# THE ACTION of OXALATES UPON, and THE RELATION OF CALCIUM SALTS TO MUSCLE.

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Salts of Calcium are found in the ash of all tissues subjected to analysis whether they be bone or some of the softer tissues. So constantly are they present, that it is not surprising to find that investigations, carried out during many years by many observers, have assigned a very important part to this metal in several vital and chemical phenomena. Ringer's experiments, carried out a number of years ago, proved conclusively that the presence of Ca. in some form was essential to rhythmic movement, and more particularly to rhythmic contraction of the heart. It is true that he also discovered that lime salts when acting alone would eventually bring the heart to a stand--still in systole, and that this effect could be counteracted by the presence of a minute quantity of potassium. The main fact, however, remains that Ca. is the only inorganic substance which does maintain rhythmic movement and that without it such movement will not continue (Journal of Phys. III. 380; IV. 29 & 222; VI. 361.)

Again, it has been shewn that when milk coagulates Ca. combines with the soluble caseinogen to form insoluble casein (Schäfer's Text Book of Physiol. Vol I. p.135.); and in the case of blood coagulation, where its presence has also been proved necessary, it is supposed that a similar combination takes place (G.N.Stewart, Manual of Physiol. p.43. 1899).

Muscle substance also coagulates, and experimental investigation shows that the cause of the changes and the changes themselves are in many respects like those which bring about and result from coagulation of blood. It is reasonable therefore to suppose that the resemblance may go the length of requiring the presence of Ca. salts here also. Of this, however, there is no proof; indeed there is some ground for believing that they are not necessary in this case.

Hermann has put forward the view that contraction of muscle and rigor mortis involve practically the same changes and that the two conditions vary only in degree. In fact, contraction he would consider merely a mild and transient form of rigor. Following this view, and considering for the meantime that Ca. is necessary for the formation of a muscle clot, one would naturally conclude that at the moment of contraction some chemical change occurs involving the interaction of the lime salt with the muscle substance and the formation of some Ca. compound.

Hermann's view as to the identity of contraction and rigor are not however accepted as proved, although in the two conditions we have the formation of the same substances, and have some similar physical changes. It was however from these considerations that this investigation was first undertaken as they seemed to indicate that the Ca. present might be involved chemically, and that some form of combination might exist at one time or other.

There is next the question of the existence of calcium proteid compounds. The difficulty of purifying proteids from the small amount of Ca. which they contain has been held to indicate that the two substances may be combined, and the changes in milk and blood coagulation already mentioned tend to strengthen this view. If then such compounds exist in a dead condition, may they not also exist in living form?.

In order to test this point ordinary analytical methods are practically useless as the living tissue becomes so rapidly destroyed.

For this purpose living tissues must be employed, and, if possible, the lime salts removed by some suitable means. Any change in their functions is noted at the time; and again when the tissues have been once more supplied with lime salts, in solution.

With these objects in view soluble neutral oxalates of sodium or Ammonium were selected as the most suitable decalcifying agents.

There are therefore three points to be considered

1. The action of the oxalates upon the muscle.

2. Do they produce their effects by the removal of Ca.salts?.

3. Do the results of the experiments give any indication as to the relation of the protoplasm and the Ca?

Before going on to describe the experiments it will be as well to review the literature that exists dealing with the action of oxalates upon muscle as it affects rigor mortis and the irritability of muscle.

Cavazzanni (Arch:Ital:di Biologie XVIII.156) held the opinion that by the action of oxalates upon muscle, rigor mortis was delayed or prevented.

(This paper I have not been able to procure, but it is quoted by each of the following writers).

W. H. Howell (Journ:of Phys.XIV p.219)

In a note appended to this paper it is recorded that when blood, decalcified by the addition of sodium oxalate, is perfused through a frog's heart the rhythmic contractions gradually disappear. They may however be caused to return by adding a trace of a soluble Ca.salt to the blood.

Howell. (Journof Phys.XVI. p.476)

In this paper Howell deals with the effect of oxalates upon rigor mortis and muscular irritability. Nerve is also dealt with but may be left out of account here. The conclusions are that oxalates do not hinder the onset of rigor mortis, even after they have been perfused through the muscles of the frog for a very considerable time. Irritability to electric stimuli is, he says, destroyed.

There are described also twitchings and tonic spasms of the muscles whilst the oxalate solution is passing through the animal.

It is also of interest to note that Prof:Howell believes that the action of these salts is due to their power of precipitating the Ca: present. He admits that they may possibly have some other independant action; but sees no special reason for believing that they have.

F. S. Locke. (Journ of Phys.XV.119)

Locke describes here a sort of rhythmic contraction that occurs when an excised muscle (sartorius) is immersed in a·7% solution of sodium oxalate. He also records his belief that irritability is destroyed.

(Locke. Journ.of Phys.XVII 293.)

Rigor mortis is dealt with in this paper, and the conclusion is that it not prevented by oxalates.

Locke however, entirely changes his views about irritability and holds now that it is not and cannot be destroyed by oxalates.

It will thus be seen that the balance of opinion is against the prevention of rigor and this would almost require to be taken as an indication that Ca is not necsssary for this change. None of my own experiments deal with the question of rigor so that no criticism can be given.

On the question of irritability however, opinions are evenly divided. This question will therefore be discussed after the description of my own experiments has been given.

## DESCRIPTION OF EXPERIMENTS:-

Frog's heart and skeletal muscles, which were the two forms of muscular tissue experimented upon, were treated with solutions of either sodium or ammonium oxalate, the tissue being either immersed in, or perfused with the fluid. After these solutions had produced their effect, a solution ~~of~~ containing Calcium chloride was substituted, and observations upon the alternate action of these solutions were made several times upon the same heart. In the case of skeletal muscle however, the oxalate solution in the perfusion experiments was not replaced by Calcium Chloride solution, as after the muscle had been excised no ~~pro~~perfusion was possible.

The solutions employed were .75% Na. Cl. Solution containing .1% of Sodium or ammonium oxalate; and .1% $Ca.Cl_2$ also dissolved in normal saline. In the case of skeletal muscle however, a stronger oxalate solution -.7% sodium oxalate in water - was most frequently employed.

As the results obtained with the two different forms of muscle bear upon rather different points in the investigation they will be described separately.

HEART.

a. by immersion: an excised frog's heart was connected with a recording lever, and, after a tracing of the normal contractions had been taken, was immersed in the oxalate solution already referred to.

The effect of this solution soon became apparent in a gradual diminution in size of the heart beats without, however, any other visible effect beyond it might be a very slight slowing of their rate. (Tracing 1.) When the individual contractions had become very small, Calcium Chloride solution was substituted for the oxalate. Almost immediately there was considerable slowing of the contraction rate, and at the same time the contractions gradually increased in size until they reached their initial height or became even larger. (Tracing 2.) Oxalate solution applied for the second time at once increased the rate, but as before brought about a progressive decline in size of the contractions. (Tracing 3.) By the alternate use of the two solutions these observations could be repeated several times on the same heart.

Occasionally a heart, which had been so treated several times, and which at the time was contracting feebly under the influence of the oxalate, came to a complete standstill, when that salt was replaced by the Calcium salt. Contraction, however, reappeared and with greater force than before, when Calcium Chloride was once more removed, and its place taken by the oxalate solution. (Tracing 4.)

b. By perfusion:-

Practically the same results were obtained when the fluids were perfused through the heart, though it is not surprising to find that the salts produced their effects more rapidly. It will be observed in the tracings shewn (Tracing 5.) that the recovery with Ca. does not seem to be quite the same as in the previous experiments. The heart beats at first increase in size and afterwards die away. This is due to the physiological action of the Ca. salt in producing gradually a condition of contracture of the ventricle which increases until the heart stops in systole.

A few rather interesting peculiarities have been noticed with some hearts which did not beat spontaneously when tied on to the perfusion cannula. Thus in some cases, even after the oxalate solution had been passed through for some considerable time, the heart would still respond to mechanical stimulation; but the responses to successive stimuli became smaller by degrees, until none were obtained ~~(Tracing 5.)~~ Recovery took place when the necessary lime salt was supplied, but in their presence the heart was much less easily stimulated to contract.

A few observations of this sort led me to the idea that possibly the loss of irritability of the cardiac muscle as effected by oxalate solutions would not occur in quiescent tissues, but only in those which were functionally active at the time. The experiments with skeletal muscle which will be described immediately deal mainly with this point.

The other peculiarity observed was that one or two hearts not contracting spontaneously at first began to do so after having been fed with the oxalate & Ca. solutions alternately once or twice; and contraction always commenced just when the Ca. solution was removed and the oxalate substituted.

Renewal of Ca.Solution again abolished contraction in these cases.

Skeletal Muscle.

Sodium oxalate solutions act upon this form of muscle very much as they do upon cardiac muscle. For these muscles solutions of .7% Sodium oxalate were employed.

At first there seems to be an increase in the excitability of the muscle. I have repeated Locke's experiment of placing an excised sartorius in .7% oxalate solution, and have obtained the semi-rhythmic contraction described by him. This movement must be due to the action of the salt upon the muscular substance as it may be obtained just as readily in a curarised muscle as in a normal one.

When a frog is thoroughly washed out with the oxalate solution injected from the heart, the fluid being allowed to circulate for some time until at least 100 c.c. have been passed through, both muscular twitchings and sometimes tetanic spasms are to be observed. Both of these I have observed in frogs, which had been previously curarised and completely pithed. After the animal had been thoroughly washed out, prepar ations were made of the gastrocnemii which were then stimulated and tracings of their contractions obtained.

Usually the first contraction was fairly good, the succeeding ones being progressively smaller, and disappearing entirely after the fourth or fifth. (Tracing 6.)

Two or three muscles failed to respond at all when stimulated, and it is interesting to record the fact, that they had been taken from frogs that shewed the violent tetanic spasms already alluded to.

Such being the experimental results, it then falls to be considered what bearing they have upon at the three points already indicated.

1. The action of the oxalates upon muscle.

At first sight it might appear that Howell's statement, that oxalates cause loss of irritability in muscle, is correct. This statement hhwever cannot pass without some modification.

It is true that in the majority of heart experiments, that is in all cases where the organ was beating spontaneously, there was exhibited a gradual decline of excitability.

It must be remembered however, that these tissues were functionally active, and also one must keep in view the fact that skeletal muscles and quiescent hearts, even when oxalated for a considerable length of time were still able to respond when stimulated.

One should notice also that this power of responding soon dies away in such muscles when stimulation is continued.

Locke, when he recanted his original view that irritability was destroyed, did so on very similar grounds.(Journof Phys vol.XVII.293/.

Howell's statement must therefore be modified to this extent that an oxalated heart or muscle rapidly loses its irritability when in a state of activity, but not at all, or only very slowly, when it is at rest.

Howell, in his account of his experiments describes the muscles as being perfused until they no longer responded to electric stimulation. Evidently the tissues were tested from time to time in this way, and from what has been already said, it will be seen that the test applied may have had a very considerable share in abolishing the excitability under the circumstances.

2. How do oxalates produce their effects?

It seems practically certain that the action of the oxalates is largely due to their power of precipitating Calcium, and so rendering it useless to the tissues. That

Ca. is essential for the proper functional activity of the heart we know from Ringer's work; and when we find that the addition of Ca to an oxalated heart restores its activity we have no other option than to believe that the poisonous action of the oxalates is largely due to their power of precipitating that metal.

There still remains the possibility of some other action in addition to the precipitation. Of this however, there is no evidence in the experimental results either one way or the other. If we suppose that such action exists, then we must also credit Ca. with a corresponding power of repairing the damage so inflicted, as well as with its known power of rendering the oxalic acid harmless by combining with it.

Although this point cannot be proved either one way or the other, yet the existence of such extra action seems very improbable.

3. Is there any indication of the relationship of the Calcium to the muscle substance?.

The lime salts of course exist in the fluid which permeates the tissues, and the question is therefore, whether they are only in this position relatively to the tissue, or whether they also exist combined in the living substance.

If they exist merely in the fluid and not as part of the tissue, their use would appear to be limited to acting either by their presence in some unknown way, or possibly they may be somehow connected with the removal of some deleterious waste product.

If merely the presence of lime salts is necessary the perfusion of an oxalate would both wash away and precipitate those salts. Muscles so treated should therefore be incapable of responding to stimulation even if they have been previously at rest, which however, has been found not to be the case.

If removal of some waste product be the object of the Calcium salts, the gradual loss of irritability might be explained by accumulation of such product coincident with the gradual removal of the metal. It is difficult to imagine however, that any waste product could accumulate sufficiently to entirely abolish excitability in a muscle after four or five contractions. This is not a very likely supposition, but it is as well to have it in mind as possible

Similarly the formation of a Calcium compound at the moment of contraction as a necessary part of the contraction phase is negatived by the occurrence of contraction in a thoroughly oxalated muscle, in which there would be no available Ca. left.

There remains however, the possibility of such a compound in muscle when at rest, its presence being necessary to the display of irritability.

From the way in which resting muscles (cardiac & skeletal) respond after being oxalated, it might be supposed that this substance is of too stable a nature to be decomposed by the oxalate, but that it breaks down when a stimulus is supplied and the liberated Ca.is then seized upon and precipitated. From this point of view it is evident that the whole of the muscle substance does not break down at each contraction, and that the muscle is therefore in a* position to contract, although with decreasing force, as this material is gradually lost. Addition of Ca.to the tissue supplies it with the material necessary for repair, after which it is again in a position to resume activity.

It is not advisable however in a research of this sort to speculate too far upon the existence of such compounds, for it cannot be proved by experiments of this class alone. The point which my results seem to indicate is that contraction is associated with the removal of Ca rather than with its addition.

Loeb in some recent papers upholds the existence of ion proteid compounds. He associates rhythmic action with the entrance and exit of Ca ions into and from the the proteid molecule (American Journ.of Phys.Vol III p.327 and Vol III p.383.)

Rhythmic contraction is brought about, according to him, either by the entrance of Ca ions or by their removal, the contractions continuing until too many ions have been added or removed.

My perfusion experiments with the heart would seem to indicate that he is correct that rhythmic contraction is associated with both entrance and exit. At the same time the fact that in quiescent heart oxalates will at times bring about rhythmic contractions which are again abolished when Ca is added rather suggests that the removal of the Ca ions is a more powerful stimulus to the muscle than the addition of the same ions.

## SUMMARY.

The conclusions therefore arrived at, are these:-

1. That oxalates acting upon either cardiac or skeletal muscle in a state of activity soon cause an entire loss of irritability.

Resting muscles are not so affected, or only very slowly, although it is to be noted that an oxalate may act to some extent as a stimulus to muscle, as evidenced by the occurrence of twitching whilst such solutions are being perfused.

2. That these effects are very largely due to the precipitation of Ca. which deprives the tissues of this necessary material.

That there is no evidence of any other action.

3. That although there is no absolute proof that Ca is combined in the living protoplasm, it is nevertheless extremely probable that such conbination does exist.

That whilst the addition of Calcium to the living proteid molecule may cause rhythmic contraction, removal of Calcium is a much more potent cause.

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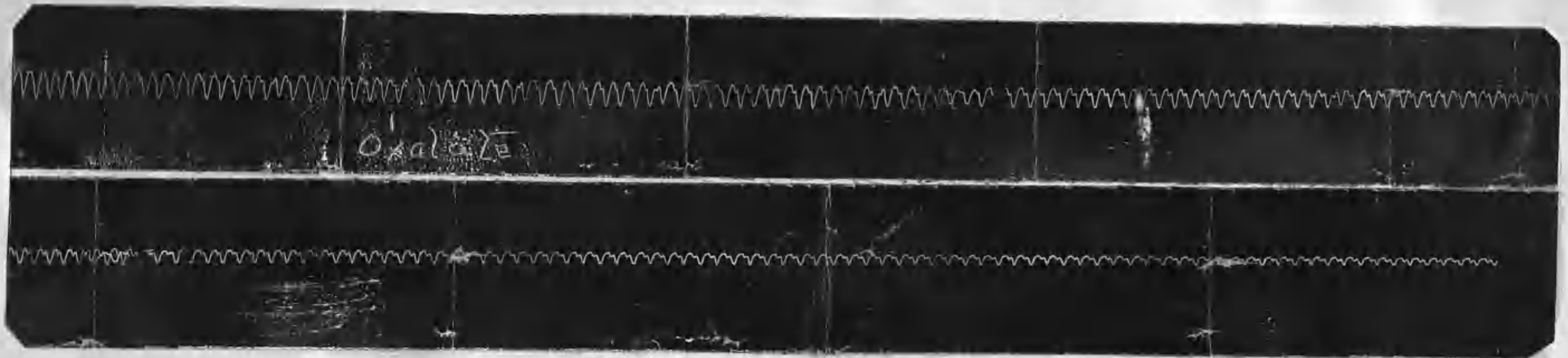
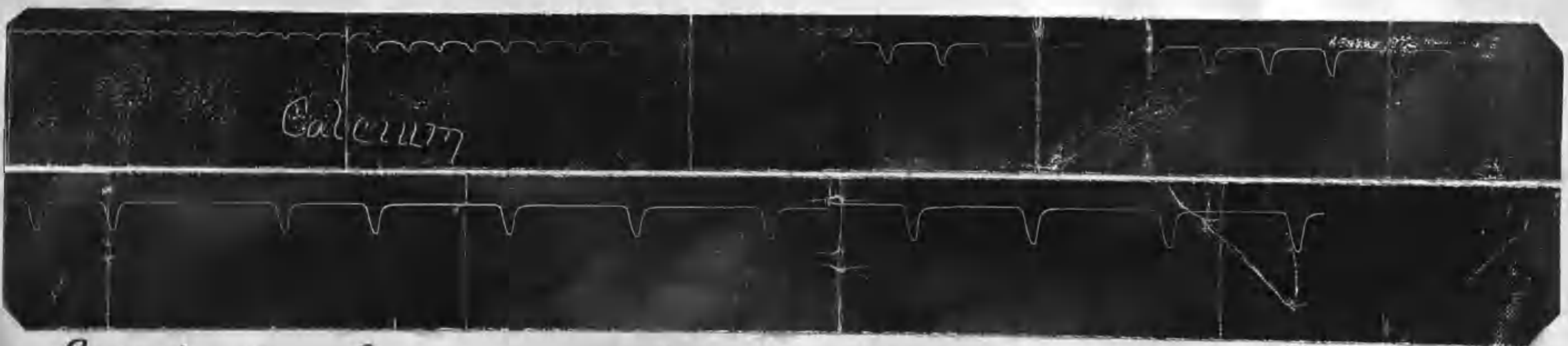
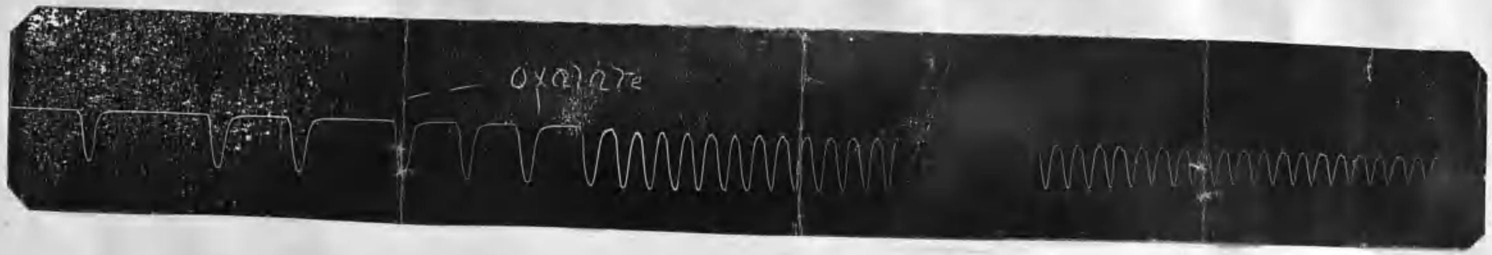



Tracing 1. The effect of an oxalate upon a Frog's heart (immersion method).



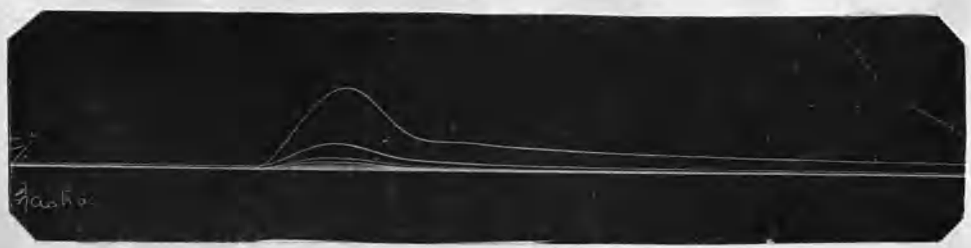

Tracing 2. Recovery with Calcium chloride. (immersion)




Tracing 3. - The effect of an oxalate following a Calcium salt.


Tracing 4. - Stoppage of the heart caused by Ca salt. Recovery on the addition of oxalate.


Tracing 5:– Perfusion of the heart with an oxalate and Ca salt alternately. Note the prolonged systole caused by Ca. (Kronecker's apparatus).



Tracing 6.– The decline of irritability of a muscle (gastrocnemius) after being perfused with an oxalate. Stimulation effected always at the same relative moment by the "flying wire".