THE EFFECTS OF DIFFERENT SALTS ON THE HEAT-PRODUCTION OF MUSCLE.

By E. SERENI (Rome).

(*From the Department of Physiology, University College, London.*)

LITTLE attention has been paid to the effects, on the energy processes in muscle activity, of varying the chemical conditions of the medium. The fundamental work of A. V. Hill and others, on the differences resulting from the presence or absence of oxygen, is one important aspect of this problem: others are (*a*) the researches on the action of veratrine, from the earliest work of Fick(1) to the more recent work of Hartree and Hill(2): (*b*) the work of Weizsäcker(3) and quite recently of Gasser and Hartree(4) on the action of alcohol: and (*c*) the researches on the action of caffein both on the heat-production(5) and on the chemical exchanges(6). These latter researches, however, deal with substances which are not naturally present in, or around, the muscle. Concerning the action of such substances as are to be found in the muscle itself, or in its surrounding medium—namely, the ions—there is little information. Apart from the recent paper by Hartree and Hill(7) on the effect of a change of hydrogen ion concentration, there are merely some researches by Weizsäcker(3) and by Gasser and Hartree(4) on the action of hypotonic solutions. In a short communication, Weizsäcker(8) states that increasing the amount of potassium produces the same effects as a hypotonic solution: *i.e.* a parallel irreversible diminution both of heat-production and of tension: in his fuller report, however, no further information is given. Quite recently Embden(9) has published some researches on the action of different sodium-salts on the lactacidogen exchanges.

In an analogous field some interesting facts are to be found in a paper by Locke and Rosenheim(10). These authors, working with the surviving mammalian (rabbit) cardiac muscle, succeeded in demonstrating that, when circulating a Ringer's solution deprived of calcium and potassium (by which mechanical action is completely, or almost completely suppressed) the consumption of dextrose and the production of CO_2 still go on, though at a diminished rate. This diminution is mainly due to the lack of potassium, the omission of which very greatly decreases the dextrose consumption which is reduced but little by the removal of Ca.

In one of his earliest papers A. V. Hill (11) refers to some experiments conducted in NaCl instead of in Ringer; I shall deal later with these experiments, which, though planned from quite a different standpoint, bear a close relation to mine.

In my experiments the sartorius muscles of the frog (both *Rana temporaria* and *esculenta*) were used; in some of the experiments the animals were winter frogs from Holland and France, which had been kept in the tanks for some months; in the majority of the experiments the frogs were summer frogs and arrived in the laboratory only a few days before the experiment. Much depends upon the quality and breed of the frogs. Both males and females were used. The experiments were performed from July to September.

The general course of the experiments was as usual. A pair of sartorii were mounted on the thermopile (Fenn's type), placed in a glass-tube, and the tube sunk in a Dewar flask, filled with water, with air bubbling to keep the temperature uniform. The temperature in the Dewar flask varied from 13° C. to 16° C. in different experiments; during the course of each experiment it remained practically constant.

A Broca galvanometer was used for the measurements of the heat-production. This was surrounded by two cylindrical iron shields. The sensitivity of the galvanometer was about 1 mm. = 10^{-10} ampère on a scale at a distance of three metres. In some experiments (the earliest) the sensitivity was less. The maximum deflection was reached in about 15 secs.

The muscles were tied to a platinum ring (one of the electrodes), soldered to an iron wire, which was attached to an isometric lever of the type described by Hill (12).

The stimuli used were short tetani from a coil, the duration being determined by means of a Keith Lucas rotating drum. In each experiment, the muscle was stimulated six times for 0·1 sec. the successive stimuli decreasing in strength from supra-maximal to weak, then three times for 0·1 sec with stimuli either maximal or just sub-maximal, and finally three times for 0·5 sec. with the same stimuli. Each stimulation was separated from its neighbours by an interval of three minutes.

All the experiments were performed with the muscle immersed in an oxygenated solution, *i.e.* in Ringer, or variously altered Ringer. This has some advantages: *e.g.* the saving of time needed for the galvanometer to settle down after the solution has been withdrawn. On the other hand the solution is still allowed to act between the stimulations; thus successive readings of the same "series" are not strictly comparable one to another as to their conditions. As, however, the readings for the different stimuli have always been performed in the same order, the readings for one and the same stimulus were always taken at the same time after the change of the solutions; so that it is safe to compare, one with another, the results for the same stimulus in different solutions. The amount of solution was always the same (200 cc.).

The first series (*i.e.* the series of 12 stimulations described above) was always taken in Ringer (NaCl 0·63 p.c., KCl 0·03 p.c., $CaCl_2$ 0·025 p.c., $NaHCO_3$ 0·015 p.c.); a few preliminary stimuli were given, which seemed to make successive readings more regular. As soon as the series was finished, the solution was changed by means of a rubber tube running through the top of the muscle chamber, and 15 minutes was generally found sufficient for the galvanometer to settle down. At the end of this time, a new series of stimulations was completed; and so on, till the most altered solution was reached. When the series in this solution was finished, a return was made to the previous solution, and so on, in the reverse order, until Ringer was again reached. In these return series, however, owing to lack of time, the stimulations were limited to two of 0·1 sec. and two of 0·5 sec. with the moderately strong stimulus (see above); and often also to a single one of 0·1 sec. with the strongest stimulus. In some experiments the same stimulus, in strength and duration, was used throughout, to investigate the gradual changes occurring. At the end of the experiment the muscle was killed with chloroform and, some time after, controls performed with the same stimuli; the results of these, if any, were subtracted from the previous readings.

The following results are based mainly on the average of the three readings taken with the same moderately strong stimulus (of 0·1 sec. duration); the individual readings do not vary very much from one stimulus to another, and almost always only in a quantitative, and not in a qualitative sense. The reason why these particular readings were chosen rather than others, are: (1) the fact that they are more reliable, because it is possible to take an average value, which diminishes the effect of occasional variations; (2) the fact that they were taken after the solution to be investigated had acted for rather a long time (33 mins.), and (3), what is still more important, after some previous contractions. In every case, reference will be made to the readings obtained with other stimuli where there has been any important difference.

The effect of pure NaCl solution. In a first group of experiments the effect of NaCl solution (0·7 p.c.) with the normal amount of $NaHCO_3$ was tried. The muscle was first changed to a solution of 50 p.c. Ringer and 50 p.c. NaCl 0·7 p.c.; next to a solution of 25 p.c. Ringer and 75 p.c. NaCl 0·7 p.c.; and at the end to pure 0·7 p.c. NaCl. This means that the salts of the medium other than NaCl were reduced first to a half, afterwards to a quarter, and at the end were eliminated. In the reverse series the muscle was first returned to 25 p.c. Ringer—75 p.c. NaCl 0·7 p.c., afterwards to 50 p.c. Ringer—50 p.c. NaCl 0·7 p.c., and at the end to Ringer.

The effect of these changes on the height of the tension curves (Curve A, Figs. 1–3) is very distinct and sufficiently regular. The height

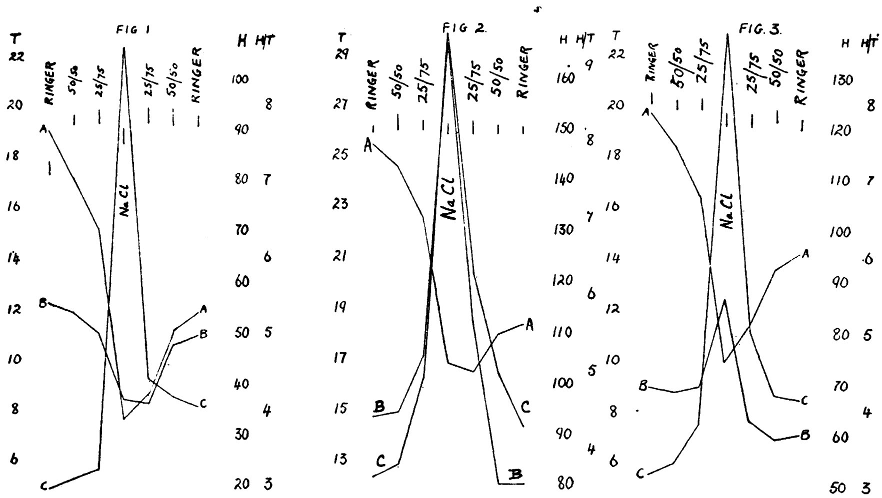


Fig. 1. Effect of NaCl solution on tension (A), heat-production (B), and heat/tension (C): mainly *Rana esculenta*. Average of several experiments.

Fig. 2. Effect of NaCl solution on tension (A), heat-production (B), and heat/tension (C): mainly *Rana temporaria*. Average of several experiments.

Fig. 3. Effect of NaCl solution on tension (A), heat-production (B), and heat/tension (C). Average of *all* experiments.

falls slowly in the solutions of 50 p.c. Ringer—50 p.c. NaCl and of 25 p.c. Ringer—75 p.c. NaCl; after that, in the pure NaCl solution, there is a sudden and more abrupt fall; and the height (in the reverse series) never attains its original value, at least within the time limits of these experiments. In some cases, when the muscle was changed back from pure NaCl solution to 25 p.c. Ringer—75 p.c. NaCl, the height of the tension curve did not rise, or only to an unimportant degree: indeed in some cases there is a further decrease. This seems to point to the fact that sensitivity to the deprival of certain salts—or perhaps better, permeability to them—is subject to individual variations in the muscles. In some of them, an equilibrium is reached within the time limit of an experiment (50 mins. for each solution); in some it is not. Another explanation might be that salts other than NaCl, when reduced to $\frac{1}{4}$ of the original concentration are unable to exert any action. Such a limit is well known for the action of NaCl on the excitability of muscle (13). Curve A, Fig. 3, represents the mean of 23 experiments, all yielding the same type of result with slight individual variations.

The change from Ringer to NaCl affects not only the height of the tension curve, but, as others have found before (*e.g.* Ringer (14), Locke (15)) also its shape. As these authors pointed out, this effect is rather variable and inconstant. Almost all these authors appear to have worked with *Rana temp.*, and it was found in the present experiments that in this frog the effect (which, in many features, recalls the action of veratrine) is much more easily produced than in *Rana esc.* This statement, however, has only a relative value: some *Rana temp.* have been found in which the effect of NaCl in giving a more or less distinct residual contracture was rather weak, while on the other hand some *Rana esc.* were used in which the effect was large. In a general sense it is possible to confirm almost completely the conclusions of previous authors. The effect is more evident with the stronger stimuli and sometimes appears only with these. An increase in the duration of the stimulus also favours the production of the effect: thus in some series contracture appears with the first strong stimulus, and then only with the last stimuli which are weaker but of longer ($\frac{1}{2}$ sec.) duration. When a stimulus has produced a very intense prolongation of the response, the following one is likely to produce less, while the next will give a greater one again. This fact, which appears very clearly and without any doubt in the experiments in which the stimulus was kept constant, gives a suitable explanation of a phenomenon which would otherwise appear to be opposed to what has been said already about the action of the strength of the stimulus: this is, that sometimes, after a very intense contracture with a strong stimulus, the next or the two next stimulations, both with weaker stimuli, fail to give any contracture, or give only a small one: but then a third and still weaker stimulation gives again a more intense contracture.

When, after the action of NaCl, the muscle is brought back to Ringer, it recovers the normal shape of the tension curve. If we change it again to NaCl, we often fail to see any change in the shape of the curve. This may be due to fatigue of the muscle: or we may accept the hypothesis of Cushing(16) who was the first to note this phenomenon, that $CaCl_2$, in these conditions, undergoes some more stable combination: and we know that it is $CaCl_2$ which antagonises the action of NaCl.

Another phenomenon is that very often the residual contracture makes its appearance not only in pure NaCl solution, but also in the solution of 25 p.c. Ringer—75 p.c. NaCl 0·7 p.c., and even of 50 p.c. Ringer—50 p.c. NaCl. Individual differences may play an important part in the genesis of the phenomenon, and lowering of the concentration, without disappearance, of the salts other than NaCl may profoundly affect the muscles.

The effect of NaCl solution on the heat-production is also distinct (Curves B, Figs. 1–3). It appears that the effect may be of two alternative types. In the majority of cases (and they generally are of *Rana esc.*) the heat-production goes slowly down in 50 p.c. Ringer—50 p.c. NaCl and in 25 p.c. Ringer—75 p.c. NaCl: and falls more rapidly in pure NaCl solution, to rise again when changed back to Ringer. Curve B, Fig. 1, shows this type of result, mainly on *Rana esc.*, averaged for a number of experiments. As opposed, however, to what happens with the tension, the heat-production nearly recovers its original value or even sometimes surpasses it on return to Ringer. In some experiments, however (generally with *Rana temp.*), the course of the heat-production is completely reversed. Curve B, Fig. 2, shows the type of result on *Rana temp.*, also averaged over a number of experiments. After falling, or rising, more or less intensely in the first two solutions, in pure NaCl solution the heat-production suddenly increases, sometimes to very high values. All these are cases in which there was a very evident residual contracture: the reverse, however, is not true; all cases with a very evident contracture (and some of them with the most intense observed) do not present the rise of the heat-production.

NaCl solution has a considerable effect also on the ratio between heat-production and tension (Curves C, Figs. 1–3). In one sense this is the most important effect, because it is directly related to the "efficiency" of the muscle in producing tension. In a general sense, and excepting some few experiments, in NaCl solution the muscle is *less* efficient. This must obviously be the case when there is an appreciable rise of the heat-production and at the same time a diminution of the tension; it is no less true also for experiments in which the heat-production is diminished. This diminution is always proportionally less than the diminution of the tension. Naturally, the rise in the ratio between heat-production and tension is larger in the former experiments than in the latter: but with few exceptions the rise is always quite evident. When the muscle is brought back to Ringer, the H/T ratio diminishes again: and, within the time limits of these experiments, it sometimes reaches or even falls below the initial value. Sometimes the maximum is not reached in NaCl, but in 25 p.c. Ringer—75 p.c. NaCl solution. This is clearly related to the similar fact in the case of the tension. A second change to NaCl solution often does not affect (or only slightly) the H/T ratio; and this fact, when compared with the parallel failure of NaCl solution in these experiments to produce a residual contracture, seems to indicate that the rise in the H/T ratio is intimately related to the production of the residual contracture. On the other hand, there are some experiments in which the H/T ratio rises without any, or only little, appearance of residual contracture: pointing to an action of NaCl on the mechanism itself which transforms the chemical energy into the mechanical one.

In order to decide upon these points it was necessary to analyse the time-course of the heat-production after the action of NaCl solution. Mr W. Hartree kindly undertook this analysis in the physiological laboratory at Cambridge: and I wish to express to him my best thanks for his kindness in allowing me to publish his results. In his experiments the conditions were slightly different from those in mine. After dissection (using Ringer) the muscle was brought directly to NaCl solution (with the normal amount of $NaHCO_3$), which was allowed to act for about one hour: then the solution was withdrawn and the records (stimuli: 0·1 and 0·2 sec.) were taken as soon as possible. Strong stimuli were used for the records (12·5 volts). The muscle was then left for three hours in Ringer, which was then removed and "normal" contractions were taken; afterwards controls were made as usual (17). The results after NaCl were corrected for the heating effect of the stimulating current, which was comparatively large (17 p.c. of the whole). The tension curve after NaCl presents a distinct residual contracture. The analysis of the heat-production shows that, under the effect of NaCl, the heat developed in the first half second is about 30 p.c. greater than in the normal muscle (although the maximum tension is 8 p.c. less); and in the subsequent prolonged relaxation an amount of heat appears equal to about 4 times the normal initial heat-production. The first fact confirms what was assumed from the experiments which presented an increase of the H/T ratio, without any contracture, *i.e.* that the NaCl probably affects the mechanism which transforms chemical energy into mechanical. The second fact shows that, in the experiments in which a contracture appears, a part, and a large part, of the heat recorded in my experiments may be associated with the maintenance of a contracture; so that the apparent decrease in the "efficiency" of setting up a tension is less than that shown by the H/T ratio, since a large part of H is due to the prolongation of the contraction.

The ratio $\frac{\text{heat rate during contracture}}{\text{(actual tension)} \times \text{(length)}}$ is comparable (0·45) with that observed by Hartree and Hill(18) at the same temperature, in a tetanus and a veratrine contraction (about 0·60): the contracture in this case is maintained with slightly more than the usual economy.

An examination of the records obtained with the other strengths of stimulus gives practically the same results. The only thing worth noting is that, with the stronger stimuli or with the longer ones, the action of NaCl solution is often more intense. The rise of the H/T ratio is larger; and the heat-production rises in many cases in which with the moderately strong stimuli there was a decrease. This fact seems to indicate that the difference noted between *Rana temp.* and *esc.* is possibly only a difference of the stimuli needed to produce the effect.

The experiments recorded above cannot give completely satisfactory evidence about the time-course of the action of NaCl solution. For this purpose some experiments were performed in which the stimulus was kept constant and maximal, and repeated every three minutes. After some records in Ringer, the muscle was changed to pure NaCl solution, and records were taken as soon as the galvanometer was sufficiently settled (generally between 10 and 15 minutes). The results of these experiments were interesting. The tension went down with the well-known "irregularity" of muscles in NaCl solution. The heat-production in some cases goes down from the beginning, but very often it increases a certain amount at first, and then, if the experiment be continued for a sufficient time, diminishes again. Also in the course of the heat-production there are some "irregularities," probably due to the irregularity of response mentioned above (p. 5) which commonly follow strong contracture.

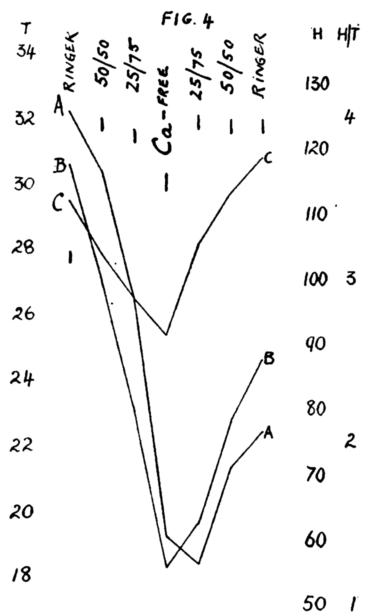
The heat/tension ratio rises steadily: and, strangely enough, its rise is not greatly affected by the irregularities of the tension and the heat-production. This is another argument for the hypothesis that the rise is due not only to the residual contracture, but also to some more intimate action of NaCl. In almost every case, in the first or in the two first records after the change to NaCl solution, the H/T ratio, instead of increasing, decreases, sometimes considerably. It is true that these first readings are not always reliable, because the galvanometer is not quite steady; but the differences are sometimes of such an order, that, if not in a quantitative, at least in a qualitative sense, it can be safely assumed that in the very first moment of the change (this means, probably, the moment when the ions are passing the superficial layer) the H/T ratio diminishes instead of rising.

Altogether NaCl seems to affect first the mechanical response of the muscle, and only later the chemical processes. When, moreover, the muscle is brought back from pure NaCl to mixtures of NaCl solution and Ringer, then on giving two successive equal stimuli the response to the second stimulus gives more tension with the same or even less heat-production than does the response to the first stimulus. Apparently the return to more normal conditions of salt-content first restores the chemical processes, and only after a further delay can these be transformed into mechanical effects.

Professor Langley was kind enough to point out to me an effect of NaCl solution which might perhaps complicate the results of these experiments. Not only may spontaneous twitchings occur in muscles suddenly subjected to NaCl, but sometimes even when these are absent similar twitches may appear, following treatment with NaCl, immediately after a muscle has been stimulated. Obviously such fibrillar twitchings would lead to heat-production which would be summed with that resulting from the contraction and would lead to a greater apparent total heat production. Since returning to Rome, therefore, I have made about twenty-five experiments upon *Rana esc.* under conditions as similar as possible to those previously made in London. The muscles were observed by eye and through a lens. In some experiments the muscles were mounted on a thermopile in case contact with shellac might affect the result. It was impossible to observe any spontaneous effect in the muscle connected with a lever: in muscles not so connected, but lying freely, there was often, but not always, a short period of spontaneous twitches immediately after the change from 25 p.c. Ringer solution, 75 p.c. NaCl, to pure NaCl. These twitches, however, ceased very soon, that is, in about five minutes, and always before the beginning of the series of stimuli, which began only after 15 minutes from the change of the solution; nor did any spontaneous fibrillar twitches reappear after the stimuli, except in two experiments in which three or four twitches appeared after the strongest stimulation. Under the conditions of my experiments, therefore, it would seem that spontaneous twitchings occur only as an exception, and that we may assume the increase in H and in H/T to be due not to them but to some more fundamental alteration in the processes of activity.

The effect of Ringer deprived of $CaCl_2$. In these experiments the altered solution was represented by NaCl 0·65 p.c., KCl 0·03 p.c., $NaHCO_3$ 0·015 p.c.; this is the usual Ringer deprived of $CaCl_2$. In this case also the change was a gradual one: first to a solution of 50 p.c. Ringer—50 p.c. Ca-free Ringer, then to a solution of 25 p.c. Ringer—75 p.c. Ca-free Ringer, and then to the Ca-free Ringer.

The effect on the tension-height is large (Curve A, Fig. 4). The



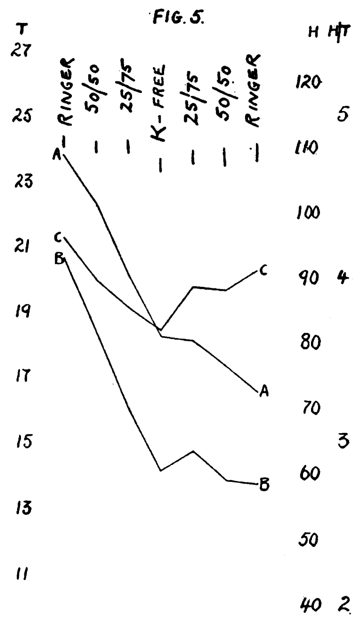


Fig. 4. Effect of Ringer's solution deprived of Ca on tension (A), heat-production (B), and heat/tension (C). Average of 12 experiments.

Fig. 5. Effect of Ringer's solution deprived of K on tension (A), heat-production (B), and heat/tension (C). Average of 11 experiments.

tension diminishes rapidly, much more than in NaCl solution; when the muscle is brought back to 25 p.c. Ringer—75 p.c. Ca-free Ringer it rises only very little, and sometimes even shows a further descent; the rise is much more evident after return to 50 p.c. Ringer—50 p.c. Ca-free Ringer and to 100 p.c. Ringer; but, within the time-limit of these experiments, the initial value is rarely attained. The shape of the tension-curve is generally unaffected; but in some experiments (*Rana temp.* only) there is, though rarely, the appearance of a residual contracture. Previous authors are of different opinions about the action of KCl on the residual contracture produced by pure NaCl. According to Ringer(13, 14) KCl increases the effect; according to Fahr(19) and Mines(20) it abolishes it.

The heat-production also is much impaired by the deprival of $CaCl_2$ (Curve B, Fig. 5). It quickly decreases in the solutions with a diminished amount of $CaCl_2$, and rises again as soon as $CaCl_2$ is increased: indeed it sometimes reaches the initial value again.

The ratio between heat-production and tension is diminished (Curve C, Fig. 4). The diminution and the increase which follow when $CaCl_2$ is respectively reduced and restored proceed almost at the same rate, and the final value not only reaches but often exceeds the initial value. This means that in this case also, when coming back to normal conditions of salt-content, the recovery is at first stronger and quicker in the chemical processes.

The decrease of the H/T ratio occurred in all but two experiments (*Rana temp.*) and in these there was a prolonged relaxation. In one of them there was practically no change: in the other there was quite a notable rise. A second transference to Ca-free Ringer often leaves the H/T ratio unaffected; in this case also there seems to have been some irreversible change.

Some experiments were made with a regular series of constant stimuli. The tension comes down, more rapidly than in the NaCl experiments; the heat-production comes down also, excepting when there is a residual contracture, which is accompanied by a rise, sometimes large. In these cases there is also a rise of the H/T ratio; in the other cases there is a progressive decrease of the ratio. It is interesting to note that in this case also, in the first records after the change from Ringer to Ca-free Ringer, the change of the H/T ratio is very often opposite to the change we observe later—there is a rise instead of a fall.

The effect of K-free Ringer. The solution used in these experiments contained NaCl 0·66 p.c., $CaCl_2$ 0·025 p.c., $NaHCO_3$ 0·015 p.c. The change from Ringer to this solution was as usual gradual; the intermediate solutions were like those in the former experiments. The effect on the tension height (Curve A, Fig. 5) is to diminish it, but the diminution is less abrupt than in the case of NaCl solution or Ca-free Ringer. The fall is much more gradual, without any sudden change, and, what is still more important, when the muscle is brought back to Ringer, the recovery is quite small, or often absent altogether, and the tension still goes down. The deprival of KCl, if it affects the muscle much more slowly and apparently less intensely, does it in an irreversible manner, at least within the time limits of these experiments. The deprival of KCl does not affect in any way the shape of the tension-curves.

The heat-production also goes down (Curve B, Fig. 8). As in the case of the tension-height, the decrease is much more gradual than in NaCl solution or Ca-free Ringer, and the recovery is small: indeed in some experiments the heat-production decreases steadily until the end, and the lowest value is attained in the final immersion in Ringer.

With these much less distinct alterations in the tension and in the heat-production, it was inevitable that the changes in the H/T ratio should also be less obvious (Curve C, Fig. 5). On the whole there is a slight tendency for the H/T ratio to decrease, as an effect of the K-free Ringer. After the minimum has been reached in completely K-free Ringer, the course of the H/T ratio varies greatly. In some cases there is a regular rise; in some first a rise, then a fall. Sometimes there is no rise at all, and the ratio goes on falling. On the average there is a rise. No satisfactory explanation can be offered of these differences.

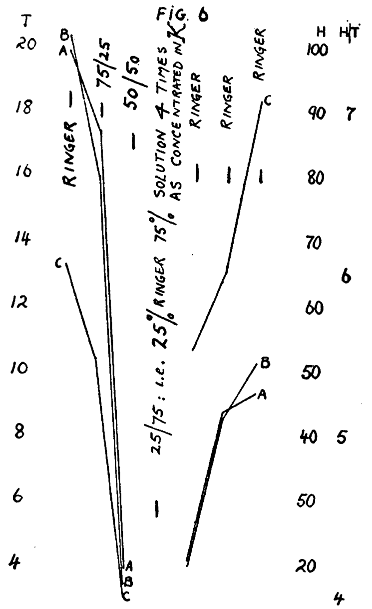
An interesting fact is that, as opposed to what happens in the experiments with NaCl solution and Ca-free Ringer, the second contraction with the same stimulation, in the return-series, gives almost invariably less heat-production and less tension than the first one.

The experiments in which the stimulation was kept constant throughout gave practically the same results. Heat-production and tension ran down rather slowly, and there was also a distinct though small decrease of the ratio between them. An increase of the ratio in the first contractions after the change of the solution is often evident, and it lasts longer than in the cases previously described. Sometimes this initial increase is rather large, and the later decrease only brings the ratio back to its initial value, or a little below.

In some experiments the action was tested of K-free, followed by Ca-free Ringer. The decrease of both heat-production and tension is steady and becomes more rapid when the muscle is changed from the K-free to the Ca-free solution. Also the ratio between heat-production and tension, which diminishes only a little in the K-free Ringer, shows a further decrease in the Ca-free Ringer.

The action of Ringer with an increased amount of K. The stock solutions for an increased K concentration were the usual Ringer and a modified Ringer containing four times the normal amount of KCl and (for isotonic reasons) rather less NaCl (0·55 p.c.). These solutions were mixed in the same proportions as in the other experiments. The muscles became inexcitable in 25 p.c. Ringer and 75 p.c. modified Ringer and sometimes in 50 p.c. Ringer + 50 p.c. modified Ringer.

The effect on the tension is striking (Curve A, Fig. 6). The tension-height falls very quickly. It reaches zero in the 25 p.c.—75 p.c. solution,



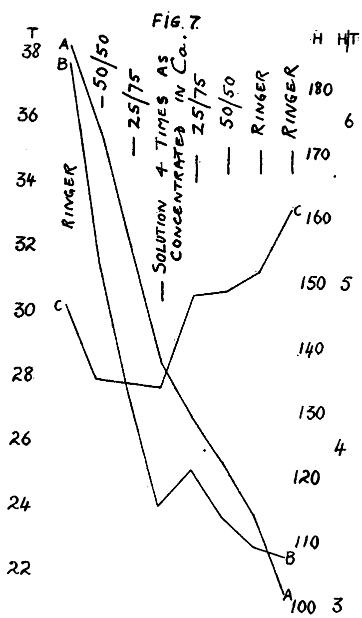


Fig. 6. Effect of an increased proportion of K on tension (A), heat-production (B), and heat/tension (C). Average of 9 experiments.

Fig. 7. Effect of an increased proportion of Ca on tension (A), heat-production (B), and heat/tension (C). Average of 10 experiments.

or, sometimes, even in the 50 p.c.—50 p.c. solution; and it remains at this point when the muscle is brought back to the less concentrated solutions. If the immersion in the 75 p.c. Ringer—25 p.c. modified Ringer solution be prolonged, there is a slight rise in the tension; when the muscle is brought back to the Ringer solution the rise is at first very rapid: after some time it becomes less. A further rise can be observed for a long time after the muscle has been changed back to Ringer. Sometimes, after a longer immersion in Ringer, the tension decreases a little as if, in addition to paralysing the muscle, the excess of KCl had a poisoning effect.

The effect on the heat-production is similar to that on the tension (Curve B, Fig. 6). Here also the zero line is reached in the 50 p.c.—50 p.c. or in the 25 p.c.—75 p.c. solution; and there is only a small amount of recovery after a longer immersion in 75 p.c. Ringer—25 p.c. modified Ringer. The heat-production, just as the tension, rises again only when the muscle is brought back to Ringer. It is interesting to note that the rise of the heat-production is quicker than that of the tension. In this case also it seems as if the chemical processes reappear first, and only afterwards the possibility of their transformation into mechanical processes. The heat-production, as well as the tension, after a longer immersion in Ringer, sometimes tends to decrease slightly.

The changes in the H/T ratio are very large (Curve C, Fig. 6). The effect is the same as in the case of the Ca-free Ringer, that is a decrease, but larger. The ratio decreases rapidly in the 75 p.c.—25 p.c. and 50 p.c.—50 p.c. solutions, and rises again, but less rapidly, when the muscles are brought back to Ringer. The recovery of both tension-height and heat-production which takes place in Ringer continues with a steady increase of the ratio.

It is interesting to note how the recovery proceeds in Ringer. By comparing the two records with the same stimulus in the same series and in different series, it appears that the stimulation itself greatly quickens the recovery process. The increase in the tension and in the heat-production between the first and the second record of every series (that is, in three minutes) is often only slightly less than the changes from the second record to the first of the next series (that is, in 21 minutes). This effect is more evident for the tension than for the heat-production.

In some experiments the muscle was changed directly from Ringer to 25 p.c.—75 p.c. or 50 p.c.—50 p.c., or 75 p.c.—25 p.c. solution and a regular series of maximal stimuli applied. As soon as the muscle had become inexcitable for the stimulus given, it was changed back to Ringer. Readings were taken as soon as possible. Inexcitability is reached after about 30 minutes in the 25 p.c. Ringer—75 p.c. modified Ringer solution, only after about 60 minutes in the 50 p.c.—50 p.c. solution. The heat/tension ratio falls from the first contraction in the 25 p.c.—75 p.c. solution; in the 50 p.c.—50 p.c. solution it remains almost constant, with a tendency to decrease, for some contractions; then it begins to fall more rapidly. As soon as the tension and the heat-production rise after restoration to the normal Ringer's solution, the H/T ratio begins to rise steadily, till it reaches, and sometimes surpasses, its initial value. At the end there is a tendency of the H/T ratio to fall a little.

The recovery takes more time than the paralysis, and the degree of recovery depends not so much on the amount of KCl as on the time this salt had been allowed to act. For instance, there has been a more complete recovery after complete inexcitability produced by 25 p.c.—75 p.c. solution than after complete inexcitability produced by 50 p.c.—50 p.c. solution: presumably because the first, having a more rapid action, was allowed to act on the muscle for a shorter time. After the muscle has recovered in Ringer, a second change to the modified Ringer reproduces the same effect.

It is also interesting to observe that in no case was there any sign of residual contracture. The presence of a normal amount of $CaCl_2$ was apparently sufficient to check the tendency of NaCl to give the contracture, which KCl alone cannot always antagonise, as shown in the experiments with Ca-free Ringer.

The action of Ringer with an increased amount of $CaCl_2$. The modified solution contained four times the normal amount of $CaCl_2$. Solutions with different increased amounts of Ca were prepared and used in the way described above.

The tension-height continually diminishes from the beginning to the end of the experiment (Curve A, Fig. 7). The decrease is generally more rapid in the first changes until the completely modified Ringer is reached; then it becomes slower, but becomes more rapid again during the prolonged final immersion in Ringer. In one case only was there a little recovery: in this, after returning to Ringer and after a first series of records had been obtained, the muscle was left unstimulated for more than two hours, before the observations in question.

The heat-production in a general sense presents the same decrease from the beginning to the end of the experiment (Curve B, Fig. 7); this decrease is less regular than was that of the tension, and there are some variations during its course. So, for instance, there is often a little rise in the heat-production when the muscle is brought back from the completely modified solution to the 25 p.c.—75 p.c. solution, as if there were the beginning of a recovery-process. Very soon after this rise the decrease begins again, and with some little irregularities it continues to the end. An interesting feature is that, as in the case of KCl-free Ringer, but much more obviously, the second contraction in the return-series with the same stimulus almost invariably gives less tension and much less heat than the first one. This effect is so distinct that, when at the end of each of the return-series (this means, after four moderately strong stimuli, two of $\frac{1}{10}$ sec. and two of $\frac{1}{2}$ sec.) the muscle was stimulated with the strongest stimulus ($\frac{1}{10}$ sec. duration), the record for both tension and heat-production was often less than in the two $\frac{1}{10}$ sec. stimulations with the moderately strong stimulus. In the same case in which, after a longer immersion in Ringer, without any stimulation, there was a rise in the tension, there was a larger increase in the heat-production. The rise in the heat-production in the first stages of the return-series can be perhaps assumed to indicate that $CaCl_2$ has a double action, first an inhibition, then a poisonous effect.

The H/T ratio presents an initial decrease (Curve C, Fig. 7), followed by a rise, which is rather irregular in its course, and reaches values beyond those at the beginning. The decrease sometimes appears only in the first change, sometimes there is a further decrease in the 25 p.c.—75 p.c. solution and in the completely modified Ringer. In no case, however, is there a further decrease in the next changes: the rise is generally more rapid than the descent, and it reaches high values, which means a low "efficiency" of the muscle.

Discussion.

The experiments described above show, that by changing the saline content of the medium it is possible to affect profoundly not only the tension, but also the heat-production of the muscle. That the changes in this latter are not merely due to the changes in the former is proved by the variations in the H/T ratio; these show that the variations of the saline content affect the "efficiency" of the muscle in developing tension, which is apparently not at its highest point with the "normal" proportions of the different salts.

Before we attempt to explain these phenomena, it is advisable to ascertain if there is any possibility of attributing them to purely technical factors. It was first pointed out by Weizsäcker (3) that, in such experiments as these, it is possible that the heat-production recorded is mainly due to the superficial layer of the muscle, *i.e.* to the layer on which any alteration of the medium is allowed first to act. It is possible, therefore, that the heat-production may appear to be altered at a moment when the tension (which is the effect of all the fibres) is still nearly unchanged. The same considerations might also give a simple explanation of the fact recorded above, that the heat-production, in the return-series, appears to be restored before the tension. It is improbable that this possibility is of any great importance when working with such a slow-moving galvanometer as that used: the temperature of a thin sartorius muscle should be fairly well equalised in 15 secs. If, moreover, the effects recorded were only, or mainly, due to this simple fact it would be impossible to explain why there is (after restoration to the normal solution) a gradual rise of the H/T ratio after the former decrease. As the superficial fibres are restored first, so there must be a moment when only these fibres are restored, and both heat-production and tension are due only to them; at this very moment the H/T ratio must be at its maximum, to descend afterwards, when the more remote fibres are restored, the tension of which is recorded but not the heat-production. This is not so, and can be safely assumed that, whatever its importance may be, this possibility cannot give a full explanation of the whole of the results.

The experiments of A. V. Hill(10) on the effect of NaCl were made with the purpose of showing that "tone" produces less heat than "contraction." By giving a stimulating current of three shocks per second, he found that the "tonus" set up in NaCl solution was greater than in Ringer, and the heat-production less. These results are not comparable with mine. In the same paper, however, Hill noted that sometimes (and more often with weaker stimuli in NaCl solution) the muscle shows a prolonged heat-production, accompanied by a contracture. The effect is weakened when many stimulations are given; but it reappears after a long rest. All these facts are in perfect accord with the results of my experiments with NaCl solution.

Summing up the results of my experiments, it has been shown that the different ions not only regulate the processes of excitability of muscle, but also modify the utilisation of the energy which the stimulation sets free. Varying the proportions of different salts influences the working conditions of the muscles. Considering the results of the variations, we see that all the changes diminish the absolute value of the response of the muscle to a constant stimulus, both as regards the tension and heat-production (excepting, as regards the last, some frogs (usually *temporaria*) in the NaCl experiments).

On the other hand, important variations are observed in the H/T ratio. The "efficiency" of the muscle is decreased (*i.e.* the H/T ratio is increased) by pure NaCl: but the "efficiency" is increased by excess of KCl (Ringer without $CaCl_2$ or with increased KCl) and to a less extent by excess of $CaCl_2$ (Ringer without KCl or with increased $CaCl_2$).

It is strange that the effects of excess of KCl and of excess of $CaCl_2$ are both in the direction of a decrease of the H/T ratio. It is true that the effect is more considerable for KCl than for $CaCl_2$, but it is often possible to demonstrate a distinct effect also for $CaCl_2$. Therefore as regards the action on the energy processes in muscle there is no antagonism between KCl and $CaCl_2$, but rather a synergism in antagonising the action of NaCl.

This makes a new element in our knowledge of the balance of salts, and the researches of Locke and Rosenheim demonstrate that its value is perhaps not confined to the striated muscle. These workers, however, as has already been mentioned, found that deprival of calcium almost abolished the mechanical response of the heart, while leaving the dextrose consumption almost unaltered. My experiments do not show any such dissociation between the action of lack of Ca on chemical and mechanical responses. Lack of Ca reduces both the chemical and mechanical processes of striated muscle and actually reduces the former more than the latter.

It is rather difficult as yet to make any suggestion as to the intimate mechanism of these effects. A. V. Hill (21) has recently demonstrated that the effect of lactic acid during muscular activity cannot be due to a charge of surface-tension: rather it must be assumed that it acts by producing some colloidal change. It is perhaps at this point that the salt-action might be supposed to intervene *e.g.* by changing the initial colloidal state of the muscle fibre.

The last point to discuss is why KCl and $CaCl_2$, which both increase the "efficiency" of the muscle although diminishing its response, are less active when working together. The normal amount of salts is that by which the different salt antagonisms have a sort of mean optimum. This may not be the optimum for each of them, as in our case. On the other hand, as the mechanisms of KCl and $CaCl_2$ in increasing the efficiency of the muscle are probably different, we can assume that each of them partly antagonises the effect of the other.

Summary.

1. If frog's muscles be transferred from Ringer to 0·7 p.c. NaCl solution, the height T of the tension curve diminishes and a residual contracture often appears. The heat-production H sometimes (mainly in *R. esculenta*) decreases, sometimes (mainly in *R. temporaria*) increases. The H/T ratio is always increased.

2. If the $CaCl_2$ content of Ringer is lowered or the KCl content increased, there is a rapid reversible decrease both of tension-height and of heat-production, accompanied by a reversible decrease of the H/T ratio. The effect is larger and more rapid in the second solution than in the first.

3. If the KCl content of Ringer is lowered, there is a small irre-

versible decrease of tension-height and heat-production, accompanied by a small decrease of the H/T ratio. If the $CaCl_2$ content of Ringer is increased, there is a more intense and irreversible decrease of tension-height and heat-production; the H/T ratio first decreases, and then increases beyond the initial value.

4. These results show that the ions affect not only the excitability of the muscle but also the energy processes during its activity.

In conclusion I should like to take this opportunity of expressing my gratitude to Prof. A. V. Hill for his invaluable and never-failing advice during the course of this research, to Mr W. Hartree for his kindness in undertaking the experiments quoted above, and to Mr J. L. Parkinson, who instructed me in the technique of these experiments.

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