HEAT RIGOR IN VERTEBRATE MUSCLE

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The experiments described in the following paper were commenced in the winter of 1905-06 by Dr. Kenny. His work was not brought to a conclusion, though the results he arrived at were to a great extent in conformity with those about to be described.

I have to express my thanks to Professor Vincent for the use of his laboratory and for his assistance throughout the course of the work.

The generally received view as to the chemistry of muscle (so far at any rate as concerns a study of the proteins contained in saline extracts of the tissue), is that we have to deal with two muscle proteins, viz., paramyosinogen (V. Fürth's myosin) and myosinogen (V. Fürth's myogen).¹ These are probably identical or closely related in both striped and unstriped muscle.

The earliest attempt to which a detailed reference need be made to study the protein constitution of muscle by means of a graphic record was that of Brodie and Richardson.² These observers using

1. References upon the more purely chemical aspect of the question are as follows:—Halliburton, *Text-Book of Physiology*, ed. by Schäfer, p. 99, 1898; Kühne, *Lehrbuch d. Physiol. Chem.*, S. 272; Untersuch., u. das Protoplast, Leipzig, 1864; Halliburton, *Journ. of Physiol.*, Vol. VIII, p. 133, 1887; V. Fürth, *Arch. f. exper. Patb. u. Pharmacol.*, Leipzig, Vol. XXXVI, S. 231, 1895, Vol. XXXVII, S. 389, 1896; Stewart and Sollman, *Journ. of Physiol.*, Vol. XXIV, p. 427, 1899; Velichi, *Centralbl. f. Physiol.*, Vol. XII, p. 351, 1898; Vincent and Lewis, *Journ. of Physiol.*, Vol. XXVI, p. 445, 1901; Vincent, *Hoppe-Seyler's Zeitschr. f. physiol. Chem.*, Vol. XXXIV, S. 417, 1902.

2. *Phil. Trans.*, 1899, pp. 127 and 146. The following are references to other papers (cited by Brodie and Richardson), which may be consulted:—Engelmann, *Pflüger's Archiv.*, Vol. VII, 1873 (*vide* footnotes on pp. 177 and 179); Hermann, *idem*, Vol. VII, p. 417, 1873; Pickford, *Zeitschr. für rat-Medicin.*, N.F.I., p. 110; Schiff, *Lehrbuch der Physiologie*, p. 44; Wundt, *Die Lehre von der Muskelbewegung*, p. 66; Kühne, *Myologische Untersuchungen*, p. 173, Leipzig, 1860; Schmulewitsch, *Compt. Rend.*, Vol. LXVIII, p. 936, 1869, and *Centralbl. f. d. med. Wissenschaften*, Vol. V, p. 81, 1867; *Medicin. Jahrbucher*, Vol. XV, p. 3, Wien, 1868; *Journ. de l'Anat. et de la Physiol.*, 1868; Marey, *Du mouvement dans les fonctions de la vie*, p. 354, Fig. 3, Paris, 1868; Bondet, *De l'élasticité musculaire, Thèse pour le doctorat en médecine*, p. 31, Paris, 1880; Moriggia, *Molescott's Untersuchungen*, Vol. XIV, p. 386, 1892; Gotschlich, *Pflüger's Archiv.*, Vol. LIV, p. 109, 1893.

the living sartorius of the frog found four distinct contractions. 'The first contraction commences at 28° C., its rate is much accelerated at 32° C., and it is completed shortly after 40° C. is reached. It is then followed by a fairly rapid and extensive relaxation.

The second contraction commences at 44° C., its main effect is produced at a slightly higher temperature and it is completed at about 40° C.

The third begins at 55° C. and is complete soon after 60° C.

The fourth commences at 63° C.'

They give a tracing in support of these conclusions which, however, to me, seems not very convincing. It appears, however, to show three contractions; the fourth, they admit, is not very prominently marked. Some criticisms of the apparatus employed by these observers have been offered by Vernon¹ and certainly the results obtained (so far as we can interpret the tracing) do not correspond to those obtained by the present writer. In mammalian muscle these observers found only two contractions, but instead of considering these, as seems to be actually the case, to be due, respectively, to muscle protein and to connective tissue elements, they attribute them to the two muscle proteins.

Vernon² performed an elaborate series of experiments upon the heat rigor of cold-blooded animals. In the striped muscle he found three contractions, but the highest of these was certainly due to blood. In unstriped muscle the first contraction, he states, is absent.

Vincent and Lewis³ found also two contractions both in mammalian and amphibian muscle, but they refer also to 'a tendency to contract at about 56° C.' in the case of mammalian tissue. The first of these two contractions, they conclude, is due to the coagulation by heat of the protein substance present in the muscle fibre during life, the second to changes in the connective tissue elements in the muscle. Curiously enough, they, like Vernon, could not record the first contraction in amphibian unstriped muscle. There can be no

1. *Journ. of Physiol.*, Vol. XXIV, p. 241.

2. Vernon, *loc. cit.*

3. Vincent and Lewis, *loc. cit.*

doubt from my own experiments that this was an error of observation, and was due to one or other of two causes—either the tissue was dead (rigored), and so the first contraction would be absent (*vide infra*), or the material used was so delicate that the counterpoise employed was too great to allow of a contraction, and caused relaxation instead. The figure they give, Fig. 9 of their paper, seems to confirm this latter hypothesis. These observers prove conclusively that the second contraction is due to connective tissue.

Brodie and Richardson state that ‘a muscle which has passed into rigor mortis, when treated as in our experiments, will give heat contractions, though, as we shall show in a paper we hope to publish shortly, the form of these contractions is greatly affected by the stage which the muscle has reached in rigor mortis.’ I have not been able to find any further publication on this subject by the authors in question, but Vincent and Lewis state definitely that a muscle which has gone into complete rigor mortis gives no trace of contraction on being heated to about 47° C. ‘The only change observed in this case is the contraction at about 63° C. (due as we show to changes in the connective tissue).’¹ They give a tracing in support of this statement, and, as will be seen below, it is entirely borne out by my own experiments.

Since the paper of Vincent and Lewis a communication by Inagaki has appeared on this same subject, but which I have been unable to consult.²

In a communication on heat contraction in nerve Brodie and Halliburton³ have incidentally made some observations on the corresponding phenomena in muscles. These observers appear to be satisfied that the successive steps of heat rigor both in nerve and muscle occur at temperatures corresponding to the points of coagulation of the proteins that can be extracted by saline solutions from the tissues in question.

1. Vincent and Lewis, *loc. cit.*

2. *Zeitschr. f. Klin. Med.*, Vol. XLVIII, pp. 313-339, 1906. From a reference, however, to this work by V. Frey (*Physik.-Med. Gesellsch. z. Würzburg. Sitz.*, Vom VIII, Nov., 1906; *Münch. Med. Woch.*, Jan. 29, 1907) it seems that this author has reached conclusions very similar to my own.

3. *Journ. of Physiol.*, Vol. XXXI, p. 473, 1904.

RESULTS OF HEAT RIGOR EXPERIMENTS

Little need be said about the apparatus employed. The method adopted has been practically identical with that previously used by Brodie and Richardson,¹ Vernon,² and Vincent and Lewis.³

‘An iron bar clamped in a stand at one end, and bent down at the other into a beaker, has a small metal hook soldered to it. This forms the attachment at the lower end of the piece of muscle under investigation. The upper end is suspended by another hook attached by a very fine copper wire to the recording lever. The resistance employed was in some cases a fine spiral spring, in others a weight passing over a small frictionless pulley. The waxed thread employed by Brodie and Richardson was shown to be disadvantageous by Vernon, who, however, left a small length of this in the apparatus he employed. We have completely eliminated any defect from this source by using metal throughout. The metal hooks have, moreover, a great advantage in point of simplicity of manipulation.

‘Our recording lever has, as a rule, magnified the extent of change of length in the muscle ten times.

‘We have always taken care that the load should be small, so as to obviate any fallacy from increased extensibility on raising the temperature. Further, to completely eliminate other sources of instrumental error, and to check the results obtained by the above apparatus, we have performed a series of experiments in which we have taken long strips of the muscle and directly observed the changes in length at the different temperatures. The muscle was suspended in a large beaker of normal saline solution with a light load attached, and placed by the side of it was a millimetre scale. The changes in length at different temperatures could then be readily and directly observed.’⁴

In the present series of experiments all these precautions have been again taken. In order to guard against any possible fallacy

1. *Journ. of Physiol.*, Vol. XXI, p. 353, 1897, and *Phil. Trans.*, Vol. CXCI, p. 127, 1899.

2. *Journ. of Physiol.*, Vol. XXIV, p. 239, 1899.

3. *Loc. cit.*

4. Vincent and Lewis, *loc. cit.*

arising from inertia or undue weight of moving parts, rather than reduce these to their smallest dimensions, I have preferred to bring about the desired result by using only large and powerful strips of muscles. In some experiments I have employed a device for recording the precise vertical movement of the muscle, without the use of the magnifying lever. (See Fig. 2.)

In many experiments the metal attachments at the ends of the strip of muscle have been insulated, and attached to wires leading from the secondary coil of an inductorium, so that during the course of a heat contraction the irritability of the muscle could be readily tested. (See Figs. 1 and 7).

In experimenting thus with mammalian muscle I have taken the material direct from the living animal so as to be reasonably certain that the loss of irritability was due to the change of temperature, and was not spontaneous owing to lapse of time after removal from the body.

1. *Effects of temperature on mammalian striped and unstriped muscle, living.*—On testing mammalian striped or unstriped muscle I find that the contractions obtained differ according as to whether the tissue is living at the beginning of the experiment or is dead. On gradually heating from room temperature up to 70° or 80° C. we find in living muscle two contractions. *First* at about 47° C. (46° to 50°). *Second* at about 62° C. (61° to 64°). (See Figs. 1 and 2).

As mentioned above Vincent and Lewis describe three contractions, viz., (1) 45° to 49° , (2) 55° to 58° , (3) 61° to 64° C. In regard to the second contraction they note:—‘A very faint indication of contraction or, more frequently, rather a cessation of relaxation at about 55° to 58° C.’ Professor Vincent tells me he is now doubtful about this result, and the tracings given in his paper with Mr. Lewis do not lend much support to the view that a contraction occurs at this temperature.¹

Causes of the first contraction.—In the opinion of the present writer this contraction is due to the coagulation by heat of the complex protein present in muscle fibre at the moment of death. Stewart and Sollmann¹ suggest ‘that paramyosinogen does not exist as such

1. *Journ. of Physiol.*, Vol. XXIV, p. 427, 1899.

in living muscle,' but, as pointed out by Vincent and Lewis, if either of the described muscle proteins (which can be separated by the heat coagulation method from a saline extract¹), be absent at the moment of death—it is the one coagulating at 56° C. and not that which comes down at 47° C. These authors further point out that, 'The fact that the contraction occurs regularly at this temperature in the heat-rigor method (in which the muscle is in the best possible state for investigation, and in which its constituents are probably less injured or changed than in any extraction method) seems strong evidence in favour of this view.'

FIG. 1.—Dog's striped muscle. Note contractions due to electric stimuli, ceasing at 48° C.

FIG. 2.—Dog's striped muscle, living. Apparatus used in this experiment shows length of contraction without magnifying. A large strip of muscle was used. A represents length of muscle at end of experiment.

1. Halliburton, *Journ. of Physiol.*, Vol. VIII, p. 133, 1887.

We may then provisionally conclude that the cause of the contraction at about 47° C. is due to the coagulation by heat of the muscle protein.

In the experiments in which I started with living muscle it has been found that loss of irritability occurs at or immediately after the temperature at which this first contraction begins.

Cause of the Contraction at 63° C.—This is due, as first clearly pointed out by Vincent and Lewis, to some change in the connective tissue elements of the muscle substance.¹ Tendon and skin show a marked contraction at this temperature.

EFFECTS OF HEAT RIGOR ON AVIAN MUSCLE

The results I have obtained point to the conclusion that the same contractions occur in avian as in mammalian muscle, and that the same modification is determined by the presence of rigor mortis or death of the muscle. The results are shown in Figs. 3 and 4.

FIG. 3.—Pigeon's striped muscle, living.

1. Vincent and Lewis, *loc. cit.*

FIG. 4.—Pigeon's striped muscle, dead twenty-four hours. Rigor mortis present.

EFFECTS OF TEMPERATURE ON AMPHIBIAN STRIPED AND UNSTRIPED MUSCLE (LIVING)

On testing the striped muscle of frogs living at the beginning of the experiment, we find (1) a marked contraction at about 39°C . (38° to 41°); (2) a contraction at about 50°C . (49° to 51°). These results are shown in Fig. 5.

FIG. 5.—Frog's gastrocnemius, living. Note contractions due to electric stimuli up to 42°C .

In unstriated amphibian muscle the same two contractions occur, but in this case there is always considerable relaxation between the two contractions. (See Fig. 6.)

FIG. 6.—Frog's stomach, living. Marked relaxation after the first contraction is well shown in this tracing.

Thus it will be seen that in amphibian as well as mammalian muscle of both kinds living at the beginning of the experiment we get two contractions, and we may assume that in both cases the first is due to the muscle protein, the second to the connective tissue.

FIG. 7.—Rabbit's striped muscle, dead one day. Rigor mortis present.

It is stated by Vernon, as quoted above, that the 37° to 40° C. contraction is absent in amphibian unstriated muscle, and this was

the opinion also of Vincent and Lewis.¹ There can, however, be little doubt that this contraction occurs, as I have obtained it in five successive experiments, and it is well shown in Fig. 6. The only differences, then, between amphibian and mammalian muscle are (1) that the two contractions occur at a lower temperature in the former case than in the latter (47° and 62° C. in mammalian and 39° and 50° C. in amphibian); (2) the marked relaxation after the first contraction in the case of amphibian muscle.

FIG. 8.—Rabbit's bladder, dead two days. Rigor mortis present.

HEAT CONTRACTIONS IN RIGORED MUSCLE

It has been stated¹ that 'if a muscle which has been passed into a state of rigor mortis be subjected to a rising temperature the above contractions are modified according to the stage the rigor has reached. When this is complete the lever writes a straight line until a temperature of 63° C. is reached, when it suddenly drops and falls off the drum.' My own experiments fully confirm this observation.

On gradually heating mammalian muscle, striped or unstriped, which is either in a state of rigor or has passed beyond this stage,

1. Vincent and Lewis, *loc. cit.*

we find that the first contraction, viz., at 47°C . never occurs. One contraction, and one contraction only, is obtained, viz., at about 63°C .

FIG. 9.—Dog's striped muscle. Rigor mortis present but not very pronounced.

FIG. 10.—Rabbit's striped muscle, dead three hours. Rigor not yet present. No reaction to electric stimulus.

On similarly testing rigored or dead amphibian muscle, striped or unstriped, we find analogous conditions. The first contraction

(at 38°C.) does not occur. Here again we get one contraction only, viz., at about 53°C.

FIG. 11.—Frog's gastrocnemius, dead ten days.

FIG. 12.—Frog's stomach, dead three days.

In the three groups of vertebrates, then, we obtain in rigored muscle only one heat contraction, due to effects upon connective tissue elements. That previous observers have not noted this in the case of frog's muscle is probably due to their having underestimated the lapse of time necessary to bring on rigor mortis in this animal. In many instances we have obtained the first contraction when the animal has been lying in saline solution apparently dead for several days, but in all these cases, on testing the matter, we have found that the muscles would respond to an electrical stimulus. If still further time

be allowed to elapse, so that no reaction will occur on stimulation, then the muscles will give only one heat contraction, viz., that due to connective tissue at 52° C. Further, if the animals are killed by chloroform, rapid rigor, as is well known, will ensue, and in the muscles from an animal so killed we get only the 53° C. contraction.

FIG. 13.—Frog's gastrocnemius, animal killed by chloroform. Rigor mortis very marked.

We have seen that when muscles are in a state of complete rigor mortis at the beginning of the experiment the first (protein) contraction is entirely absent. It must now be added that when the muscle is in a state of partial rigor a modified or slight degree of contraction may be expected at about 47° C.—see Fig. 10 and compare with Figs. 9 and 7 and 8.

The usual teaching in regard to this subject may be illustrated by the following quotation from a recent text-book.¹ 'It is found that the first shortening occurs at the coagulation temperature of paramyosinogen (47° to 50° C.), and if the heating is continued a second shortening occurs at 56° C., the coagulation temperature of myosinogen. If frog's muscles are used there are three shortenings, viz., at 40° , 47° and 56° C.'

It will readily be seen how widely this differs from the results detailed above. It is particularly to be noticed that no mention is made of any connective tissue shortening.

1. Halliburton, *Essentials of Chemical Physiology*, p. 177, London, 1904.

SUMMARY OF CONCLUSIONS

1. Both striped and unstriped mammalian living muscle on being subjected to a gradually rising temperature undergo two marked and comparatively sudden contractions: First, at about 47°C . (46° to 50°); Second, at about 62°C . (61° to 64°).

2. Striped and unstriped amphibian muscle under similar conditions likewise undergoes two contractions: First, at about 39°C . (38° to 40°); Second, at about 50°C . (49° to 51°).

3. Any muscle, striped or unstriped, mammalian, avian, or amphibian, taken from an animal in a state of rigor mortis, or from one which has passed beyond this stage, does not show on being subjected to heat, any primary contraction.

4. A muscle in a state of partial rigor will exhibit a lessened degree of the primary heat contraction.

5. The first contraction is due to the coagulation by heat of the protein present in the muscle fibre during life, the second to changes in the connective tissue elements in the muscle.

6. It thus appears very probable that the different protein substances which can be obtained from a saline extract of muscle are not actually present as such in the living tissue, and the probability exists that at the moment preceding death the muscle fibre contains as its characteristic protein constituent one complex body.