulière[5]). This process is attended by, if it be not actually a part of, the oxidative removal of lactic acid, and hence an improvement of the state of the muscle might be expected. Sometimes, and at first, this may appear to be the case, but, after a period, usually of an hour or two (varying between 30 minutes and a day, although rarely longer than 5 hours), the muscle ceases to respond to stimulation, although it still conducts electricity as can be shown by including an excitable muscle in series with the inexcitable one.

A muscle which has just become inexcitable to a stimulus previously maximal will sometimes respond to an increased stimulus, but after a further interval in the gas, the muscle fails to respond at all. The phenomenon appears to be unaffected by the type of exciting current used: direct current (2–4 volts) gave similar results. In general, a one-second tetanus was employed for testing the excitability, the strength

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being adjusted initially to be just maximal and the stimulus applied by electrodes placed directly on the muscle.

The amount of phosphagen in these muscles may be very high, as might be expected from the oxygen treatment, and the inexcitability may be maintained for at least 20 hours in oxygen, without affecting the phosphagen content. Prolonged stimulation of an inexcitable muscle (for example, 300 one-second tetani at three-second intervals, sufficient under ordinary circumstances to destroy all the phosphagen) is entirely without effect on the phosphagen content. Hence it appears that the breakdown of phosphagen is conditioned either by the excitability or by the contractility of the muscle, and not by the application of an electrical stimulus as such.

C. The use of gases other than oxygen.

Since the oxygen treatment involves the removal of traces of lactic acid initially present, and since in a thin muscle carbon dioxide would be expected to diffuse out, alkalinity progressively increasing might be supposed to be the cause of the inexcitable state which supervenes. That this is not the case is shown by the appearance of the phenomenon when air, commercial nitrogen, or 5 p.c. carbon dioxide in air is used instead of oxygen. Prof. A. V. Hill has verified the fact that a sartorius muscle becomes inexcitable in moist nitrogen which has been purified from oxygen by passing it slowly over heated copper. Since in these cases the alteration in phosphagen content is comparable with that for excitable muscles (Eggleton and Eggleton(6) have shown that an increase occurs in aerobic, and a decrease in anaerobic conditions) changes in phosphagen associated with the respective actions of these gases are not connected with the production of the inexcitable state.

D. The water content of the muscle.

In the earlier experiments the stream of gas, which was maintained for the whole duration of the treatment, was bubbled through water or Ringer's solution; it might not have been completely saturated and hence might have been able to remove a small amount of water from the muscle, though, in fact, the latter appeared to be quite wet at the end of the treatment. In later experiments use was made of a bottle containing oxygen and about 50 c.c. of Ringer's solution, completely sealed up; the muscles were suspended in the gas above the solution by silver wires running through the stoppers and were tested without opening the bottle. Others were treated with oxygen which had been

completely saturated with water by passing through a cylinder packed with wet cotton wool and then through Ringer's solution. It appeared in some of the earlier experiments of this type that the time required for the onset of inexcitability had been increased by the change in conditions, but further controlled experiments, in which two muscles of the same frog were used, showed that in fact no real difference existed.

The independence of the phenomenon in question of the water content of the muscle was finally proved by the success of experiments made with mercury and with thick paraffin oil. Sartorii immersed for 5 minutes in Ringer's solution were surrounded with mercury or paraffin. In the latter case a control muscle was inserted in the electrical test circuit to check the possibility of the muscle being insulated from the silver electrodes and hence appearing inexcitable when it was really unstimulated. In the case of the mercury experiments the test for excitability was made by removing the mercury from the muscle by tilting the tube containing it. Contact with the muscle was established by iron wires tied to it. In both cases the muscles became inexcitable in about an hour. Hill and Hartree ((7), p. 93) noticed in 1921 that liquid paraffin "acts unfavourably on the condition of the muscle."

E. The restoration of excitability in Ringer's solution.

It is a matter of common knowledge with those who have worked with isolated muscles that a failing muscle may be revived by immersing it in Ringer's solution. Thus, for example, Hill and Hartree ((7), p. 92) record that it is best, "for the purpose of maintaining the condition of the muscle, to fill the (thermopile) chamber with Ringer's solution" and to replace this by oxygen only when the actual measurements of heat production are to be made. In the type of non-irritability examined by Foster and Moyle(8), to which further reference will be made below, immersion in Ringer's solution will restore the irritability of muscles previously entirely unaffected by a stimulus. In the present case the effect of immersion in Ringer's fluid is striking and may be very rapid. The solution employed was of the composition: NaCl 0·65 p.c., KCl 0·014 p.c., CaCl 0·012 p.c. The solution used need contain neither phosphate, nor bicarbonate, nor oxygen. A resting muscle, which has become inexcitable by standing in a gas, may return to a condition in which the tension developed in response to a stimulus may be as large as, or even larger than, it was initially. No permanent damage appears to result from a period of inexcitability. Muscles rendered inexcitable by immersion in paraffin are revived without any treatment designed

to remove the oil, which seems to have no effect on the restorative action of the solution.

It may be recalled that Gottschalk[9] found that nerves rendered inexcitable by deprival of oxygen can be restored in part, either by readmission of oxygen, or by washing with oxygen-free Ringer's solution, but that complete recovery requires both oxygen and washing.

F. THE RESTORATION OF EXCITABILITY BY SOLUTIONS OTHER THAN RINGER'S.

The solutions capable of restoring excitability to an inexcitable muscle may be divided into two classes: (*a*) those in which a muscle progressively regains its excitability, and (*b*) those in which a muscle at first regains a certain degree of excitability which it loses completely on further immersion. A muscle allowed to remain inexcitable for long may not be revived at all by solutions (*b*).

Effect of various solutions on inexcitable muscles.

Permanent recovery	Temporary recovery	No recovery	Subsequent action of Ringer's solution
Ringer's solution			
Ca-free ditto			
Isotonic NaCl			
Isotonic NaNO₃ (one exp. only)			
Ringer's solution with 0·05 p.c. or 0·1 p.c. NH₄Cl*			
50/50 p.c. mixture of Ringer's solution and isotonic glycerol			
	Isotonic glucose (one exp. only)		Complete recovery in approx. 15 mins.
	Isotonic Na₂SO₄		Ditto
	Isotonic LiCl (one exp. only)		Ditto
	25/75 p.c. mixture of Ringer's solution and isotonic glycerol†		
		Isotonic glucose (four cases)	Ditto
		Isotonic LiCl (three cases)	Ditto
		Isotonic NaHCO₃	Ditto
		Distilled water	Ditto
		Isotonic NH₄Cl	No recovery

* This was used to test the suggestion that inexcitability might be due to the accumulation of traces of ammonia.

† Subsequent recovery in a 50/50 p.c. mixture of Ringer's solution and isotonic glycerol.

G. Permanence of Excitability and Inexcitability.

While a muscle will remain inexcitable for 20 hours if left in oxygen and will then recover if placed in Ringer's solution, a long preliminary treatment with this solution appears to render a muscle permanently excitable in the sense of the present experiments. A. V. Hill ((10), p. 134) has found that for myothermic experiments muscles "behave best and survive longest if, after dissection, they are soaked for some time, one to three hours, in Ringer's solution before stimulation is begun." In our experiments it appears that a series of changes from the excitable to the inexcitable state, and *vice versa*, with treatment by Ringer's solution, is possible only until the sum of the times of immersion in Ringer's fluid has reached a critical value. Prof. A. V. Hill informs us (see also (10), p. 157) that a muscle treated with Ringer's solution for a period, sufficient in ordinary circumstances to ensure its permanent excitability, may become inexcitable when allowed to recover in oxygen after having been stimulated to exhaustion in nitrogen; this inexcitability can, as in our experiments, be removed by treatment with Ringer's solution.

H. Temperature.

Results obtained early in July appeared to indicate that inexcitability could not supervene at temperatures above 25° C. Sartorii of *Rana esc.* (Hungarian) did not become inexcitable until the temperature was reduced to below 20° C. Attempts to confirm this in September failed. In one case a frog was kept at 27° C. overnight before killing. The muscle was rapidly excised and placed in oxygen at 27° C.: it became inexcitable in about one hour. Raising the temperature of inexcitable muscles from 20° to 27° C. was without effect. The divergences found are probably to be attributed to seasonal and other variations in the muscles employed.

I. Types of Muscles and Frogs.

During the course of the work the gastrocnemii and sartorii of *Rana temp.* and *esc.* (English, Dutch and Hungarian) have been employed and have shown the same phenomenon of inexcitability. No regular difference between the species can be stated, though occasional differences occur. In general sartorii become inexcitable far more regularly and quickly than do gastrocnemii. In some cases this has been particularly striking; sartorii after careful dissection have become inexcitable

in from 20 minutes (the shortest time noted) to 2 hours, while gastroc-
nemii from the same frog have shown signs of bacterial decomposition
before the onset of inexcitability. Similarly the skinned legs of a frog
from which sartorii have been removed will remain excitable for hours
after the dissected muscles have become inexcitable, even when they
have been partly dried by remaining on the bench without precautions.
Gastrocnemii may show all the phenomena described; more than 20
definite cases have been recorded. The occurrence however is not regular
as in the case of sartorii, and inexcitability, when it occurs, may take
longer to come on. Only sartorii are now being used for this work.
Removal from a normal environment in contact with other muscles
seems to have some effect on the phenomenon. That the sartorii are
not seriously injured by the dissection is shown by the fact that after
treatment with Ringer's solution they may remain excitable and in
good condition for days. The difference between individual frogs is par-
ticularly marked. Two frogs which appear to be equally active and
which are of the same size may provide muscles which will differ several-
fold in the time taken to become inexcitable (*e.g.* 40 minutes and 5 hours
respectively). Many of the frogs used have been extremely fresh and
active; the effect cannot be attributed to poor condition in the animals.

J. TORTOISE MUSCLES AND CRAB NERVES.

The biceps cruris muscle of the African land tortoise rapidly became
inexcitable (September) and behaved in a manner closely resembling
that of frog's muscle. Furusawa (private communication) has recently
found that the limb nerve of *Maia*, which is non-medullated, becomes
inexcitable if exposed for a very short time to moist air immediately
after removal from the animal. It may be recovered entirely by im-
mersion in sea water for 30 minutes or less, and will then remain excitable
for a long period. Isotonic glucose will not restore it.

K. THE TIME RELATIONS OF EXCITATION.

Preliminary experiments indicate that the value of the chronaxie
increases gradually with the onset of inexcitability. There is a con-
siderable rise in the value of the rheobase. It is of interest to note that
Lucas ((11), p. 471) found a most striking alteration in the time value
of excitation, in a sartorius muscle immersed in Ringer's fluid, the
change reaching completion in a few hours. The chronaxie decreased
enormously as the result of soaking, and the minimal current gradient
largely increased. Lucas attributed this to the presence of free calcium

ions in the Ringer's fluid. It might equally well be due to the washing away of excess of potassium ions (see below). The similarity in the times of immersion required (*a*) to produce the effect described by Lucas, and (*b*) to render a sartorius permanently excitable in the present sense, suggests that the loss and recovery of excitability described here are due to changes in the excitatory (as distinguished from the contractile) process, and may, perhaps, be attributed to progressive alterations in the concentrations of Ca or K ions in the muscle fibres or the spaces between them.

L. The production of reversible inexcitability by solutions.

Cooke[12] has shown that inexcitability may be obtained by immersion in hypertonic solutions. This fact was confirmed. A solution of double the concentration mentioned above, and a Ringer's fluid having twice the usual concentration of sodium chloride, caused inexcitability in several muscles tested. Immersion for 10 minutes in normal Ringer's solution restored excitability in all cases.

It is well known that an increase of potassium or calcium in Ringer's solution may lead to the onset of inexcitability in a muscle immersed in it. See, for example, Sereni ([13], p. 13), where full references can be found. According to Sereni a muscle may become inexcitable when immersed in Ringer's solution containing rather more than three times the usual potassium concentration, sometimes in a solution containing only $2\frac{1}{2}$ times the usual concentration. Recovery is possible in such cases by washing in ordinary Ringer's fluid. Higher concentrations of potassium (0·2 p.c. KCl) may cause contracture of the muscle. It seems conceivable that the onset of the inexcitable state may be due to small quantities of potassium diffusing out from the interior of the fibres to the interspaces[1] between them, a possibility that is borne out by the experiments of Ernst and Scheffer[14] who found that potassium does, in fact, leave an isolated muscle perfused with Ringer's solution.

Prof. Lovatt Evans, who called our attention to this possibility, has made experiments (unpublished) in which a state of tonus occurring in a smooth muscle suspended in oxygen could be abolished by immersing the muscle in oxygenated Ringer's solution. As is well known, addition of a small quantity of a potassium salt will cause a contracture in many types of plain muscle.

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[1] The effect of high K values in the Ringer's fluid must be due to changes produced in these interspaces: for the K concentration inside the fibres is far higher than in the Ringer's solution which produces the inexcitability.

M. Discussion.

Reversible inexcitability, therefore, may rapidly be induced in frogs' muscles by dissecting them, preferably without the use of Ringer's fluid or with minimal use thereof, and placing them in a gas, or in an environment, such as mercury or paraffin, which is free from electrolytes. Sartorius muscles, perhaps because they have a greater surface per unit volume, perhaps because they require more dissection, become inexcitable more quickly and more regularly than do gastrocnemii. Hermann[15] in 1867 showed that while gastrocnemii retain their irritability better in oxygen than in air, the reverse was the case for sartorii. He advanced a surface theory to account for this. Fletcher[16] in 1902 found that loss of irritability is delayed by oxygen in both cases. The apparent contradiction between the two results may be explained on the grounds that the loss of irritability in the experiments of Hermann was a reversible one in the sense of the present work, while the maintenance of irritability found by Fletcher referred not to excitability as such, but to the oxidative removal of lactic acid and to the other reactions which occur in a muscle when its life and normal condition are maintained by oxygen. Owing to the very great differences which exist between individual frogs, and to the possibility that Hermann did not use any saline solution when dissecting, it is easy to see how one set of muscles might not become inexcitable while the others did.

Reference has already been made to the observations of Foster and Moyle[8]. A variety of experiments conducted in the Biochemical Laboratory at Cambridge (see *e.g.* [8], [17], [18]) have shown that when frogs' muscles (gastrocnemii, or the whole musculature of the hind limbs) are kept at 0° C. in oxygen or air, they become non-irritable after a period varying from 1 to 2 weeks. At 0° C. the rate of metabolism is so reduced that diffusion of oxygen is fast enough to prevent any increase in the concentration of lactic acid. The condition of non-irritability in question could thus be sharply distinguished from that associated with the familiar events which, when oxygen is deficient, lead gradually to rigor and death. In these experiments at Cambridge, all at 0° C. on the whole legs or the gastrocnemii of English fen temporaria frogs, in no single case (so Sir F. G. Hopkins informs us) has non-irritability been observed to occur in less than 5 to 6 days. When a condition of non-irritability is established, the lactic acid content is that of the "resting minimum" of fresh muscles (Foster and Moyle[8], Lascelles, quoted by Thomson[17]).

If such non-irritable muscles be transferred to an atmosphere of nitrogen the lactic acid increases normally. They display normally the phenomena of heat rigor, and, under the influence of chloroform or toluene they behave like fresh muscles. They show a normal increase in lactic acid as the result of injury or disintegration.

The effect of ions in reducing the concentration of "inorganic"[1] phosphate is the same in preparations of these non-irritable muscles as in preparations from fresh muscles (Edsall(18)).

On the other hand, the osmotic properties of the non-irritable muscles studied at Cambridge are abnormal; a solution which is isotonic to the normal muscles is hypertonic to a non-irritable one. The internal resistance after 2 hours' washing with isotonic cane sugar solution is increased (Thomson(17)).

A non-irritable sartorius was found to give no action current, but a definite, though diminished, injury current (Foster and Moyle(8)).

Such muscles, even when their non-irritability has been long maintained, are readily restored by immersion in Ringer's solution.

The fundamental difference, therefore, between the experiments in the Biochemical Laboratory at Cambridge and ours, lies in the time required for the production of inexcitability; in those, of the order of a week, in ours of the order of an hour. It will have been noticed that Foster and Moyle employed mostly the complete legs of English frogs, which we have never observed to become reversibly inexcitable; they employed also the gastrocnemii of English frogs, which rarely have become inexcitable in our experiments. The rapid onset of reversible inexcitability at room temperature is most obvious in the thin dissected sartorius. Whether the Cambridge phenomena and those we have described are essentially the same only future study can decide. They are alike in that the chemical state of the inexcitable muscle is in either case analogous to that of a resting excitable one.

One of us (W. D.) has shown(19) by perfusion of the hind limbs of *Rana temp.* with calcium-free Ringer's solution that inexcitability may occur without the phosphagen content being affected. While this case differs from the others in that calcium has been removed and that therefore a chemical change is involved, it resembles them in that from the chemical point of view the muscle is otherwise in a resting condition. The criterion of rest in the experiments of Foster and Moyle was the lactic acid content, in the present work the phosphagen content, checked

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[1] This included the "phosphagen" phosphate.

by the ability of the muscle to perform work after recovery of its excitability.

When a muscle is removed from its normal environment it is conceivable that changes may take place in the membranes surrounding the muscle fibres, or in the relative volumes of fibres and interspaces. Hopkins(20), in discussing the work of Foster and Moyle, suggested that a dislocation between stimulus and energy discharge had occurred, and that associations between colloids and electrolytes may have assumed increased stability in the time required for the onset of non-irritability; in either case changes might result in the relative concentrations, inside and outside the fibre, of potassium, calcium or other substance. One outstanding difficulty in this respect is that a muscle which has been soaked in Ringer's solution for an hour or more may remain permanently able to withstand contact with an environment free from electrolytes. Gottschalk(9) working with the sciatic nerves of frogs found that the time required for a nerve to become non-conducting when deprived of oxygen was lengthened by preliminary treatment with hypotonic sodium chloride, and decreased with hypertonic sodium chloride. He suggested that the reason for this was a change in metabolic rate caused by alterations in the water content and not by changes in specific electrolytes.

No explanation can at present be advanced of why a sartorius removed from a leg will become inexcitable while the leg remains excitable. On the whole we are inclined to attempt a further investigation on the lines indicated by Prof. Lovatt Evans' suggestion of the importance of potassium ions. Owing either to leakage outwards of potassium, or to osmotic diminution of the volume of the interspaces, potassium ions become concentrated in the interspaces, and, since immersion in Ringer's solution containing a small excess of potassium causes inexcitability to appear, such an increase in the potassium concentration of the interspaces might be expected to have the same result. The phenomenon, therefore, would become one related to excitability as such, a subject which has been widely investigated by those who have studied the excitation process in muscle and nerve. The rapid recovery of excitability in Ringer's fluid of a muscle which has just become inexcitable reminds one of a diffusion process in that it is rapid at first and then more slow as time goes on, and it may be that the recovery is due simply to the diffusion of potassium ions from the interspaces into the Ringer's solution[1].

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[1] Preliminary experiments show that excitable sartorius muscles will become inexcitable in about an hour if placed in Ringer's solution containing four times the normal

The fundamental difficulty of this general explanation is that it is not clear how a preliminary treatment with Ringer's solution prevents any subsequent accumulation of potassium in the interspaces when the muscle is suspended in a gas. Possibly the permeability of the membranes of the fibres is affected by the "shock" of dissection, however careful, and only returns gradually to its normal condition after prolonged exposure to a normal environment of electrolytes.

In later work it is proposed to investigate further the changes in chronaxie associated with the onset and removal of inexcitability, which may throw some light on the excitation process, and also to determine whether a muscle which does not respond to electrical stimulus will respond to chemical treatment. A resting excitable muscle if ground up slowly in 4 p.c. trichloroacetic acid gives an extract, which, on analysis, resembles that obtained from a fatigued muscle. It is proposed to treat inexcitable muscles in the same manner. Preliminary observations of this kind suggest that no difference will be found between muscles of the two types. In this connection Mines and Dale[21] found that the skeletal muscles of the ray, when made non-irritable to electrical stimulation by treatment with dilute ether, respond to the application of acid, alkali and salt solutions.

SUMMARY.

1. In the estimation of phosphagen and inorganic phosphate in muscle care must be taken to avoid the "stimulus" provided by dissection and extraction. A thin muscle suspended at rest in oxygen for some time, and ground up in the cold, gives lower values of inorganic phosphate, and higher values of phosphagen, than ordinary "resting" muscle.

2. Isolated frogs' sartorius muscles, dissected without the use of Ringer's solution, and suspended at room temperature in moist oxygen, nitrogen, air, air with 5 p.c. CO_2, paraffin oil or mercury, rapidly become potassium content, and that muscles which have become spontaneously inexcitable, or those rendered inexcitable by the above treatment, will recover their excitability if placed in Ringer's solution containing three times the normal amount: spontaneously inexcitable muscles, placed in Ringer's solution containing four times the normal amount of potassium, remain inexcitable and do not show even a temporary recovery of excitability.

The inexcitable condition induced, by immersion in carbon dioxide, in a muscle previously soaked in Ringer's solution, remains after the carbon dioxide has been replaced by oxygen: the muscle, however, may then be made excitable by soaking in Ringer's solution.

reversibly inexcitable. Gastrocnemius muscles may do the same, but the occurrence is neither frequent nor regular.

3. Such inexcitable muscles do not appear to differ chemically from excitable ones.

4. Excitability may be rapidly restored by immersion in Ringer's fluid, or in various solutions. The period of inexcitability may be extended over many hours without affecting ultimate recovery on treating with Ringer's fluid.

5. The onset of inexcitability may be prevented by previous prolonged soaking in Ringer's fluid. Onset and recovery may be repeated several times, until the total period of the immersion reaches a critical value, after which the muscle will remain permanently excitable in the sense of these experiments.

6. The phenomena are not due to drying, to changes in the total water content, to temperature, to the condition of the animals, or to gross injury.

7. Leg muscles of the African land tortoise and limb nerves of the spider crab show similar reversible effects.

8. The chronaxie increases gradually with the onset of the inexcitable state; according to Lucas it decreases during soaking in Ringer's fluid.

9. Reversible inexcitability is readily produced by soaking a muscle in Ringer's solution containing three times the normal potassium ion concentration (Sereni). Potassium is known to escape from the fibres of a perfused muscle (Ernst and Scheffer). It is suggested that an increase in the concentration of the potassium ions in the interspaces between the fibres may be the basis of the phenomenon described. The washing away of these ions would, in that case, be the cause of the restoration.

10. A comparison is made of the present results with those obtained in the Biochemical Laboratory at Cambridge. It is not certain that the two sets of phenomena are the same. They are similar in that no chemical differences have been found between excitable and inexcitable muscles of either type. They are different in the times involved in the onset of the inexcitable state (an hour and a week respectively).

In conclusion we desire to express our gratitude to Prof. Lovatt Evans and Prof. A. V. Hill for their advice and criticism during the progress of the work.

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