THE EFFECT OF POTASSIUM ON THE ACID METABOLISM
OF SURVIVING SKELETAL AND CARDIAC
MUSCLES OF THE FROG.

BY FRED R. GRIFFITH, Jr.

(From the Laboratories of Physiology of the University of Buffalo, Buffalo, and
Harvard Medical School, Boston.)

(Received for publication, May 5, 1924.)

Studies have been made of the effect of potassium on the oxygen
consumption of developing eggs (1) and the carbon dioxide production
of bacteria (2), but, apparently, its pronounced effect on the con-
tractile process in muscle (3) has not been correlated with changes
of metabolism of any kind.

This paper describes the effect of potassium on the acid production
of surviving skeletal and cardiac muscles of the frog.

I.

Skeletal Muscle.

A. Total Acid Production.—In these experiments, sartorius muscles
were used and their rate of acid production was measured by the
indicator method of Haas (4).

Immediately after pithing a frog, its sartorius muscles were dissected out and a
ligature tied around the tendon of insertion of each. This was done in order to
have something by which the muscles could thereafter be handled without danger
of stimulating them to contract. If a muscle was not to be used immediately it
was left in a bath of several cc. of Ringer solution in a large watch-glass until
needed.

In all experiments the "normal" rate of acid production was determined for
each muscle in Ringer solution before subjecting it to the action of potassium.
The Ringer solution used in this work had the following composition: sodium
chloride, 0.763 per cent; potassium chloride, 0.014 per cent; calcium chloride,
0.012 per cent. The potassium effect was obtained with an isotonic, 1.08 per
cent solution of potassium chloride. The pH values of the Ringer and potassium
chloride solutions, as thus made up in distilled water, were 6.8 and 6.6, respec-
tively, using brom-thymol blue as indicator.
In preparation for an experiment, a sample of each of these solutions was made alkaline with sodium bicarbonate. For this purpose, an approximately 0.5 M sodium bicarbonate solution was used in the proportion of 3 drops for each 100 cc. of Ringer or potassium chloride. Phenolsulphalein (5 drops of a 0.02 per cent aqueous solution per 10 cc.) was also added to each sample. The Ringer and potassium chloride solutions were then brought to the same hydrogen ion concentration (always pH 7.5±) by titration with 0.002 N hydrochloric acid. The samples, as thus prepared, were then allowed to stand in loosely stoppered Pyrex flasks for at least a day so as to permit them to come into equilibrium with the carbon dioxide tension of the laboratory air. Just before an experiment, any inequality of hydrogen ion concentration (as judged by the tint of the indicator in the solutions) was further equalized by titrating again with 0.002 N hydrochloric acid. The identical pH values and buffer capacities of the solutions thus prepared is evident from the fact that they maintained identical tints when exposed to laboratory air for several days; or when equilibrated with alveolar air (using methyl red as indicator); or when treated with equal amounts of 0.002 N hydrochloric acid. This last test was made as follows: 2.5 cc. of each solution were placed in small test-tubes, of the kind used in these experiments, and to each was added one drop of the acid; the test-tubes were then stoppered with paraffined corks and shaken. The same change of pH in the solutions of both tubes showed that they were identical as regarded their original hydrogen ion concentrations and buffer values.

The rate of acid production of the muscles and the effect of potassium on it were then determined as follows: 2.5 cc. of the adjusted Ringer solution were measured by a pipette into a small, hard glass test-tube of 3 cc. capacity; a muscle was lifted by its ligature from the bath of Ringer in which it was being kept, dried as much as possible of all adhering moisture by contact with filter paper, and dropped in the 2.5 cc. of Ringer solution in the test-tube; the tube was quickly stoppered with a paraffined cork and the time required to change the pH of the solution between standard end-points of pH 7.381 and 7.168 determined with a stop-watch. During the determination the tube was continually tipped from end to end, holding it always by the stopper so as not to affect the temperature of its contents. At the end of a determination the contents of the tube, including the muscle, were poured into a watch-glass; the muscle was quickly picked up with forceps (by means of its tendon ligature), dried as before, and immediately placed in another 2.5 cc. of the Ringer solution and another determination made. This was repeated until the rate of acid production in Ringer solution was established. The muscle was then placed, in the same way and without any delay, in 2.5 cc. samples of the potassium chloride solution (previously adjusted as to pH and buffer values as described above) and determinations of the rate of acid production made under this condition. The transference of the muscle from one tube to another

1 Phosphate standards prepared as described by Clark (Clark, W. M., The determination of hydrogen ions, Baltimore, 1920, 81) were used in this work.
required only about 30 seconds so the measurements of the rate of acid production made in this way give a fairly continuous picture of the variations in metabolic rate.

Twenty-six experiments, of which the two shown in Fig. 1 are typical, were all concordant in showing that the acid production of surviving sartorius muscles is increased when the muscles are transferred from Ringer to isotonic potassium chloride solution. The average, maximum increase for the entire series was about 200 per cent. This figure should be accepted, however, merely as indicating the qualitative nature of the effect because certain precautions that would have been necessary in order to make the experiments absolutely comparable quantitatively were not taken. For example, they were carried out at various room temperatures, which, though sufficiently constant during any one experiment, varied widely (14°-27°C.) on different days; also, in all but two of the experiments (those given in Fig. 1), no account was taken of the weight of the muscles so that the results could not be reduced to the common denominator of acid production per gm. of tissue; and finally, the potassium effect was tested at various times—15 minutes to 3½ hours—during survival. Yet the results are valuable just because they show that even under such diverse conditions potassium invariably increases the rate of survival acid production.

In all cases this increase attains its maximum promptly and is followed by a return to "normal." If the last determination in Ringer solution is taken as the "normal" rate it will be seen from Fig. 1 that the increased rate persists for 35 to 40 minutes. This, however, is probably too short a time; for it is not improbable that the course of total acid production (which may consist chiefly of carbon dioxide) follows approximately the course of survival carbon dioxide production; and this latter we know from the work of Fletcher (6) (see also Fig. 2) falls off rapidly during the first 2 hours of survival and then more slowly until death. The first part of the curve of Fig. 1 does indicate such a similarity between total acid production and carbon dioxide production during the 1st hour of survival and it is not unreasonable to suppose that the relationship would hold for the remainder of the survival period. In that case the curve representing the "normal" course of survival acid production would
intersect the curve of Fig. 1 sometime between 60 and 120 minutes, i.e. the increased rate of acid production caused by potassium persists for such a time.

**Fig. 1.** The curve represents the average of two experiments in which sartorius muscles of the frog were treated with 1.08 per cent KCl near the end of the 1st hour of survival. Abscissa, time in minutes; ordinates, rate of acid production expressed as per cent of the last determination in Ringer (100 per cent). Temperature throughout both experiments 19°-20°C. The complete data in regard to each experiment follow: in each case the figure in parenthesis is the time in seconds required for 1 gm. of muscle to produce an increase in acidity from pH 7.381 to 7.168; the preceding figure is the number of minutes before or after immersion of the muscles in KCl (i.e. the abscissae); the figure following the parenthesis is the rate of acid production calculated as a percentage of the final rate in Ringer solution (i.e. the ordinates).

**Experiment 25.**—Weight of muscle, 0.103 gm.; 34 (21.3) 298; 27 (33.2) 192; 19 (56.7) 111; 6 (63.7) 100; KCl; 3 (21.5) 296; 9 (20) 318; 14 (25.8) 246; 22 (34) 187; 32 (50) 127; 45 (69.2) 92; 60 (65.7) 98; 130 (123.6) 51; 310 (329.6) 19.

**Experiment 26.**—Weight of muscle, 0.083 gm.; 38 (14.9) 315; 33 (18) 216; 25 (28.15) 165; 15 (36.8) 127; 6 (46.8) 100; KCl; 3 (13.7) 342; 8 (15.8) 296; 14 (25.1) 187; 25 (38) 123; 38 (52.2) 90; 62 (123.8) 38; 128 (201.5) 23.
These results in regard to the effect of potassium on total acid production receive confirmation and are to some degree explained by the experiments that follow.

B. Carbon Dioxide Production.—The following experiments afford evidence that the increased acid production, recorded in the previous section, may consist entirely or at least in large part of carbon dioxide. These experiments were performed with a modified Osterhout apparatus which has previously been described (5) and by which carbon dioxide can be distinguished from other acids being produced at the same time. The technique was identical with that used in the work just referred to and therefore need be only briefly recapitulated here.

After pithing or decapitating a frog it was skinned and both hind legs retained after being cut off from the trunk. The leg muscles were cut loose at their insertions and separated from each other so as to permit as free access of the solution to all of them as possible. They were then immersed in 25 cc. of Ringer solution (adjusted for pH and buffer effect as described in the previous section) and the rate of carbon dioxide production measured, i.e. the time required to produce a change in acidity of the indicator solution (phenolsulfophthalein) from pH 7.381 to 7.168 was determined until the “normal” rate was established. The Ringer solution was then drained off and the muscles were treated with 25 cc. of 1.08 per cent potassium chloride solution, which, as described in the previous section, had been adjusted to the same hydrogen ion concentration and buffer capacity as the Ringer solution for which it was being substituted.

Ten experiments of this kind were performed at various room temperatures and at different times during survival, and all agreed in demonstrating an increased carbon dioxide production as a result of the potassium treatment. The data of four experiments are expressed graphically in Fig. 2.

The rate of carbon dioxide production by surviving frog muscles is known (6) to fall off rapidly during the first 2 hours to a fairly steady rate which is maintained with only a very gradual decrease thereafter. In all but three of the experiments of this series the potassium effect was tested at different times during this period of fairly uniform carbon dioxide production. Fig. 2, Curve II represents the only experiment of this type in which the normal course of carbon dioxide production was followed carefully from the beginning of survival; and it shows the effect of treating the muscles with potassium during the 5th hour of survival. In the three experiments referred
to above the muscles were treated with potassium at the end of the 1st hour of survival, i.e. while the "normal" rate of carbon dioxide production was rapidly declining; on account of the similar procedure in each case the results have been averaged and expressed as a single curve (Curve I) in Fig. 2. A comparison of these two

![Fig. 2. The effect of potassium on the carbon dioxide production of the leg muscles of the frog.](image)

Curve I represents the average of three experiments in which the muscles were immersed in isotonic potassium chloride solution at the end of the 1st hour of survival.

Curve II shows, in the first part of the curve, the normal rate of survival carbon dioxide production during the first 4 hours of survival; the latter part of the curve represents the effect of immersing the muscles in isotonic potassium chloride solution at the end of 4 hours and 10 minutes of survival. The data are from a single experiment.

In both cases the reciprocals of the actual times in seconds required to produce an increase in acidity from pH 7.381 to 7.168 are taken as ordinates.

curves fails to reveal any significant difference in the potassium effect, especially if it is borne in mind that the "normal" rate of carbon dioxide production during the first 4 hours of survival would, in every case, follow approximately the same course as the first part of Curve II. That is, in both cases the increased rate of carbon dioxide production is similar in magnitude and is maintained for approximately
the same length of time above what would have been the "normal" rate in Ringer solution. The significance of this similarity in the two cases will appear in what follows.

The evidence of the two preceding series of experiments shows that under the influence of potassium, in the concentration used in this work, the total acid production and the carbon dioxide production of surviving skeletal muscles of the frog are increased. The magnitude and duration of this increase in both cases correspond sufficiently closely to justify the inference that carbon dioxide may be the principal if not the only acid involved in the reaction. Granting this, it is probable that the figures in regard to carbon dioxide production do not give as true a picture of the real magnitude and time relations of the potassium effect as the data in regard to the total acid production. For the latter were obtained with single sartorius muscles, with which the rate of diffusion of the potassium ion would not be so significant a factor. The data in regard to carbon dioxide production, on the other hand, represent, at any given time, the average effect on all of the leg muscles—both large and small—so that differences in the rate of penetration of the potassium ion might be important. The justification of the method used in the measurement of the rate of carbon dioxide production lay in the necessity of using a relatively large mass of tissue in order that the carbon dioxide production would be great enough for rapid determination with the apparatus used.

The question of most interest in connection with these results is whether the increased acid production is an indirect effect of the contraction which the potassium also causes, or whether potassium acts more directly on the fundamental metabolic processes in the muscle. Experiments are being planned to test this point. But in this connection it is worth while calling attention to Fletcher's conclusions in regard to the effect of electrically stimulated contractions on the carbon dioxide production of surviving muscles.

He says, "The survival discharge of CO₂ from an excised muscle is increased during periods of contraction in the presence of abundant oxygen, the increase being proportionate, or roughly so, to the number and degree of the contractions. In agreement with observations already published, this additional yield of CO₂ accompanying contraction is absent or incomplete in the case of a muscle made to
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contract in air or in nitrogen. The preceding observations here referred to, were to the effect "that, under suitable conditions, the occurrence of active contractions in an excised muscle is not accompanied by an increase in the rate at which carbon dioxide is yielded by the muscle. The conditions are fulfilled when the stimulation causing contraction is not so severe as to produce marked fatigue and permanent shortening, and when it is applied during an early survival period. Stimulation during a late period may cause a rise in the rate of carbon dioxide discharge, even though the contractions evolved are much feebler than previous ones not accompanied by a rise."

The bearing of these observations on the results recorded in this paper is sufficiently obvious when the following facts are kept in mind. In the first place, the increased carbon dioxide production caused by potassium seems to be the same in both early and late periods of survival (see Fig. 2); secondly, in this work the muscles were supplied only with air and probably insufficiently with that, because they were immersed throughout the experiments in solutions (Ringer and KCl) that no precaution was taken to keep well aerated; and finally, as is well known, the potassium contraction is not a permanent shortening but is very brief; tests carried out under the conditions of these experiments showed that it had a duration of only 2 to 3 minutes with sartorius muscles. No attempt is being made to suggest that the acid production and contraction are not related; but until further work can be done, these considerations, together with those to be mentioned in the following section, indicate that potassium does affect, directly, the tissue metabolism.

II.
The Effect of Potassium on the Total Acid Production of Surviving Cardiac Muscle.

Whereas potassium causes a brief contraction of skeletal muscle, its immediate effect on cardiac muscle is well known to be exactly the opposite in nature. A comparison of its effect on the metabolism of both tissues is, therefore, of interest.

The acid production of cardiac muscle was determined in the same manner and using the same precautions as described in the

Fletcher (7), p. 497.
Fletcher (6), p. 79.
first section of this paper regarding the acid production of skeletal muscle.

After killing a frog, the heart, including the sinus, was quickly removed and placed in a bath of Ringer solution; the auricles and ventricle were slit open in order that no solution could be carried over from one test-tube to another, inside the cavities. Between each determination the heart was freed as much as possible from all adhereing solution by contact with filter paper. The whole heart was used, on account of its small size, rather than ventricular or auricular tissue alone, in order that the acid production would be great enough for rapid determination with the method used.

In all but two of the experiments nothing was done to prevent the beating of the heart during the preliminary determinations of the rate of acid production in Ringer; in fact, as was mentioned above, the sinus was left intact to facilitate this, in order to have something by which to judge the functional integrity of the tissue when treated with potassium. This might be thought, however, to invalidate the comparison of the effect of potassium on this tissue with the effect produced on inactive skeletal muscle. So in two of the experiments the hearts were rendered quiescent at the beginning by cutting off the sinus and it was observed that there was no resumption of beating during the preliminary determinations in Ringer. A comparison of the results of these two experiments with the others, indicated that this was not a vital matter. Another experiment, also interesting in this connection, was one performed with a strip of turtle ventricle, which although beating slightly at the time it was treated with potassium, was not beating either as rapidly or as regularly as the intact frog hearts. And this experiment concurred with the two mentioned above in indicating that the effect of potassium on acid production is not dependent on the simultaneous inhibition of contraction. Further considerations will be mentioned later which also tend to the same conclusion.

Altogether nineteen experiments were performed, including the one with the ventricular strip of the turtle; and, with the exception of a few determinations that were within the limit of error of the method, the rate of acid production was found to diminish progressively after treatment with potassium. Unfortunately, nothing seems
to be known as to the normal course of acid production by surviving heart muscle; but on the assumption that it might be similar to the course of survival carbon dioxide production in skeletal muscle (6) (see also Fig. 2), it became apparent that it would have to be determined with some accuracy before the potassium effect could be evaluated. Six experiments of this series that were carried out in such a way as to throw light on this question furnished the data which are represented graphically in Fig. 3. Precautions were observed which make these six experiments quantitatively comparable; they were all carried out at a temperature of 19°–20°C.; in each case the hearts were weighed so as to be able to compute and compare the rates of acid production per gm. of tissue; and finally, the experiments were run in pairs so that one could serve as a control for another—that is, two frogs were killed at the same time, their hearts removed, and determinations of the rate of acid production of each made alternately so that one might be serving as a control in Ringer solution at the time that the other was being subjected to potassium. Thus (Fig. 3) Curve I (or to be more exact, the heart which furnished the data shown in Curve I) served as a control for one of the experiments incorporated in Curve III; Curve II served as a control for another of the experiments incorporated in Curve III; and the third experiment of Curve III served as control for Curve IV. The potassium effect shown at the end of Curve II was not controlled, except by the careful determination of the preceding normal rate in Ringer, but the result is unmistakable.

Curves I and II and the first part of Curve III show that the rate of survival acid production by cardiac tissue is similar to the course of survival carbon dioxide production, as we know it for skeletal muscle; that is, the rate diminishes rapidly during the first 2 hours of survival and then continuously but more gradually thereafter—at least to the 8th hour of survival, which is as long as any of these experiments were followed. These experiments also show that potassium causes an abrupt and marked diminution in the rate of survival acid production. Curve IV illustrates the necessity of such controls as were used, especially if the potassium effect is to be tested and evaluated during the first 2 hours of survival. Most of the experiments of this series were of this kind, i.e. the hearts
were treated with potassium after two or three "normal" determinations had been made in Ringer. And until the evidence of Curves I, II, and III was at hand there was no way of appreciating the magnitude of the potassium effect.

Fig. 3. The effect of potassium on the total acid production of surviving cardiac muscle of the frog.

Curve I represents the normal rate of acid production of an isolated, surviving frog heart in Ringer solution.

The other curves show the effect of potassium chloride on the acid production of surviving frog hearts after varying preliminary periods in Ringer.

Curve II shows a single experiment in which the heart was treated with potassium at the beginning of the 8th hour of survival.

Curve III shows the average of three experiments in which the hearts were treated with potassium at the end of the 3rd hour of survival.

Curve IV represents a single experiment in which the heart was treated with potassium during the 2nd hour of survival.

In each case the ordinates represent the reciprocals of the time required for 1 gm. of tissue to produce an increase in acidity from pH 7.381 to 7.168.
Again the question arises as to whether this depression in acid production is due to the cessation of contraction which the potassium also causes or whether the effect is primarily on the metabolic processes within the tissue. Some evidence has already been advanced in favor of the latter assumption and it is strengthened by the following considerations. Thus, the heart which furnished the data of Curve I (Fig. 3) stopped beating at some time between the last two determinations without producing any apparent effect on the curve; also, one of the hearts whose data are incorporated in Curve III stopped beating after the first determination in Ringer and resumed activity toward the end of the 2nd hour of survival (still in Ringer) without producing any noticeable change in the curve of acid production. And, finally, results which are the converse of these but which also show that the acid production may vary independently of the activity of the heart have been reported by Weizsäcker, who found (8) that excised frog hearts might have their carbon dioxide output (and also their oxygen consumption) reduced to zero by treatment with cyanide, without any alteration in the rate of contraction. It would seem, therefore, that the mere inhibition of contraction need not be expected, in itself, to account for the marked depression of metabolism which potassium also causes.

SUMMARY.

The potassium contraction of skeletal muscle and relaxation of cardiac muscle have been correlated with the carbon dioxide and total acid production of these tissues.

1. The immersion of surviving sartorius muscles of the frog in isotonic potassium chloride solution causes a marked increase in the rate of acid production.

2. It is probable that carbon dioxide is the principal acid involved in the above effect.

3. The immersion of surviving cardiac muscle of the frog in isotonic potassium chloride solution causes a pronounced depression in the rate of survival acid production.

4. Reasons are given for believing that these changes in metabolism may be independent of the stimulation and inhibition of contraction which potassium simultaneously produces in these tissues.
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