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THE EFFECT OF POTASSIUM ON THE EXCITABILITY AND RESTING METABOLISM OF FROG'S MUSCLE.

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Frog's muscle soaked in Ringer's solution containing from three to eight times the normal K-ion content becomes inexcitable, the effect being reversed by washing in normal Ringer. Dulière and Horton [1929] linked this effect with the spontaneous and reversible inexcitability which occurred if the muscle was not bathed in Ringer's fluid following dissection. In this case the K freed from the injured fibres produced the inexcitability.

Having cause to render muscles inexcitable with K, it was observed that the effectiveness of K, as an agent for producing inexcitability in frog's muscles, underwent marked seasonal variations. Further, increasing the K content of the Ringer's fluid bathing a muscle was found to produce a great increase in its resting metabolism, as measured by the resting heat production. Fenn [1930] has recorded an increase in oxygen consumption under various conditions of inexcitability. The present paper deals with the experimental investigation of K inexcitability and K increment of resting metabolism and their possible correlation.

Method.

The studies on excitability and on resting metabolism were usually carried out simultaneously on the same preparation. Excitability was tested after the value for resting metabolism had been obtained.

The double sartorius preparation from the frog, usually *Rana temporaria* (Belgian or English), but occasionally *R. esculenta* (Hungarian), was used. It was mounted on a Downing-Hill muscle thermopile specially designed for resting heat measurements. The instrument resembles the usual muscle thermopile [Hill, 1928], but has only fifteen

constantan-manganin couples of wire 0·0006 mm. in diameter. These are held between very thin wafers of mica. Thus heat conduction between the junctions in thermal contact with the muscle and the "cold" junctions is minimized. The instrument is housed in a brass chamber into which solutions may be run for bathing the muscle. A thread leads from the muscle to a lever outside the chamber. Stimulating electrodes are in permanent contact with the muscles when they are mounted on the thermopile. The apparatus, set up as described, was immersed in a water-bath thermostat at least 1 hour before any readings of resting heat production were made.

A Downing moving-coil galvanometer was used. The apparatus was calibrated in absolute units of heat by passing condenser discharges through a killed muscle, or Ringer-soaked filter paper of similar dimensions, set up on the thermopile in the usual way. The energy, which could be calculated from the capacity and the charging voltage, was dissipated as heat over the length of the muscle, which was of such a high resistance that other resistances in the circuit could be neglected. Discharging the condenser through the muscle at a steady rate (say sixty alternating discharges per minute), the steady reading of the galvanometer was noted. The heat production per minute which this reading represented was calculated. The linearity of the response of the thermopile-galvanometer system was tested by finding the rate of heat production required to maintain various deflections covering the entire scale used in the experiments.

When frequent readings had to be made between applications of a solution it was withdrawn from the thermopile chamber into an adjacent receptacle which was also immersed in the water bath. When the reading had been made the fluid was returned directly into the thermopile chamber. In this way readings could be repeated every 10 min.

In all cases, unless otherwise mentioned, oxygen was kept running slowly through the thermopile chamber.

The Ringer's solution used as normal contained 6·25 g. NaCl, 0·15 g. KCl, 0·20 g. $CaCl_2$, made to 1·0 litre with distilled water. Any other salts introduced were added to this Ringer in the form of isotonic solutions. This involved a small decrease in the percentages of the normal contents of the Ringer. However, these slight changes were reproduced independently, and no resulting alteration in either excitability or resting heat production could be detected.

Results.

Excitability. Table I shows the results of the investigation of seasonal changes in K-induced inexcitability in frog's muscle. *R. temporaria*

Table I. Production of inexcitability in frog's sartorius muscle.

Frog (*R. temporaria*, English and Belgian. *R. esculenta*, Hungarian, gave similar results)	Onset of inexcitability after dissection: Spontaneous (not bathed in Ringer)	Bathed in Ringer with 4 × normal KCl	Bathed in Ringer with 8 × normal KCl
Summer frog (May–August)	1–2 hours	1–2 hours	Approx. 1 hour
Winter frog (September–April)	4–6 hours	8–10 hours	6–8 hours

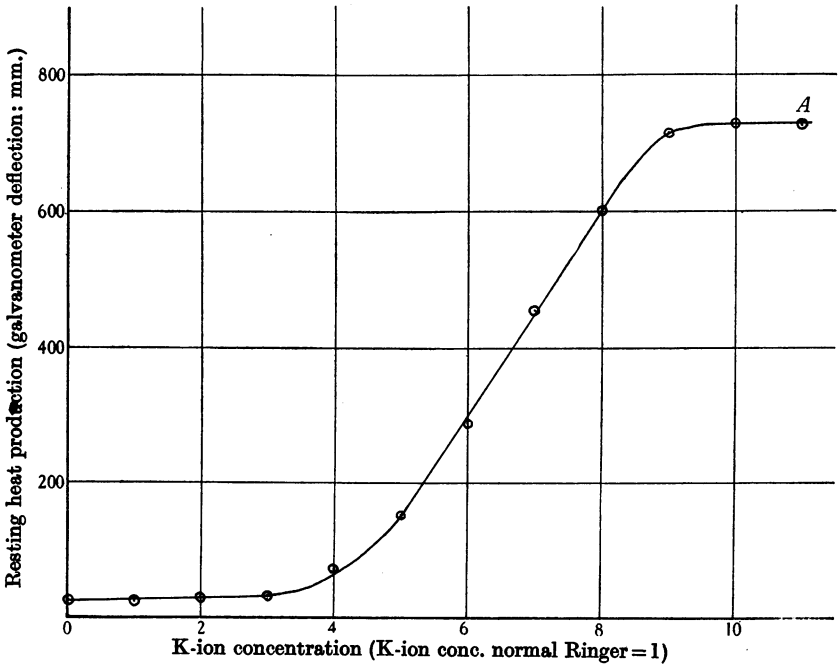

Fig. 1. Resting heat production of frog's (*R. temporaria*—Belgian) sartorius muscle with Ringer's fluid containing various concentrations of K ions. Curve constructed from several interlocking series. Increment falls off rapidly above point A, till none can be detected with K concentrations above fifteen times normal.

(Belgian and English) and *R. esculenta* (Hungarian) were used and all gave the same results. Muscles from "winter" frogs (September to April) were consistently more resistant to K-induced inexcitability than those from

"summer" frogs (May to August). This was true for spontaneous inexcitability or that induced by bathing the muscle in K-rich Ringer.

The time of change from "winter" to "summer" response coincided with the breeding season. The onset of the change was rapid and was coincident with the onset of breeding. The change from "summer" to "winter" response was more gradual and was apparently in progress from August to October and possibly later.

Resting metabolism. Increasing the K content of the Ringer's solution bathing a frog's sartorius produced a marked increase in resting heat production. In oxygen the rate of resting heat production with K may be over twenty times the normal value in the case of *R. temporaria* and about half this for *R. esculenta*. The increment was approximately halved in nitrogen. Fig. 1 shows that eight to ten times the normal K gave the maximum increment. Concentrations of K up to three times the normal gave no increase in resting heat production; in a number of experiments the resting heat value in three times normal K was actually lower than in normal Ringer. Concentrations over fifteen times the normal gave no increase in resting heat production and irreversibly damaged the muscle. A concentration of about thirty times (approximately the concentration of K inside the muscle fibre) produced contracture.

The K increment of resting heat was readily reversed by washing the muscle in normal Ringer's solution. Indeed, the increment and its reversal could be accomplished several times on the same preparation.

The increment in resting heat produced by stretching the muscle [Feng, 1932] is obtained normally in the presence of the K increment (five experiments).

The maximum K increment of resting heat was reached between 2 and 3 hours after the solution has been applied. Fig. 2 shows how the increment rose rapidly and dropped off slowly with time. The condition of the muscle at the end of such a long exposure could not be determined because it was quite inexcitable.

The K increment in resting heat was found to approximately the same degree in "summer" and "winter" frogs (ten determinations).

In absolute units the normal rate of resting heat production of *R. temporaria* sartorius muscle was about 160 g. cm. (3·75 millicalories) per g. of muscle per minute at 18° C. (six determinations). Hill [1928] gives a value of 180 g. cm. (4·2 mcal.) per g. of muscle per minute at 17·6° C. After soaking the muscle in Ringer with eight to ten times the normal K, values as high as 3600 g. cm. (84 mcal.) were obtained. The average of sixteen experiments gave a resting heat value, after exposure to Ringer

plus eight times K, of 2430 g. cm. (57 mcal.) per g. of muscle per minute at 17–19° C.

Table II shows the effect of various inorganic ions on the resting metabolism of muscle. Ca antagonizes the K increment when added in a

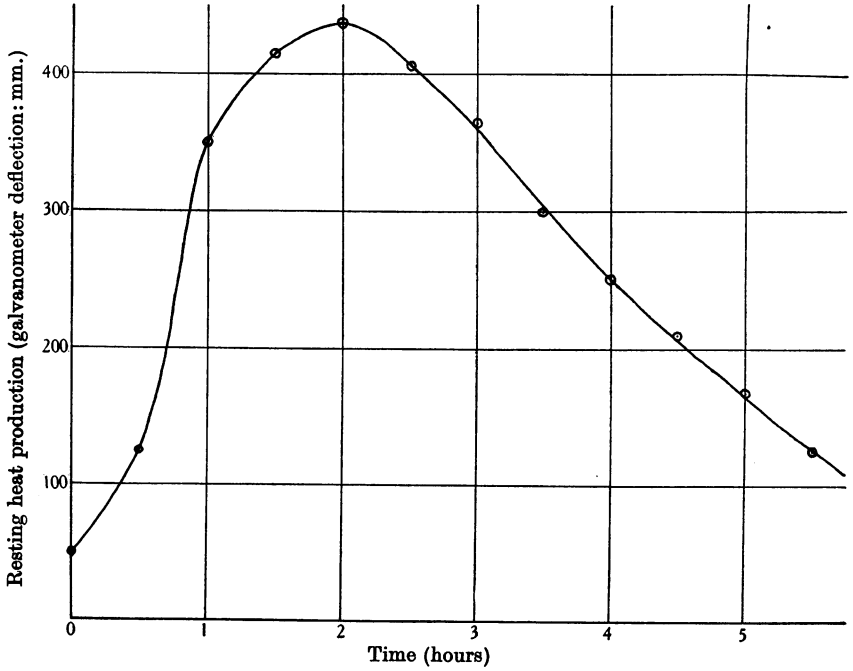

Fig. 2. Increment in resting heat production of frog's (*R. temporaria*—Belgian) sartorius muscle on exposure to isotonic Ringer's solution containing eight times the normal K-ion concentration.

TABLE II. Resting metabolism of frog's sartorius muscle (*R. temporaria*). Effect of various inorganic substances in the Ringer's solution bathing the muscle.

Substance	Concentration	Effect on resting metabolism	Effect on K increment of resting metabolism	No. of experiments
KCl	3 or 4 ×–10 × conc. in normal Ringer	Increased up to 20 × normal		40
RbCl	0·24 g./100 c.c. (equivalent to 8 × KCl)	Increased up to 20 × normal	Adds till equivalent of 10 × normal KCl reached	4
$BaCl_2$	0·1 g./100 c.c. (equivalent to 2 × KCl: greater concentrations too toxic)	Increased up to 2 × normal	Adds till equivalent of 10 × normal KCl reached	5
$CaCl_2$	2 ×–10 × conc. in normal Ringer	Nil	Opposes action of KCl in Ringer proportions	5
$SrCl_2$	0·25 g./100 c.c.	Nil	Opposes action of KCl	5

quantity sufficient to keep the Ca : K ratio as in normal Ringer. Reducing either the Ca or K content of normal Ringer had no detectable effect on resting heat production. Removing both these constituents resulted in twitching which made true resting heat measurements impossible. Rb and Ba act like K, although Ba is so toxic that it could only be used in low concentrations. Sr acted like Ca in opposing the K increment.

Table III shows the effect of various organic agents on the resting metabolism of muscle. Acetylcholine was tried because of the suggestive relation between its production and K in some situations [Beznák, 1934; Brown and Feldberg, 1935].

TABLE III. Resting metabolism of frog's sartorius muscle (*R. temporaria*). Effect of various organic agents in the Ringer solution bathing muscle.

Agent	Concentration	Effect on resting metabolism	Effect on K increment of resting metabolism	No. of experiments
Acetylcholine	1 : 2 million to 1 : 50,000 (at *p*H 6·2 with 1 : 500,000 eserine)	Nil	Nil	7
Sodium iodoacetate	1 : 200,000	Nil	Nil	7
	1 : 100,000	Nil	Contracture	
Curare-like substance (trimethyloctyl ammonium iodide)	7 mg./100 c.c.	Nil	Nil	4
Dextrose	4·0 p.c. solution	10–20 × normal		4
Sucrose	6·5 p.c. solution	10–20 × normal		

In amounts short of the contracture-producing dose it had no effect either with or without eserine. Sodium iodoacetate had no effect in sub-contracture doses. Preventing nerve-muscle conduction with the curare-like trimethyloctyl ammonium iodide had no effect on either resting heat production or its increment by K. Isotonic sugar solutions (no salt content) simulate K. The increment due to sugar solutions is antagonized by Ca, K or Na [Fenn, 1931].

Changing the *p*H between 6·0 and 8·0 of the Ringer's solution bathing a muscle had no effect on resting heat production (seven experiments). Changing the tonicity of the Ringer (by alterations in the NaCl content) between 0·6 and 0·8 p.c. NaCl equivalent had no effect (four experiments). Changes in tonicity or *p*H much beyond these limits caused twitching or contracture which made resting heat measurements impossible. However, it is safe to assume that any tonicity or *p*H changes which might occur in the actual solutions used would have no effect on resting heat production.

Searching for a possible explanation of the apparent K increment of resting heat, efforts were made to rule out the possibility of contracture. A lever carrying a mirror which operated an optical lever 3 metres in length was connected to the muscle. No change in muscle length could be detected on producing or reversing the K increment. Indeed, no shortening occurred until K concentrations about thirty times normal produced the typical K contracture.

It was thought that a submaximal fibrillar contracture [Gelfan, 1930] might account for the K increment of resting metabolism. Such contracture occurs only at and around the neuromuscular junction. The non-neural pelvic third of the frog sartorius muscle was bathed in Ringer solution containing extra K and the characteristic increment of resting heat production was obtained.

Could it be that the K only acts on a muscle damaged by immersion in a highly artificial medium such as normal Ringer solution? Such a solution has been shown to cause loss of nitrogen and gain of chloride in frog muscle, and these changes are affected by the K content of the Ringer. They are at a minimum at four to six times the K concentration of normal Ringer [Fenn, 1935]. However, muscles dissected and mounted on the thermopile with no initial soaking in Ringer's solution, hence bathed only in their own serum, showed a progressive rise in resting heat production. This, we conclude, was due to the K leaking from injured muscle cells which Dulière and Horton [1929] showed to be the cause of the spontaneous inexcitability taking place under these circumstances. The K apparently can affect resting heat production even when its vehicle is the muscles' natural perfusate.

Discussion.

From the foregoing it is apparent that the resting metabolism of frog's sartorius muscle, as measured by the resting heat production, is greatly increased by exposure to a Ringer's solution containing from four to ten times the normal K concentration. Fenn [1930] has noted that the oxygen consumption of frog's muscle, rendered inexcitable with sugar or K, or becoming spontaneously inexcitable, is markedly increased. Studying particularly the case of sugar inexcitability Fenn [1931] suggests that the increased oxygen consumption might be due to alterations in the cell membrane changing the Na : K balance between the muscle cell and its environment. He finds, also in the case of sugar, a slight contracture and suggests that, if this be inefficiently maintained, it might account for the increased oxygen consumption. This latter

suggestion does not help to explain the increase in resting metabolism induced by K for in that case there is no evidence of contracture; indeed, concentrations which produce contracture give no increment of resting metabolism.

K goes into the muscle cell when the K concentration in the Ringer is over four times normal [Fenn and Cobb, 1934]. This is in spite of the fact that the K concentration in the muscle cell is over thirty times that of normal Ringer. This suggests a secretion of K by the muscle cells. If we assume that the degree of secretion depends on the ratio of K outside to K inside, then by raising the K outside we might force the muscle to secrete more K. This secretion against a high K gradient might account for the increment of resting metabolism. Fenn and Cobb [1934] have shown that high external K concentrations damage the muscle cell, producing irreversible inexcitability and loss of the power to concentrate K. This would explain the falling off in resting heat increment at external K concentrations of over ten times the normal.

The present experiments indicate that K inexcitability and K increment of resting metabolism differ in so many ways that it is possible that they are quite separate processes. The former shows a seasonal variation and the latter none. The former is unaffected by Ca ions [Horton, 1930]; the latter may be completely prevented by Ca in the proper amount. In some "winter" frogs a marked K increment of resting metabolism may be observed in muscles showing unimpaired excitability.

Against there being no fundamental connection between inexcitability and increment in resting metabolism is the fact that sugar and K each produce both effects. It is possible, however, that the increment in resting metabolism is produced in different ways by the two substances, as the inexcitability probably is. In the case of K, we find the K moving into the muscle cell, and in the case of sugar, K (as K phosphate) moves out of the muscle cell [Fenn, 1931]. Possibly in the first of these increment in resting heat is caused by the active secretion of K as previously described. In the case of sugar the increment might be due to work done by the muscle in retaining its K under a large K gradient and an altered permeability of the membrane. The fact that K does escape under these conditions is evidence that this altered permeability exists.

Summary.

Experiments are described dealing with the inexcitability and the increment in resting metabolism induced in frog's muscle by an increased K-ion concentration in the environment. A seasonal variation in the

production of inexcitability was demonstrated. The relation of the increment in resting metabolism (as measured by resting heat production) to K concentration, to time of exposure to K, and to other inorganic and various organic substances has been studied. Possible explanations of the increment in resting metabolism, and its relation to inexcitability, are discussed.

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