The effect of nitrate, iodide and bromide on the duration of the active state in skeletal muscle

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If a frog's skeletal muscle is soaked in modified Ringer's fluid, containing bromide, nitrate or iodide in place of chloride, the tension and duration of a twitch, and its heat production, are largely increased, in the order Br < NO₃ < I. In a tetanic contraction, however, the tension, the heat production and the velocity of shortening under any load are unaffected. The primary effect of these anions, acting on the excitable surface of the muscle fibres, is to increase the duration of the active state after a shock; the increased tension and heat in a twitch result from this. The rate at which the effect comes on after sudden immersion of a muscle in iodide-Ringer depends solely on the rate at which iodide diffuses into the interspaces between the fibres; the rate of penetration of iodide into the fibres is very slow. The effect is not due to any direct action of the anion on the contractile mechanism, for (1) it passes off rapidly, when a muscle after long soaking in iodide-Ringer is transferred to chloride-Ringer, although iodide remains inside for a long time, and (2) the tetanic contraction is unaffected.

If the fundamental 'unit' of contraction were a cycle of events triggered by excitation and then running its own course to completion, the duration of the active state would be an intrinsic property of the contractile mechanism and would not depend on something outside it. But if the active contractile state is set up and maintained in the interior by some influence transmitted from the excitable surface outside, its duration would depend on the time course of the responsible process at the surface. The facts (a) that the duration is greatly affected by the presence of, for example, iodide in the interspaces between the fibres, at a time when there is practically none of it inside the fibres, and (b) that the effect rapidly passes off as iodide is removed from the interspaces although a large amount of it may still be inside, is decisive evidence for the second view. These abnormal anions somehow affect the time course of the process, started by excitation, in the surface of the fibres from which the active contractile state is transmitted into and maintained in the interior. This exposes a new link in the chain of events connecting excitation with contraction.

INTRODUCTION

According to Kahn & Sandow (1950) a frog's muscle soaked in Ringer's fluid, in which chloride is replaced by nitrate, shows the following effects during the isometric twitch evoked by a single supermaximal shock: (1) the peak tension is greatly increased; (2) the latent period is slightly decreased; (3) the fall of tension during 'latency relaxation' is somewhat increased (though not proportionally as much as the peak tension); (4) these effects come on quickly after the change from normal Ringer; (5) they are rapidly reversed when the muscle is restored to normal Ringer; (6) the action potential is the same after chloride- as after nitrate-Ringer; and (7) excitability is somewhat increased. As regards (7), Kahn & Sandow do not define the duration of the stimulus used, but Chao (1935), whom they quote in this connexion, used currents of long duration.*

* Inserted in proof 2 October 1954. Professor Sandow informs us that the shocks were of short duration, 0·1 or 0·2 ms. For threshold response there was no increase of excitability in nitrate-Ringer, but maximal response was obtained with a shock strength only about 50% of normal.
In later notes (Kahn & Sandow 1951; Sandow & Mauriello 1953) further results were reported: (8) bromide and iodide produce effects similar to those of nitrate, in increasing the twitch tension and prolonging its duration, in the order \( \text{Br} < \text{NO}_3 < \text{I} \); (9) the tetanus tension was found to be greater in nitrate- than in chloride-Ringer (but see (13) below); (10) twitch tension in a series at 40/min is reduced (by fatigue) more quickly in nitrate-Ringer; and (11) the nitrate effect can be simulated in chloride-Ringer by spacing two shocks 20 ms apart. In a recent letter, however, Professor Sandow tells us, (12) that nitrate considerably reduces the fusion frequency in a tetanus; and (13) that result (9) above was due to using too low a frequency, and that, provided fusion is complete, the tetanus tension is the same in nitrate- as in chloride-Ringer. The evidence has led Sandow and his colleagues to conclude that nitrate, bromide and iodide produce their effects by prolonging the active state, and that these results are of interest chiefly in the light they may throw on the connexion between excitation and contraction.

Some time ago Professor Sandow suggested to one of us that similar experiments should be made on the heat production. This was not done at once because of other commitments; and when time was available it was thought better at first to gain experience with the effect of these ions on the mechanical response. Later the heat also was examined: in an isometric twitch due to a single supermaximal shock the heat production after nitrate- (or iodide-) Ringer was considerably greater than after chloride-Ringer, just like (and nearly in proportion to) the peak tension; while in an isometric tetanus, with high enough frequency to provide complete summation and long enough in duration to give maximum tension, the heat production was the same in both. The effect, therefore, of nitrate-Ringer on the heat production was exactly similar to its influence on the tension (which is also unaffected in a tetanus). These effects on the heat were easy to establish. There may possibly be small secondary effects, though of this there is no present evidence; but the main results of heat measurements were merely to confirm those of mechanical measurements, and, since the latter are simpler to make, further investigation was concentrated on them.

We have confirmed the following results of Sandow and his colleagues: (1), (4), (5), (8), (12) and (13). We have made no experiments on (2), (3), (6), (7), (10) and (11); though as regards (7), with pulses of very short duration, such as we generally used, there was no noticeable change of excitability, while (10) is an inevitable consequence of each twitch producing more heat. We have made numerous experiments on the rate at which the characteristic effect of nitrate or iodide comes on, and the results show that this is determined merely by the speed of diffusion into the interspaces. We agree with Sandow’s conclusion (13) that it is easy to be led into error by not using a high enough frequency of stimulation, in fact for a time the same happened to us; with a frequency sufficient to ensure adequate fusion the tetanic contractions in nitrate-, iodide- and chloride-Ringer are identical (i) in maximum isometric tension and rate of rise, (ii) in velocity of shortening under any load, and (iii) in heat production.

That the effects are not due to any direct action of the abnormal anion on the contractile mechanism inside the fibres is shown by several things: (a) they occur,
after replacing chloride- by iodide-Ringer, far more rapidly than the penetration of the anion into the interior; that has a half-time of about 90 min; (b) when a muscle long exposed to iodide- is put back into chloride-Ringer the effect of the abnormal anion rapidly falls—although the interior of the fibres has by then accumulated a considerable amount of iodide, which comes out only very slowly; and (c) the characteristic properties of a tetanic contraction are quite unaltered.

We agree, therefore, with Kahn & Sandow's conclusion that the immediate effect of the anion is located at the surface of the fibres, i.e. at the excitatory membrane.

The reason why the tension in an isometric twitch may be so much less than in a tetanus (Hill 1951c) is simply that the active state in a twitch lasts too short a time, and decays too rapidly, for the contractile element of the muscle to be able to shorten enough, and to stretch its series elastic component enough, to exert the full tension. Any extension of the period of full activity, and any decrease in its rate of decay, should increase both the tension exerted in a twitch, and also the heat production (which lasts until the active state has come to an end; Hill 1953b).

Assuming then (1) that the effects described are due to the extended duration, after a shock, of the fully or partially active state, and (2) that they are not caused by any direct effect of the anion on the contractile machinery inside the fibre, we are forced to conclude that the state of activity inside is determined in duration by something happening at the surface. Hitherto one has tended to regard the excitatory process at the surface as pulling a trigger that fires off an independent contractile process inside, which then runs its own course, up and down, to completion. This idea is now seen to be wrong. The duration of the contractile process inside is determined, over a fairly wide range, by something which does not occur inside but at the surface. What that 'something' is, and how it is influenced by the anion, is still unknown. It is not the action potential, which (according to Kahn & Sandow) is unaffected by the anion; nor is there, as yet, sufficient evidence of any after-potential which might be associated with the unknown process at the surface. But at least we are one stage further towards linking excitation with contraction.

1. Methods

All isometric contractions were recorded with a mechano-electronic transducer (RCA 5734). In the earlier experiments this operated a cathode-ray tube through an amplifier, and records were made photographically. This was inconvenient when a large number of twitches had to be recorded (as in §§ 5, 6 and 10 below), so the transducer was connected through a balanced d.c. amplifier to a moving-coil recorder writing with a pointer on a smoked drum. This arrangement has all the advantages of the classical method of physiology, providing a continuous record of everything that goes on and permitting the experimenter to observe what is happening. At the same time it is superior to purely mechanical methods in allowing records to be made anywhere, not in the immediate neighbourhood only of the experimental object.

The recorder (HVT1), constructed by Messrs Kelvin and Hughes (Industrial) Ltd, was slightly modified to allow it to write on a vertical smoked drum, and mounted on a large Palmer stand (D12) for vertical adjustment. The amplifier
(Type 6), also by Kelvin and Hughes, could provide an output of ±50 V proportional to the input voltage, when connected through the 5000Ω of the moving coil. The deflexion of the recorder was fairly accurately linear for 25 mm (±12.5 mm from zero); this was ample, since the line drawn on the drum was readable within 0.1 mm. So much power is used to drive the pointer that friction on the smoked surface is negligible. The frequency response of the recorder to a constant alternating voltage at the input of the amplifier was measured as follows:

<table>
<thead>
<tr>
<th>frequency (c/s)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>deflexion</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>96</td>
<td>88</td>
<td>79</td>
<td>69</td>
<td>58</td>
<td>48</td>
<td>42</td>
</tr>
</tbody>
</table>

The deflexion, therefore, was rapid enough to allow twitches to be recorded with negligible distortion at the highest temperature used.

For isotonic contractions a photoelectric method was used (Hill 1951b), with a lever of small equivalent mass. Since records of shortening had to be made at room temperature, where contraction is rapid, it was important to keep the equivalent mass as small as possible; this was assisted by interposing a weak, long spring between the lever and the weight providing the constant force. For the heat production, normal methods were used, the output of the photo-electric amplifier of the galvanometer being recorded either photographically or on a smoked drum.

The solutions used (containing nitrate, iodide or bromide) were the same as the Ringer’s fluid generally employed (NaCl 6.75 g, CaCl₂ 0.20 g, KCl 0.15 g, H₂O 1000 g), but with NaNO₃, NaI or NaBr in equivalent amount replacing the NaCl. Originally the Cl of the CaCl₂ and KCl also was replaced; this, however, was found unnecessary, the effect of the anion increases with its concentration ($§$ 4 below), but there is no virtue in excluding Cl altogether. In many experiments (in practically all with iodide) the solution bathing the muscle was made up by mixing (say) iodide- and chloride-Ringer in the desired proportion; thus, for example, 67% iodide-Ringer is a mixture of 67 parts of iodide- and 33 parts of chloride-Ringer.

In the experiments in which the time course of the effect of iodide was followed, from the moment when the new solution was introduced, the new solution had first to be adjusted to the same temperature as the old. The old solution was withdrawn, the new solution was run in, and at the moment when the new solution reached the muscle a stop-watch was started; it was stopped at the first subsequent twitch, in a regular series which had been going on all the time except during the short interval between the two solutions.

In the experiments made to determine the speed of onset of the effect, iodide had a special advantage in the fact that its radioactive isotope could be used to study the speed of penetration of the anion. This penetration occurs at two very different rates, first into the interspaces between the fibres by simple diffusion (this is rapid) and second from the interspaces to the interior (this is very slow). Dr B. C. Abbott at the Marine Biological Laboratory, Plymouth, made special experiments on our behalf with radioactive iodide-Ringer. A single frog’s sartorius was used, exposed on both sides to the solution, and in most experiments the muscle was first soaked for several hours in the solution, and then suddenly immersed in normal Ringer;
the rate of escape of the iodide was measured by a Geiger counter. In the rapid phase of diffusion out from the interspaces the time for the process to be half completed was about 1 min; in the slow process of escape across the membranes bounding the fibres the half time was about 90 min.

2. The main effect

(a) Isometric twitches

In figure 1 are two isometric twitches of the same muscle at 18° C, one in chloride-Ringer, the other after 50 min soaking in nitrate-Ringer. The twitch tension is about doubled in the nitrate, and the duration, however reckoned, is greatly increased. The two curves start off together, but the nitrate curve soon diverges from the chloride one. The duration of the fully active state in a normal muscle at 18° C is very short (Ritchie 1954a), so divergence occurs early.

(b) Isotonic twitches without after-load

The effect on shortening in an isotonic twitch is shown in figure 2. A frog’s sartorius (l₀ = 3·25 cm, weight 0·097 g) was loaded with 0·76 g and contracted as shown, in response to single supermaximal shocks, in chloride- and in nitrate-Ringer. The height of contraction was about twice as great in the nitrate, while the duration was several times as long. In spite of this, however, in a tetanus of the same muscle the velocity of shortening under various loads was unaffected by the nitrate.

(c) After-loaded isotonic twitches

A striking comparison is shown in the following figures:

| after-load (g) | 0 | 1·87 | 3·74 | 5·60 | 7·47 | 9·35 | 14·0 |
| shortening (mm), Cl | 7·1 | 5·3 | 4·3 | 3·5 | 3·0 | 2·6 | — |
| shortening (mm), NO₃ | 11·5 | 9·7 | 9·3 | 8·9 | 7·9 | 7·7 | 6·3 |
| ratio | 1·53 | 1·83 | 2·16 | 2·54 | 2·63 | 2·96 | — |
The muscle was a sartorius at 15°C, \( l_0 = 3.5 \text{ cm} \), weight 0.148 g; the initial load was 0.71 g, single maximal twitches. Obviously the ratio of twitch height, nitrate to chloride, could be given any value between 1.5 and infinity merely by varying the after-load. The explanation is simple. The active state in nitrate lasts longer; the muscle has first to shorten and stretch its tendons (and connexions to the lever) until their tension is enough to lift the after-load; then only can shortening occur externally. If the after-load is large enough the chloride muscle has not time enough to develop the tension needed, while the nitrate muscle may still have plenty of time left to continue shortening.

It might have been thought that the same effect would be shown with a muscle at different temperatures; for at a lower temperature the active state lasts longer. But against this must be set the fact that at a lower temperature a muscle shortens more slowly and so takes longer to shorten the amount needed to raise the tension in the tendons enough to lift the load. In nitrate the velocity of shortening is the same as in chloride, so the time spent in getting up tension to lift the load is the same; the remaining time is available for external shortening.

A few experiments were made with the method described recently by Macpherson & Wilkie (1954); the time of earliest divergence was determined of the isometric contraction produced by two shocks, suitably timed, from that due to one shock only. The object was to confirm that in a muscle in nitrate-Ringer this divergence occurs later. In four experiments at 0°C the time of earliest divergence in nitrate-Ringer was on the average 1.6 times that in chloride-Ringer: in three experiments at room temperature the ratio was about 2. It was not worth while pursuing the matter further, for Ritchie (1954b) has recently shown, by an analogous but more sensitive method, that at the end of an isometric tetanus the time after the last shock at which relaxation can just be detected is increased by nitrate.

Thus nitrate not only causes a slowing of the rate at which the active state decays, as is evident from such records as those of figures 1, 2 and 3, but also prolongs the period after a stimulus during which no decay at all can be detected, even by the most sensitive methods.

3. THE EFFECT OF BROMIDE AND IODIDE

In the so-called ‘lyotropic series’, anions are usually arranged as follows: Cl < Br < NO₃ < I. In their effect on muscle the four anions lie in this order. The effect of bromide is rather small, that of nitrate is much larger, while that of iodide is larger still. According to Kahn & Sandow (1951) the twitch tensions in chloride-, bromide-, nitrate- and iodide-Ringer are, on the average, in the ratio of 100:155:235:310. In a general way our results agree with this; but the effect depends on temperature, concentration, muscle length and type of contraction, and there is no reason to assume that isometric twitch tension is a simple proportional measure of it. The fundamental phenomenon is probably an increase in the duration of the active state, and in such a complicated matter it is scarcely possible to define the effect of each ion by a single number.

At concentrations less than about 70%, iodide-Ringer produces effects exactly similar to those of bromide- and nitrate-Ringer. All seem to be completely rever-
sible. But when 100% iodide-Ringer is used, after an initial large rise of twitch tension, a fairly quick decay sets in, which is accompanied by a rise of resting tension. This continues until the muscle is irreversibly damaged. Possibly in 100% iodide-Ringer the 'door' which is 'opened' by a stimulus never 'closes' completely afterwards; an exaggerated prolongation of the active state occurs and a permanent state of activity is set up which exhausts the muscle.

4. THE INFLUENCE OF CONCENTRATION

In figure 3 are five maximal isotonic twitches at 15°C of a frog's sartorius ($l_0 = 3.0$ cm, weight 0.104 g) in concentrations of nitrate 100, 67, 44, 30 and 0%. The solutions were made by mixing nitrate- and chloride-Ringer to these proportions. The muscle was soaked first in nitrate-Ringer, then successively in the other mixtures; the initial load was 0.71 g, the after-load 9.3 g. A similar experiment with iodide, made in the opposite order, gave the following results:

<table>
<thead>
<tr>
<th>% iodide</th>
<th>0</th>
<th>17</th>
<th>31</th>
<th>42</th>
<th>61</th>
<th>87</th>
</tr>
</thead>
<tbody>
<tr>
<td>contraction height</td>
<td>100</td>
<td>158</td>
<td>199</td>
<td>226</td>
<td>254</td>
<td>263</td>
</tr>
</tbody>
</table>

After-loaded isotonic twitches, particularly with heavy after-loads, show greater differences than isometric twitches and do not provide so constant an index of the nitrate or iodide effect. Our best evidence of the influence of concentration is drawn from a large number of experiments with isometric twitches made for other purposes with various concentrations of iodide. In figure 4 the percentage increment of twitch tension is plotted against the concentration of iodide producing it.
There is wide scatter of the observed points, but this cannot be avoided, since the effect varies from muscle to muscle. The interpolated curve, nevertheless, gives a reasonable idea of the usual effect of concentration. A smaller number of experiments with nitrate confirmed the form of the relation in figure 4.

There is no 'threshold' to the nitrate or iodide effect—it increases continually with concentration. If we were to take the increment of twitch tension as an actual measure of the phenomenon, we should conclude that the effect increases with, but not in proportion to, the concentration. The increment, however, of twitch tension is due, not directly to an increase of contractile strength but to an increased duration of the active state. But there is no reason to suppose that the tension increment is directly proportional to the duration increment; to take a *reductio ad absurdum*, if the duration increased indefinitely the tension would remain finite and become that of a tetanus. It is more likely that if a linear relation does exist it is between concentration and duration; and since we cannot expect the increment of tension to be *proportional* to the increment of duration, it should not be proportional either to concentration.

The relation, therefore, of figure 4 is to be regarded as an empirical fact. In § 10 below it will be used in interpreting, in terms of diffusion, the rate at which the twitch tension increases when a muscle is suddenly immersed in a nitrate or iodide solution.
5. The Influence of Temperature

The effect of nitrate or iodide on the maximum tension in a twitch depends largely on temperature. In the experiments summarized in figure 5 a frog's sartorius, at its 'standard length' in the body, was stimulated directly with maximal condenser discharges \((R C \approx 0.3 \text{ ms})\), at regular intervals for several hours, and the isometric tension recorded. The intervals were usually of 1 min but could be less at the higher temperatures; the purpose of the regular stimulation was to keep the muscle in a steady state of limited activity. To consider a single experiment, stimulation began at 20° C in normal Ringer's fluid and continued until constant twitches were obtained. Care was taken, by trial at each temperature, that the shocks were maximal. The temperature was changed to 15° C and the same procedure followed; this was repeated at 10, 5 and 0° C. Then the chloride-Ringer was replaced by nitrate, and the muscle left to soak for 70 min, stimulation at 1 min intervals being continued. When the continuing records showed that the twitch tension was steady, the temperature was raised successively to 5, 10, 15 and 20° C, records being made at each temperature. Finally the nitrate- was replaced by chloride-Ringer, and after 80 min the series 20, 15, 10, 5 and 0° C was repeated. The agreement of the third series with the first showed that the muscle had not changed.

The order of the experiment was sometimes altered, e.g. by starting at 0° C instead of 20° C, but the general result was the same. Quantitatively the results varied from one muscle to another, for example, (a) the effect of temperature on the chloride twitch might be greater or less than shown by the lower curve in the

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**Figure 5.** The influence of temperature on twitch tension in chloride-Ringer (lower curve) and nitrate-Ringer (upper curve). The twitch tension in chloride-Ringer at 0° C is taken as 100. Muscle at length \(l_o\).
(b) the ratio of the twitch tensions in nitrate and chloride might be greater or less (in figure 5 at 18°C it is about 2.5, but in other experiments it varied from 1.8 to 2.7); and (c) the maximum tension in nitrate might occur at a rather different temperature.

In chloride-Ringer, the lower curve shows the usual effect of temperature on twitch tension (see Hill 1951c). At all temperatures the duration of the twitch was considerably greater in nitrate than in chloride-Ringer. The difference between the two curves of figure 5 must be related to the facts, (i) that the tetanic tension is the same in nitrate and in chloride, (ii) that the tetanic tension is rather greater at a higher temperature, and (iii) that the prolongation of the active state in nitrate allows the tension in a twitch to approach, more nearly than in chloride, that in a tetanus. There are good grounds for supposing (Hill 1951c) that the twitch tension comes nearer at a lower temperature to the tetanus tension because, in the twitch, the duration of the active state, and therefore the total time available for shortening, is increased more than is the time required for shortening a given amount under a given load. Since the duration of the active state at a low temperature is already relatively long, the effect of increase of duration by nitrate is less.

6. The influence of initial length

Most of the experiments described in this paper were made at an initial length about equal to \( l_0 \), the standard length in the body. At this length the full isometric tension in a tetanus is near its maximum (Hill 1953a), and the resting tension in a pair of frog's sartorii of usual size is about 1 g (Hill 1949). At shorter lengths the resting tension is less, and owing to the smaller slope of the tension-length relation of the series-elastic component the contractile component has to shorten more before it can stretch the series-elastic component to a given tension. If the series-elastic component could be abolished (as it is effectively by a quick stretch) the twitch tension would be equal to the tetanus tension, while increasing the series-elastic compliance (Hill 1951a) makes the twitch tension less. Anything, on the other hand, that increases the duration of the active state, while leaving the velocity of shortening unchanged, would allow the muscle more time to shorten and develop tension. This is the basis of the nitrate effect. It could be expected therefore that a reduction of the resting length would have less influence in reducing the twitch tension after treatment with nitrate.

Experiments showed this to be so. A muscle in chloride-Ringer was connected to a tension recorder which could be lowered or raised as required. It was stimulated by single maximal condenser discharges at regular intervals (e.g. of 30 s), and the length was reduced until only a very small twitch tension was developed. After two or three shocks at this length a steady response was obtained, then the recorder was raised successively 1 mm at a time, and at each length two or three twitches were recorded. The process was continued until the length of the muscle was 4 mm greater than \( l_0 \). The length was then reduced to something less than \( l_0 \) and nitrate- or iodide-Ringer was introduced. After an hour or so the process was repeated, from the shortest length at which a tension was developed up to 3 or 4 mm more than \( l_0 \).
Figure 6 gives the average of two such experiments at 17° C, in good agreement, one with nitrate-Ringer, the other with 50% iodide-Ringer. In the neighbourhood of \( l_0 \) the usual effect was observed, a doubling of the twitch tension. At shorter initial lengths the twitch tension was less both in normal and in nitrate- or iodide-Ringer; but the ratio of the nitrate- or iodide-tension to the chloride-tension steadily increased with diminishing length, reaching 4 with a 27% reduction of length. Above \( l_0 \) the opposite effect was observed.

**Figure 6.** The influence of initial length on twitch tension, in chloride-Ringer (lower solid curve) and nitrate- or iodide-Ringer (upper solid curve). Mean for two muscles \((l_0 = 30\text{ mm})\), in nitrate and iodide. The broken line gives the ratio of the twitch tensions.

Similar experiments were made at 0° C, and the same general result was obtained. The increase of twitch tension due to nitrate (or iodide) is much less at 0° C than at higher temperatures, and reduction of length has a smaller influence on the nitrate effect. All this, and the rise with fall of temperature of the ratio (twitch tension)/(tetanus tension), can be deduced from the assumption that the effective duration of the active state is greater at the lower temperature, so that increasing it further has less result.

7. **The tension exerted in a tetanic contraction and the influence of frequency**

If the fully active state due to a single shock lasts longer, the frequency of stimulation needed for a complete tetanus should be less. This is certainly true when the fully active state is prolonged by lowering the temperature, for the frequency of stimulation required for a complete tetanus at 0° C is only about one-
quarter of that at 20°C. The difference between the effects of nitrate and of lowering the temperature is that with nitrate the velocity of shortening under a given load is unaffected, whereas at a lower temperature the velocity of shortening is less.

Records were made with a cathode-ray tube of isometric contractions in chloride- and in nitrate-Ringer. A just complete tetanus at 18°C in nitrate-Ringer was obtained with (say) 50 shocks per second; the actual frequency depends on the temperature. When the nitrate was replaced by chloride, the same frequency gave an obvious ripple on the tension record. If, however, with both, a high enough frequency was used to give complete fusion, not only were the maximum tensions the same but the curves along which the tension developed were identical.

Ritchie (1954a) has shown that the frequency required in a tetanus for complete fusion depends on the sensitivity of the recording arrangement used. With a piezoelectric crystal, reading to 0.004% of the full tension, complete fusion did not occur at 18°C below a frequency of 125 to 150 shocks/s. At 75 shocks/s there was an oscillation of the order of 20 dynes, which our recorder (reading a total say of 50000 dynes) could not have detected. At 50 shocks/s Ritchie found an oscillation of about 100 dynes, which we could detect. At 18°C (our usual temperature) 50 shocks/s applied to a chloride muscle gave an obvious ripple and less than the full tension; in a nitrate muscle the fusion was complete and the full tension was exerted. Ritchie (1954b) and Sandow (private communication) have observed this effect of nitrate on the fusion frequency, and Sandow's conclusion is the same as ours, that, in order properly to compare the tetanic tensions in chloride- and nitrate- or iodide-Ringer, frequencies of stimulation must be used which are high enough to give effectively complete fusion in both. Frequencies which are unnecessarily high should be avoided, otherwise the contraction dies off too quickly to give its full tension.

The following experiments were made, all at room temperature (15 to 18°C). A muscle in chloride-Ringer was stimulated isometrically with short supermaximal shocks at regular intervals of (say) 20 s, until the twitch tension became steady. It was then excited by a rapid sequence of the same shocks, of high enough frequency to give full summation and long enough to allow maximum tension to be reached. The solution was then replaced by nitrate- or iodide-Ringer, and after (say) 20 min the records of twitch and tetanus were repeated. Finally, the original chloride-Ringer was put back, and records of twitch and tension were made once more. The twitches showed the usual effect of the abnormal anion, i.e. the twitch tension was increased in height and extended in time; but in the tetanus the maximum tension reached, and the time course of its rise, were unaffected. Many experiments of this kind were made; if care is taken to avoid change in the muscle by over-stimulation, and if the effect of slight progressive change is eliminated by going back to the initial conditions and taking a mean, the tetanus tensions are found so nearly the same, in spite of large differences between the twitch tensions, that on other conclusion can be drawn than that nitrate, or iodide, has no effect on the maximum force which a muscle can exert.
8. The force-velocity relation

The most general mechanical characteristic of muscle is the curve relating velocity of shortening to load; on this, the maximum tension represents a single special point, that at zero velocity. It was important therefore to find out whether the whole of the load-velocity curve for a tetanus is the same in nitrate as in chloride, not merely one point on it.

A frog’s sartorius, $l_0 = 3.25$ cm, weight 0.097 g, was set up in chloride-Ringer at 18°C under a small constant initial load of 0.76 g. It was stimulated with a short tetanus at 75 shocks/s (constant current pulses of 0.1 ms duration, 7 times supermaximal) with various afterloads (0, 5.6, 13.1, 17.6, 27.1 and 36.4 g), and records were made of its rate of shortening. The chloride-Ringer was then replaced by nitrate, and after 80 min the series was repeated. It was found that over the whole range of loads, with a nearly five-fold variation of speed of shortening, the records were identical in pairs up to the end of the stimulus. The velocities of shortening, when measured, fitted a normal load-velocity relation; but the comparison was best made simply by superimposing the records, which were indistinguishable. In order to avoid the risk of altering the muscle the full isometric tension was not recorded (this would have required a much longer stimulus); but the greatest load corresponded to $P_0/M = 1220$, and must have been 50 to 70% of the full isometric tension.

This complete identity of the force-velocity relation during a tetanus is in striking contrast to the form of the single twitch. In figure 2, for the same muscle as was used for the experiment just described, isotonic twitches are shown, for 0.76 g initial load and zero afterload, for nitrate and for chloride. It is evident that the difference lies, not in the contractile machine itself, but simply in the time during which that is brought into action.

The effect described was obtained several times and confirmed in various other ways: for example, (1) the records of twitches in figures 1, 2, 3 and 7 all start off along the same curve, diverging only as the active states begin to decay; (2) the rising curves of an isometric tetanus are identical in chloride- and in nitrate-Ringer, which could not happen if the velocity of shortening were not the same for both under all loads.

9. The heat production

The comparison of the heat production, in twitch or tetanus, after soaking (a) in chloride- and (b) in nitrate- or iodide-Ringer was made in two ways: first, by recording heat and tension simultaneously by photography; secondly, by recording heat only on a smoked drum in a series of twitches at regular intervals (1 min or less). The solution had to be removed during the actual heat measurements.

By the first method, in one experiment at 17°C, after soaking in chloride-Ringer the twitch tension ($P$) was 29 g, the heat ($H$) was $1.85 \times 10^{-3}$ cal/g, and $P_0/H$ (expressing $H$ in mechanical units) was 8.2; after soaking in nitrate-Ringer $P$ was
74 g, $H$ was $7.35 \times 10^{-3}$ cal/g and $P_l/H$ was 5.3. In another experiment at 17°C the following results were obtained:

After Cl-Ringer: at start: $P = 39.5$ g, $H = 2.31 \times 10^{-3}$ cal/g, $P_l/H = 7.0$; at end, after washing out nitrate: $P = 30.0$ g, $H = 1.88 \times 10^{-3}$ cal/g, $P_l/H = 6.6$.

After 1/3 $NO_3$-Ringer: $P = 56.2$ g, $H = 3.4 \times 10^{-3}$ cal/g, $P_l/H = 6.8$.

After 2/3 $NO_3$-Ringer: $P = 59$ g, $H = 3.4 \times 10^{-3}$ cal/g, $P_l/H = 7.1$.

After 1/1 $NO_3$-Ringer: $P = 75$ g, $H = 5.3 \times 10^{-3}$ cal/g, $P_l/H = 5.8$; after further soaking: $P = 81$ g, $H = 5.54 \times 10^{-3}$ cal/g, $P_l/H = 6.0$.

The effect of nitrate was, as usual, to increase the twitch tension; and the heat was increased rather more than in proportion to the tension, as is shown by the fall of $P_l/H$. According to Rosenberg (1934) the average value of $P_l/H$ in a twitch (at room temperature) is about 8, while in a tetanus (Hartree & Hill 1921, p. 144) $P_l/H$ steadily gets less as the stimulus is prolonged. It was natural therefore that the more prolonged contraction after treatment with nitrate should give a smaller value of $P_l/H$.

In the second series of experiments, all at 17 to 18°C, in which the twitch heat was recorded in a regular series of contractions, the following heats were obtained, in units of $10^{-3}$ cal/g.

- **Expt. 1.** Cl 0.83, $NO_3$ 1.58.
- **Expt. 2.** Cl 2.33, $NO_3$ 5.4.
- **Expt. 3.** Cl 2.3, 5/6$NO_3$ 3.5, 1/1$NO_3$ 4.0.
- **Expt. 4.** Cl 1.85, $NO_3$ 3.0, Cl again 1.4.
- **Expt. 5.** Cl 2.0, 2/3 I 3.6, Cl again 2.1.
- **Expt. 6.** Cl 2.8, $NO_3$ 4.5.
- **Expt. 7.** Cl 1.7, $NO_3$ 3.6, Cl again 1.6.

In experiment 4 the heat in a 0.25 s tetanus after Cl was $12.9 \times 10^{-3}$, after $NO_3$ $13.3 \times 10^{-3}$ cal/g. In experiment 5 the heat in a 0.25 s tetanus after Cl was $11.2 \times 10^{-3}$, after 2/3 I $12.2 \times 10^{-3}$ cal/g. In experiment 6 the heat in a 0.3 s tetanus after Cl was $16.6 \times 10^{-3}$, after $NO_3$ $17.2 \times 10^{-3}$ cal/g. In experiment 7 the heat in a 0.3 s tetanus after Cl was $14.4 \times 10^{-3}$, after $NO_3$ $15.5 \times 10^{-3}$ and after Cl again $15.2 \times 10^{-3}$ cal/g.

Thus in four muscles treated with nitrate or iodide, in which the twitch heat was about doubled, the tetanus heat was increased on the average only about 5%. A slight increase indeed could be expected, for the active state after the last shock of a tetanus in the nitrate or iodide muscle would decay more slowly than in the chloride muscle; so although the duration of the stimulus was the same the contraction would last a little longer, with slightly greater heat.

Measurements, therefore, of the heat production confirm the result from mechanical records that a considerable increase in twitch response, but none in tetanic response, is caused by these abnormal anions; they strengthen the conclusion that no change is produced in the contractile mechanism, but only in the time during which it remains in action after a stimulus.

There are various other experiments that could be made on the heat production, particularly in the analysis of the heat into its different phases. Is the heat of activation prolonged by treatment with nitrate or iodide? Is the heat of shortening...
unaffected? does mechanical work appear as a separate item in the balance sheet?
is relaxation heat absent if no mechanical energy is dissipated? It would be difficult,
however, to do such experiments except at 0°C; at 15°C everything would happen
several times more quickly, and the present myothermic apparatus has nearly
reached (for purposes of accurate resolution) its upper limit of speed. At 0°C,
however (see figure 5), the change produced by nitrate, or iodide, is not very large,
and from the experiments reported above it is clear that most of the heat must be
distributed in the usual way. The nitrate muscle shortens more in an isotonic
twitch—the heat of shortening will be correspondingly greater; the active state
lasts longer, the heat of activation will continue longer; more work can be done.
All this can be predicted on the likeness of a nitrate, or iodide, twitch to a short
tetanus. The distribution of the energy liberated between the three phases is
unlikely to be much, if at all, altered; and at 0°C, where the change due to nitrate
or iodide is rather small, the alteration, if any, would evade accurate analysis. It
would have delayed the investigation too much to have tried to tackle the technical
difficulties involved either in a closer analysis of the heat at 0°C, or in attempting
to resolve the heat into its several phases at 15°C where the nitrate effect is large.
Further experiments on the heat should be made, but they are unlikely to yield
results of special interest.

10. THE SPEED OF ONSET OF THE NITRATE OR IODIDE EFFECT

In figure 7 are six isometric twitches of a frog's sartorius (l0 = 3.45 cm, weight
0.130 g) mounted at 18°C on a multiple-electrode plate (for stimulation 'all over').
The bottom record was made in chloride-Ringer. A 67% iodide-Ringer was quickly
introduced and records were taken 25 s, 57 s, 2 min, 5.5 min and 9.5 min afterwards. The contraction rapidly increased in duration and strength. The top record
gave a twitch tension corresponding to \(P0/M = 1400\), which is 60 to 70% of that
normally observed in a tetanus.
As in figure 3 above, all the twitches started off on the same line, but those in iodide then rapidly diverged, successively more and more, from the chloride one. This experiment was not well designed to show the speed of the change after introducing iodide. The muscle was a large one, and one face of it was not directly in contact with the solution; for both reasons diffusion would take longer. If none of the solution were able to reach the surface in contact with the electrode plate, diffusion would take four times as long as if both surfaces were exposed; actually some of the solution is bound to creep round and reach the inner surface, but to an unknown extent. In this experiment the time for half the full increment of twitch tension to occur was between 1 and 2 min, which is much more (see below) than the time in a smaller muscle exposed on both sides.

In later experiments muscles of about 80 mg were used, and they were exposed on both sides to the solution; stimuli were applied through two platinum electrodes in contact with them. A muscle in chloride-Ringer was first brought to a steady condition by supermaximal condenser shocks every 20 s, records of twitch tension being made on a smoked drum. During the short time required to withdraw the chloride- and introduce the iodide-Ringer no stimuli were given. At the moment when the iodide solution covered the muscle a watch was started; it was stopped at the first subsequent twitch. Records were continued until the twitch tension became constant. The tensions were measured and the increment of twitch tension due to the iodide was plotted (as a percentage of the final increment) against the square root of the time in seconds ($t^\frac{1}{2}$) after introducing the solution. The reason for using the square root was twofold, first to condense the picture into reasonable dimensions, and secondly because, for short times, the amount of substance diffusing into a body suddenly exposed to a solution is proportional to the square root of the time (Hill 1928, p. 74), so a linear relation (see figure 8) could be expected near the origin.

Ten such experiments were made, all at 18° C, with muscles averaging 0.082 g in weight and 3.15 cm in length. Usually about 50% iodide-Ringer was used. In order to average the results, each curve of increment of twitch tension against $t^\frac{1}{2}$ was read off at the following values of $t^\frac{1}{2}$: 2.5, 5, 7.5, 10, 15 and 20. For each value of $t^\frac{1}{2}$ the mean was taken of the ten quantities so obtained. These are the solid circles of figure 8. The time for half the full increment to be reached is about 40 s.

A similar procedure was followed for the decrease of twitch tension which occurred when iodide- was replaced by chloride-Ringer. The excess of the steady twitch tension at the start in iodide-Ringer over the final value in chloride-Ringer was taken as 100, and the excess tension remaining at any time was plotted against $t^\frac{1}{2}$. Six experiments of this kind were made at 18° C, with muscles averaging 0.078 g in weight and 3.1 cm in length. The mean values of the remaining excess tension at the same six values of $t^\frac{1}{2}$ are plotted as hollow circles in figure 8. The time for the excess tension to fall to one-half is about 90 s.

The two curves of figure 8, though generally similar, have significant differences: first and most obvious, the rise of the curve for iodide introduced is quicker than the fall of that for iodide removed (corresponding to half times of 40 and 90 s); secondly, whereas the rising curve goes straight out from the origin, the falling one
bends round after the start, dropping initially more slowly than later on. The chief reason for this lies in the shape of the curve of figure 4 relating increment of twitch tension to iodide concentration, the slope of which at 50% iodide is only one-third of that at 0%. In the rising phase when iodide is beginning to go in the steepest part of the curve is involved, but in the falling phase when iodide is beginning to come out the least steep part. Although the amount of iodide entering in the former case is the same as the amount coming out in the latter, it has at the start three times as much effect on the tension.

![Graph](image)

**Figure 8.** Rising curve and solid circles, increase of twitch tension of frogs' sartorii (mean of 10) after the rapid introduction at time zero of iodide-Ringer. Falling curve and hollow circles, decrease of twitch tension (mean of 6) after the rapid replacement of iodide-by chloride-Ringer. Average about 50% iodide. The circles are means of observations; the curves were calculated for the diffusion of iodide into the interspaces between the fibres (see text).

It is possible to calculate the form of the curves in figure 8 if we assume, (a) that iodide produces its effect in increasing the twitch tension as soon as it reaches the surface of a fibre, (b) that the effect depends on the local concentration at any moment in the way shown in figure 4, and (c) that the rate at which iodide penetrates the fibres themselves is negligible compared with that at which it diffuses into the interspaces (see below). The muscle is regarded as an infinite plane sheet of thickness 2b cm suddenly exposed on both sides to the new solution. The curve of figure 4 cannot be expressed (except formally) by an equation, so it is impossible to integrate mathematically equation (40) in Hill's (1928) paper, to give the total effect on the twitch tension at any time. Numerical integration, however, can be carried out as follows. The sheet of muscle is taken as divided into ten equal zones, 0 to 0.2b, 0.2b to 0.4b, ..., 1.8b to 2.0b. Then the total amount of iodide in each zone is calculated for various times by integrating equation (40), and at each time the concentration is assumed to be uniform throughout that zone. Taking the final
concentration to be that of 50% iodide-Ringer, the effect on the twitch tension of the concentration reached at any moment in the zone is read off from the curve of figure 4. Since tensions are additive, the sum of these effects in the ten zones is taken as the total effect in the whole muscle. It is then expressed, for each time, as a percentage of the final effect after a long time.

With the symbols used in the paper referred to, the average concentration at time $t$ in the interspaces of the zone between $x = nb$ and $x = mb$ ($n < m$), expressed as a fraction of the concentration finally reached, is

$$1 - \frac{8}{(m-n)^2} \left( \cos \frac{1}{2} m \pi - \cos \frac{1}{2} n \pi \right) \left( \exp \left( - \frac{k \pi^2 t}{4 b^2} \right) + \frac{1}{6} \left( \cos \frac{3}{2} m \pi - \cos \frac{3}{2} n \pi \right) \left( \exp \left( - \frac{9 k \pi^2 t}{4 b^2} \right) \right) + \frac{1}{15} \left( \cos \frac{5}{2} m \pi - \cos \frac{5}{2} n \pi \right) \left( \exp \left( - \frac{25 k \pi^2 t}{4 b^2} \right) \right) + \ldots \right)$$

Table 1 is calculated from this equation, and the concentrations ($\times 100$) at the times shown in the first column are given in the other five columns, corresponding to five of the ten zones (the other five are the mirror images of these). For any time the concentrations are then read off for the different zones, e.g. for $k \pi^2 t/4 b^2 = 0.50$, 88, 65, 46, 31 and 24; if the solution around the muscle is 50% iodide-Ringer, the calculated concentrations are then 44, 32, 23, 15 and 12% respectively. From figure 4 these would provide percentage increments of twitch tension 109, 90, 71, 51 and 42, average 73. But the final increment, for 50 iodide-Ringer, would be 117; so at time $k \pi^2 t/4 b^2 = 0.5$ the calculated increment would be 73/117 = 0.62 of the final increment. The same calculation was made for each value of $t$ in table 1, and the result is plotted as the rising curve of figure 8. The only adjustment possible is to choose the value of $k \pi^2 t/4 b^2$ to give the best fit; the value chosen was $7.25 \times 10^{-3}$.

The average thickness ($2b$) of the muscles used in these experiments, calculated from weight and dimensions, was 0.07 cm; inserting this in $k \pi^2 t/4 b^2 = 7.25 \times 10^{-3}$,
the value of $k$ obtained is $3.6 \times 10^{-6}$ cm$^2$/s. According to Harris & Burn (1949) the diffusion constant of radioactive Na out from the interspaces of a frog’s sartorius is $2.6 \times 10^{-6}$ cm$^2$/s. According to Harris (1952) the time for the loss of half the radioactive potassium present in the interspaces of a sartorius 0.8 mm thick, suddenly exposed on both sides to normal Ringer, was 1.2 to 1.5 min. Using equation (42) of Hill’s (1928) paper this gives a diffusion constant of $3.5$ to $4.3 \times 10^{-6}$ cm$^2$/s.

The mobilities of Na and K ions in water are in the ratio of about 2 to 3, so Harris’s estimates of the diffusion constants of Na and K through the interspaces are in good agreement. Cl and I have almost exactly the same mobility, which is nearly the same as that of K; with considerable confidence, therefore, we may take the diffusion constant of iodide in the interspaces of the frog’s sartorius as about $3.6 \times 10^{-6}$ cm$^2$/s. This is not a true diffusion constant, for the fluid in the interspaces of a muscle previously soaked should be practically pure Ringer’s fluid, in which the diffusion constant of Cl or I (from tables) should be about $16 \times 10^{-6}$ cm$^2$/s; but if the length of the roundabout journey from the surface through the interspaces to any point inside the muscle were twice the direct distance the four-fold difference would be exactly accounted for.

Abbott’s estimate of about 1 min for the half-time of escape of radio-iodide from the interspaces is in reasonable agreement with Harris’s estimate for potassium; indeed, if Abbott’s muscles were 0.7 mm thick (as ours were on the average) the agreement would be exact. From figure 3 of Levi & Ussing’s (1948) paper it is possible to read off roughly the half-time of the escape of Na from the interspaces; the mean value is about 2 min. The size of their muscles was not mentioned, but Na diffuses only two-thirds as fast as K and their estimate agrees well with Harris’s. For Cl they state only that it came out from the interspaces at about the same rate as Na, but on physico-chemical grounds we should expect it rather to behave like K and I, and Levi & Ussing’s estimate was admittedly rough.

There is good evidence therefore that at room temperature the effective diffusion constant of iodide out of the interspaces of a sartorius muscle (allowing for the roundabout track) is about $3.6 \times 10^{-6}$ cm$^2$/s, the value required to fit the calculated rising curve of figure 8 to the observed points; and further evidence is at hand in the form of the falling curve (figure 8) for chloride-Ringer suddenly replacing iodide-Ringer. This curve was calculated in the same way for the same diffusion constant; it shows, as observed, the initial slower fall and the later quicker one, it predicts that the rate of decrease of twitch tension on removing iodide should be considerably less than the rate of increase on introducing iodide, and it gives not too bad a fit to the observed points. It is reasonable, therefore, to conclude that the rate at which the twitch tension rises (or falls) after the introduction (or removal) of iodide, depends solely on diffusion of iodide into the interspaces. Any possible reaction of iodide with the surfaces must be so relatively fast that the time taken by it is negligible compared with that in diffusion.

In Abbott’s experiments muscles previously soaked for a long time in radio-iodide-Ringer were suddenly put in chloride-Ringer and the time course of the slow escape of the iodide was followed with a Geiger counter. The half-time was about 90 min. If we assume that the rate of entry of iodide into the fibres of a muscle put
suddenly in iodide-Ringer is similar to that of its escape into chloride-Ringer, the amount that would penetrate the fibres in 40 s, even if entry to the interspaces were instantaneous, would amount only to 0.5% of the final amount after a very long time. Actually, since entry to the interspaces is far from complete in 40 s, the amount in the interior by then would be much less than 0.5%. Forty seconds was the time for half the full increment of twitch tension to occur after introducing iodide-Ringer. It is difficult to believe that if the iodide effect depends to any serious extent on direct reaction with the contractile material inside the fibre, half the full effect would be reached when considerably less than 0.5% of the final amount of iodide has penetrated. This supports the conclusion reached on other grounds that the abnormal anion acts on the excitable surface, not on the contractile mechanism.

Abbott’s half-time of 90 min for iodide escaping from the inside of the fibres can be compared with half-times for Na and Cl obtained by Harris & Burn (1949), Harris (1950) and Levi & Ussing (1948): Cl, room temperature, 10 min; Na, room temperature, 34, 39 and 45 min; Na, 0°C, 70 min. In Abbott’s case radio-iodide was exchanging with chloride; in the other cases the radioactive isotope was exchanging with the non-radioactive one.

An attempt was made to repeat at 0°C the experiments described above, in order to test the prediction that if the speed of onset of the effect of iodide is determined solely by diffusion it should have a rather low temperature coefficient (say 1.3 per 10°C). Three difficulties were found: (a) the expected one that the effect is much smaller, (b) that in iodide-Ringer the twitch tension fairly rapidly reaches a maximum and then usually decreases gradually, and (3) that when chloride-Ringer is reintroduced a small early increase of twitch tension often appears followed by the usual later fall. Various attempts were made (on summer frogs) to get over these difficulties, but without success; possibly winter frogs would be better. In general, however, we found it hard to get very consistent results with iodide or nitrate at 0°C.

11. Discussion

The influence of inorganic ions on cell activity is discussed by Höber in his book (1945, p. 289 etc.), where there is frequent reference to the anion series Cl, Br, NO₃, and I. These ions, in spite of very different ionic weights (35.5, 79.9, 62 and 126.9), have nearly the same mobilities (65.2, 67.4, 61.7 and 66.1) in water; from which it is deduced that, together with their associated water molecules, they make up particles in solution of nearly the same diameters (Boyle & Conway 1941, p. 42). The most hydrated is Cl, the least hydrated I. In the lyotropic series the more hydrated ions are supposed to withdraw water from colloidal systems in contact with them, the less hydrated ions to favour hydration. Whether the order of hydration of these ions has any connexion with the order of their effect in prolonging the active state of stimulated muscle is not clear.

Davson (in Davson & Danielli 1952, chapter 12) discusses the effect of the same series of anions on the penetration of K ions, and the escape of Na ions, in cats’ erythrocytes suspended in isotonic solutions of KCl, KBr, KNO₃ and KI. The
quantity of K penetrating in 1 h had the following relative values in these solutions, 100, 112, 133 and 143; but, as Davson points out, the difference of K penetration could be largely due to differences of electrical drag exerted by the anions, which themselves penetrate at rates increasing in the same order. For the escape of Na (cats' erythrocytes contain mainly Na) the differences of rate with the four anions are relatively much greater and in the opposite order, so cannot be due to the purely electrical influence of the mobility of the anion. The escape of Na was very rapid when the external medium was KCl, rapid in KBr, slower in KNO₃ and very slow in KI. According to Davson (1942) 'there is an enzyme-like mechanism in the cat erythrocyte membrane which reduces the activation energy necessary for sodium penetration': if such a mechanism exists in the excitable membrane of muscle fibres it may be influenced by the anions in its neighbourhood. These facts and considerations may be significant in view of the conclusion (Hodgkin 1951) that excitation is momentarily accompanied by a high and selective permeability to Na. If the clue is correct the rate of inrush of Na following excitation might be diminished by the presence of Br, NO₃ or I (in that order) at the excitable surface, and so the active state might somehow be prolonged.

Conway & Moore (1945) observed that, from a solution containing a large excess of K, Cl penetrates a resting muscle cell faster than Br, and Br faster than NO₃ (in the ratio 1:0·67:0·5). The order is confirmed by the difference (§ 10 above) between the half-time of escape of radio-chloride (10 min) and that of radio-iodide (90 min). During excitation K ions could come out and Na ions enter in equivalent amounts without any transfer of anions: but if the amounts were unequal then a net transfer of anions would be needed, and if Cl were replaced by I in the interspaces the inward movement of anions might be slower, as well (on the analogy of cats' erythrocytes) as that of Na. It may be interesting to test the effect on the twitch of substituting iodide for chloride (a) when part of the NaCl of Ringer's fluid is replaced by isotonic sucrose, or (b) when part of the Na is replaced by quaternary ammonium ions (Fatt & Katz 1953): but preliminary experiments on this have yielded no result.

Although the duration of the active state, whether full or partial, is clearly extended, and may be greatly extended, by the presence of these abnormal anions in the neighbourhood of the excitable surface, with the effect of increasing the twitch tension, there is no clear sign at present of any electrical after-potential which could reasonably be associated with the increased duration. The conclusion that the bromide-nitrate-iodide effect occurs primarily at the surface or membrane, not in the main bulk of the muscle fibre, was discussed in the introduction, and in several sections above. Some process in the membrane, triggered by a stimulus and then running its course to completion, must determine the time during which the active contractile state, absolute or relative, is transmitted into and maintained in the interior; and this unknown process in the membrane is extended in duration by the presence of Br, NO₃ or I ions. The link between excitation and contraction is still missing, but its neighbouring links are being found.

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