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# THE ENERGY LIBERATED IN TOTAL EXHAUSTION OF FROG'S MUSCLE.

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A SYSTEMATIC investigation has been performed on the total energy liberated anaerobically in stimulation to complete exhaustion (*a*) in normal muscles and (*b*) in muscles poisoned with iodoacetate (I.A.) in which no lactic acid is formed [Lundsgaard, 1930]. Very constant values were obtained, namely about 1·0 cal. per 1 g. of normal muscle, and about 0·42 cal. per 1 g. of I.A. muscle, each without regard to differences of temperature or of frequency of stimulation.

The same batch of Dutch frogs (*R. esculenta*) was used throughout. The sartorius muscles were mounted on a thermopile of the usual type [Hill, 1928], and put first in oxygenated Ringer's solution[1] for 2 hours, or longer, and then the oxygen was removed by bubbling purified nitrogen for at least 20 minutes through the solution in the muscle chamber. The solution was then replaced by nitrogen saturated with water vapour. To poison the muscle Ringer's solution containing 1 in 17,000 of neutral iodoacetate was introduced into the chamber, oxygen was passed for 30 minutes, then nitrogen for at least 20 minutes, after which the solution was withdrawn and replaced by nitrogen.

The muscle was stimulated directly at regular intervals (12 and 24 per minute) with single just-maximal break shocks from a Harvard induction coil. The thermopile was immersed in a thermostat either at 0° C. or at 20° C. The heat liberated by the muscle was measured by the myothermic method [Hill, 1928] by means of "area" calibration.

*Normal muscles. At* 20° C. the isometric twitches showed three stages: (*a*) of "Treppe," (*b*) of constant contractility, (*c*) of decreasing contractility tending to exhaustion. Correspondingly the deflection-time curve of the galvanometer showed at first a quicker and then a slower

[1] 0·675 g. NaCl, 0·02 g. $CaCl_2$, 0·015 g. KCl per 100 g. solution, freshly buffered with sodium phosphate mixture *p*H 7·2 (10 mg. P per 100 c.c.).

rate of rise until the end of the second stage: in the third stage the deflection gradually decreased until it reached the final base line. The latter was higher than the original base line by an amount corresponding to the increased osmotic pressure of the muscle [Hill and Kupalov, 1930; Hill and Parkinson, 1931].

The total anaerobic heat liberated in ten experiments was as follows (all in April):

| No. ... ... ... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Sex ... ... ... | M. | M. | F. | M. | M. | F. | F. | M. | M. | F. |
| Shocks per min. ... | 34 | 34 | 34 | 34 | 34 | 12 | 12 | 12 | 12 | 12 |
| Total heat, cal. per g. | 1·04 | 1·06 | 1·16 | 1·13 | 1·04 | 0·91 | 1·02 | 1·01 | 0·89 | 0·85 |
| Average... | | | 1·08 | | | | | 0·94 | | |

*Normal muscles.* At 0° C. the second stage is not distinguishable from the third, both showing a steady decrease of contractility as well as heat rate. The maximum deflection occurs later than the maximum of contractility; this is due to the time lag of the galvanometer system when used for temporarily accumulating heat. The total heat in five experiments was as follows (all in March):

| No. ... ... ... | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Sex ... ... ... | M. | F. | M. | F. | M. |
| Shocks per min. ... | 12 | 12 | 12 | 12 | 12 |
| Total heat, cal. per g. | 0·68 | 1·14 | 1·02 | 1·11 | 0·81 |
| Average... | | | 0·95 | | |

*I.A. muscles.* At 20° C. the three stages of contractility exist as in normal muscle. The second and third, however, are much shorter, and in the beginning of the third stage the first sign of contracture appears which soon develops its full strength. This applies both for frequent and for less frequent stimulation. The total heat liberated by poisoned muscle is very constant and unaffected by the rate of stimulation.

| No. ... ... ... | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Month ... ... | Feb. | Feb. | Feb. | Apr. | Apr. | Apr. | Apr. |
| Sex ... ... ... | M. | M. | M. | F. | F. | M. | M. |
| Shocks per min. ... | 34 | 34 | 34 | 34 | 34 | 34 | 34 |
| Total heat, cal. per g. | 0·49 | 0·55 | 0·44 | 0·37 | 0·55 | 0·41 | 0·46 |
| Average... | | | | 0·47 | | | |

| No. ... ... ... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Month ... ... | Mar. | Mar. | Mar. | Apr. | Apr. | Apr. | Apr. | Apr. | Apr. | Apr. | Apr. |
| Sex ... ... ... | M. | F. | F. | F. | M. | M. | F. | F. | M. | F. | F. |
| Shocks per min. ... | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| Total heat, cal. per g. | 0·37 | 0·43 | 0·49 | 0·34 | 0·36 | 0·44 | 0·46 | 0·41 | 0·45 | 0·52 | 0·46 |
| Average... | | | | | | 0·43 | | | | | |

The ratio of total heat to total tension is much larger ($Tl/H$ is smaller) in poisoned than in normal muscles. For example:

1. Normal muscle at 20° C., 12 shocks per min., total heat[1] 1·01 cal., total tension[1] 62,900 g. $Tl/H = 2{\cdot}92$.

2. I.A. muscle at 20° C., 12 shocks per min., total heat 0·41 cal., total tension 14,700 g. $Tl/H = 1{\cdot}67$.

3. Normal muscle at 20° C., 34 shocks per min., total heat 1·04 cal., total tension 47,900 g. $Tl/H = 2{\cdot}16$.

4. I.A. muscle at 20° C., 34 shocks per min., total heat 0·37 cal., total tension 7300 g. $Tl/H = 0{\cdot}92$.

This appears at first to be contrary to the statement that $T/H$ in a muscle twitch is unaffected by poisoning with I.A. [Fischer, 1931; Meyerhof, Lundsgaard and Blaschko, 1930; Cattell, Feng, Hartree, Hill and Parkinson, 1931.] The fact is that in the I.A. muscle, during the onset of contracture, much heat is liberated continuously between, as well as momentarily during the twitches. The total heat contains the former as well as the latter. This is easily shown by stopping the stimulation when the heat production is not found to cease altogether.

Intermittent stimulation produced about the same total heat as regular stimulation (all experiments in April).

| No. | Sex | Total heat, cal. per g. | Shocks per min. | Stimulated during |
|---|---|---|---|---|
| 1 | F. | 0·33 | 12 | 1st, 3rd, 5th, 7th, 9th, 11th, 13th min.—exhaustion |
| 2 | F. | 0·43 | 12 | 1st, 3rd, 5th, 7th, 9th, 11th, 13th, 15th min.—exhaustion |
| 3 | M. | 0·37 | 34 | The first 1½ min., 8th min.—exhaustion |
| 4 | M. | 0·47 | 34 | The first 2 min., 8th min.—exhaustion |

In some muscles liberation of heat and performance of mechanical work continue very much longer than in others. No difference, however, in total heat results. The initial tension, moreover, does not appear to affect the total heat.

*I.A. muscles.* At 0° C. the first stage of the heat production is similar to that of normal muscles at 0° C. The isometric record also during the first 1 or 2 minutes is similar to that of normal muscles, but later the diminution of contractility is more marked. It takes generally about 10 minutes (12 shocks per min.) for the twitches to be reduced to about one-tenth, and it is at about this time that the contracture begins to appear which, slowly increasing in strength, reaches finally a constant level. The

[1] Both reckoned per gramme of muscle.

tension of contracture (10 to 15 g.) is about two-thirds of that at 20° C. (15 to 20 g.). Contracture begins at 20° C. much earlier. The total energy, however, per 1 g. of poisoned muscle at 0° C. is remarkably equal to that at 20° C. (All experiments in March except the first two in February; all at 12 shocks per min.)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No. ... ... ... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Sex ... ... ... | M. | M. | M. | M. | M. | M. | F. | F. |
| Total heat, cal. per g. | 0·43 | 0·46 | 0·44 | 0·24 | 0·39 | 0·35 | 0·30 | 0·54 |
| No. ... ... ... | 9 | 10 | 11 | 12 | 13 | 14 | 15 | |
| Sex ... ... ... | F. | F. | M. | M. | F. | M. | M. | |
| Total heat, cal. per g. | 0·48 | 0·49 | 0·43 | 0·46 | 0·29 | 0·33 | 0·41 | |

Average 0·40.

It is possible to poison a muscle at 0° C. if only it be soaked for a sufficiently long time (2–4 hours) in I.A. Ringer's solution. Some muscles were prepared by this means, others by poisoning first at room temperature and then cooling to 0° C. No difference was noticed between these two groups.

With regard to the absolute values of the total anaerobic heat given above, it will be noticed that they are somewhat higher for the case of the I.A. muscle than those of Hill and Parkinson [1931], averaging 0·42 instead of 0·37. The total amount of phosphagen, and the stimulation maximum of lactic acid, may differ in different batches of frogs and at different times of year: and the procedure of cutting out, blotting and weighing the inter-electrode region of the muscle naturally involves some degree of personal discretion. The more careful removal, before weighing, of the solution adhering to the surface of the muscle is probably the chief cause of the difference observed.

## Summary.

1. The total heat liberated anaerobically by a frog's sartorius (March and April), in stimulation by a series of induction shocks to complete exhaustion, has been measured. It is independent of temperature and of frequency of stimulation. In normal muscles it is about 1·0 cal. per g.: in similar muscles poisoned with iodoacetate, in which lactic acid formation is impossible, it is about 0·42 cal. per g.

2. The ratio of total tension developed in a series of twitches to total heat up to the stage of complete exhaustion is considerably less in I.A. muscles than in normal ones. This is due to the fact that in poisoned muscles heat production occurs continuously during the onset of contracture, as well as during the individual twitches.

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