CXXXVI. STUDIES ON "NON-IRRITABLE" MUSCLE.

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THE condition known as "non-irritability" or "inexcitability," as distinct from fatigue, in amphibian muscle is one to which attention was drawn by Fletcher and Hopkins [1907], though Hermann [1867] had made observations which indicated that the phenomenon had to some extent fallen under his notice. In the course of the classical experiments of Fletcher and Hopkins these authors observed that occasionally the frog muscle that they were using ceased to be irritable, in spite of the absence of rigor and the fact that the lactic acid content was not at its maximum, and that the condition was therefore not one of ordinary fatigue. The nature of the phenomenon was not further studied at the time, and it was not until 14 years later that the work of Foster and Moyle [1921] was published. In their paper it was shown that the condition of non-irritability could readily be produced in frog's muscle by keeping it at a temperature of 0° in an atmosphere of oxygen, air or nitrogen, the time necessary varying according to the gaseous medium employed. Two or three days were sufficient in the case of nitrogen, whereas two or three weeks were required for oxygen, whole limbs being used in both cases. When oxygen was used there was no rigidity at the end of the period, a circumstance which is in conformity with the experiences of Fletcher and Hopkins.

Foster and Moyle then made an investigation of some of the chemical properties of amphibian muscle in the non-irritable state, and they found that heat, toluene, and mechanical injury all have the same effect as in the case of normal muscle; that is to say, all these treatments result in the production of large amounts of lactic acid. An extension of these studies to some of the physical properties led to the conclusion that the loss of irritability is associated with a redistribution of certain ions; perhaps as a result of changes in membrane permeability, or perhaps caused by adsorption on colloidal surfaces. This "ionic" theory was supported by the very important further observation that the loss of irritability was reversible; that by treating the inexcitable muscle with various solutions it was possible to recover a certain degree of irritability. Thomson's [1928] experiments on the internal conductivity of normal and non-irritable muscle, demonstrating as they do a drop

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of over 40 % in respect of the latter, furnish additional evidence that a disappearance of free ions is involved in the causation of the phenomenon. Dulière and Horton [1928], pursuing an investigation of non-irritable frog's sartorii, came to the conclusion that the loss of excitability was dependent upon the diffusion of potassium ions from the interior of the fibres to the interspaces between them.

There thus appears to be a fair accumulation of data which support the hypothesis of what Foster and Moyle called the “ionic” origin of non-irritability in muscle. Whether or not this be accepted, however, a further study of the phenomenon appealed to the present authors from the following standpoint. In view of the fact that the muscle can be restored to its original condition by immersion in certain solutions, it is obvious that it is not in any way irreversibly damaged; we have, in the circumstance that we can at will suppress the excitability of a muscle and at will restore it again, a means ready to hand of eliminating certain phases of the activity of the tissue, thereby leaving the way more open for a study of those that remain. The series of metabolic and energy-producing changes in muscle is so complicated that any technique by means of which it is possible to attain even a partial separation of these processes must be a valuable one. Any addition to the array of facts already discovered in connection with non-irritable muscle may therefore be of importance with regard to elucidation of the processes occurring in normal muscle.

The work to be described in the present paper may conveniently be divided into four sections, *viz.* A. The chemical effects of stimulation of non-irritable muscle; B. The thermal effects of stimulation of non-irritable muscle; C. The resting heat-rate of non-irritable muscle; and D. The caloric quotient of non-irritable muscle.

A. THE CHEMICAL EFFECTS OF STIMULATION OF NON-IRRITABLE MUSCLE.

As has been mentioned above, Foster and Moyle [1921] came to the conclusion that there are “no chemical differences” between non-irritable and normal muscle. They stated that “non-irritable muscle formed lactic acid in exactly the same circumstances as fresh irritable muscle,” by which they meant that the effect of heating the muscle, treating it with toluene, or subjecting it to mechanical injury was to give rise to the production of lactic acid whether the muscle was normal or non-irritable. Curiously enough, neither these nor any other workers tried the experiment of directly or indirectly stimulating the muscle electrically, in order to see whether any lactic acid was then produced. To do this becomes of importance, when it is considered that the mechanism of irritability is far from being understood. The fact that a non-irritable muscle gives no contraction on stimulation may be due to one of at least two causes, namely that (*a*) the mechanism by which lactic acid is formed from carbohydrate as a consequence of stimulation may be inhibited, or (*b*) lactic acid may still be produced in the ordinary way, but

may be unable to bring about any response in the muscle on account of derangement of the contractile mechanism.

Methods.

(i) *Production of non-irritability in muscles.* A number of skinned hind limb-pairs from frogs were suspended at 0° in a large closed jar, at the bottom of which was a layer of water, out of contact with the limbs, but whose presence was necessary to prevent desiccation of the muscles. Two limb-pairs were connected to silver wires leading outside the jar and were used as tests for irritability, but were not employed in the actual experiments. It was usually found that from 4 days to a week in air produced non-irritability, and that no bacterial or other contamination made its appearance in that time; nor was there any trace of rigidity.

(ii) *Recovery of irritability.* Non-irritable limbs were completely immersed in amphibian Ringer's solution, through which a current of oxygen was continuously bubbled. Excitability began to return as a rule within 20 minutes, as evinced by the response of the muscles to single induction shocks, and after an hour or two's treatment the muscles were in most cases strongly irritable. When the muscles were to be used for subsequent experimentation, it was naturally undesirable to give more shocks than could be helped, for fear of vitiating the results by causing accumulation of lactic acid.

(iii) *Estimation of lactic acid.* The muscles were dissected off the limbs as rapidly as possible on a cold plate and immediately ground in an ice-cooled mortar with sand and 5 cc. of ice-cold 20 % trichloroacetic acid. The pulp was washed into a measuring flask with further trichloroacetic acid and was made up with water to a total volume of 50 cc. Having stood overnight in the ice-chest, the contents of the flask were filtered, neutralised with saturated potassium hydroxide, and interfering carbohydrates removed by the usual copper-lime method. After filtering again, lactic acid was estimated by the method of Friedemann, Cotonio and Shaffer [1927].

(iv) *Estimation of total carbohydrates.* This was performed by the method described by Lohmann [1928].

Results.

Two non-irritable limb-pairs were weighed. All the muscles were then dissected off both right legs and immediately worked up as described above for lactic acid estimation. The limb-pairs were meanwhile weighed again, and the muscles of the remaining legs were stimulated for 10 minutes with continuous induction shocks. These muscles were at once dissected away after the cessation of stimulation and were also treated with trichloroacetic acid. The limb-skeletons were re-weighed; by these successive weighings the amount of muscle taken before and after stimulation was known. Two limb-pairs which had been rendered non-irritable in the same batch as the above two were allowed to regain their irritability in oxygenated Ringer's solution; they then underwent the same sequence of weighing, dissection and stimulation.

The results of the lactic acid estimations are comprised in the first experiment recorded in Table I, which contains also the protocols of other experiments of the same kind made at different times.

Table I.

		Weight of muscle (g.)	Mg. lactic acid per g.	Change in lactic acid content on stimulation
1. Non-irritable	Right legs	4.350	0.77	—
	Left "	4.581	0.76	-0.01
Recovered	Right "	3.118	0.28	—
	Left "	2.930	0.49	+0.21
2. Non-irritable	Right legs	4.59	0.90	—
	Left "	4.72	1.00	+0.10
Recovered	Right "	5.85	0.65	—
	Left "	5.80	0.89	+0.24
*3. Non-irritable	Right legs	5.732	0.87	—
	Left "	5.540	0.95	+0.08
Recovered	Right "	6.555	0.54	—
	Left "	6.391	0.90	+0.36
*4. Non-irritable	Right legs	4.224	0.65	—
	Left "	4.451	0.62	-0.03
Recovered	Right "	5.120	0.38	—
	Left "	5.340	0.64	+0.26
5. Non-irritable	Right legs	4.805	0.88	—
	Left "	4.721	0.93	+0.05
Recovered	Right "	5.004	0.58	—
	Left "	5.182	0.99	+0.41

* In these experiments the stimulation was direct; in the others it was indirect, *i.e. via the nerves.*

It is noticeable that the lactic acid content of the recovered muscles is always lower than that of the non-irritable ones. This can be ascribed partly to the diffusion of lactic acid in the Ringer's solution, and partly to a certain amount of oxidative resynthesis during recovery.

From these results the conclusion can be drawn that, although in non-irritable muscle the lactic acid-forming mechanism exists and, as Foster and Moyle showed, is able to function under a variety of conditions, nevertheless the ordinary type of stimulation is unable to bring it into play. What has been called the "trigger mechanism" seems to be incapacitated in some way, and the stimulus is unable to initiate the series of reactions leading to the production of lactic acid.

There remains however the possibility that although lactic acid does not appear, carbohydrates are nevertheless disappearing under the influence of the stimulation, but are undergoing some other transformation. To test this point it was necessary to repeat the above experiments, with the difference that instead of lactic acid, total carbohydrates were estimated before and after stimulation.

The results obtained are given in Table II.

Table II.

		Weight of muscle (g.)	Mg. carbo- hydrate per g.	Change in carbohydrate on stimulation
1. Non-irritable	Right legs	4.72	1.58	—
	Left "	4.66	1.62	+0.04
	Recovered Right "	4.90	2.01	—
	Left "	5.25	1.88	-0.13
2. Non-irritable	Right legs	3.68	1.70	—
	Left "	3.51	1.67	-0.03
	Recovered Right "	4.04	2.54	—
	Left "	4.19	2.24	-0.30
*3. Non-irritable	Right legs	4.83	1.76	—
	Left "	4.88	1.74	-0.02
	Recovered Right "	3.75	2.63	—
	Left "	3.92	2.35	-0.28
4. Non-irritable	Right legs	3.50	1.43	—
	Left "	3.61	1.45	+0.02
	Recovered Right "	4.21	2.10	—
	Left "	3.99	1.84	-0.26

* In this experiment direct stimulation was employed.

It is evident from this that in addition to there being no lactic acid production upon stimulation of non-irritable muscle, there is no disappearance of carbohydrate. After recovery in oxygenated Ringer's solution, the muscle is restored to its normal state and stimulation leads to the production of lactic acid and the disappearance of carbohydrate in the usual manner. The conclusion appears justifiable then that the phenomenon of non-irritability has its rise in a failure to act of the so-called "trigger mechanism" and not in a failure of the contractile mechanism to respond. The observation of Foster and Moyle, that stimulation of a non-irritable muscle produces no action current, is worthy of notice in this regard, for there is reason to suppose that the action current is a phenomenon associated with excitation and not with contraction.

B. THE THERMAL EFFECTS OF STIMULATION OF NON-IRRITABLE MUSCLE.

For the purposes of studying this question, use was made of the differential resistance thermometer described by McCullagh and Case [1931]. The gastrocnemius of the frog was the muscle employed, and it was dissected out from the non-irritable limb together with the sciatic nerve. The muscle was placed in the tube V [1931, Fig. 4] containing the heating coil, together with distilled water in such amount that the total heat capacity was equal to that of 3 cc. of water. (Ringer's solution could obviously not be used, as it would tend to bring about restoration of the muscle.) The nerve passed through a slot in the stopper of the tube V and lay across two small closely-placed electrodes of silver wire, which in turn were connected to two copper wires which passed up the tube 6 and thence to an induction coil. By this means the muscle could, when desired,

be stimulated *in situ* in the calorimeter without disturbing any part of the apparatus.

The technique of using the apparatus has been fully described in the previous paper and need not be enlarged upon here. After the bridge had attained equilibrium the muscle was stimulated for ten successive periods of 10 seconds, with 10-second intervals, using rapidly-recurring faradic currents from a coil. The balancing resistance values were noted at regular intervals for about an hour, at the end of which period the reading had returned to its original value. The tube containing the muscle was now removed and placed in a beaker of boiling water for 10 minutes, cooled, dried, and put back into the apparatus. When equilibrium was again reached, the stimulation from the coil was repeated in exactly the same manner as before, and fresh readings were taken. The reason for this procedure was that the passage of the stimulating current between the electrodes, although these were not actually enclosed in the tube V, might be expected to give rise to some heat production of a purely physical nature, which would be of the same magnitude whether the muscle were alive or had been killed by exposure to the temperature of boiling water.

Table III.

			Readings of shunt resistance (ohms)	
			Non-irritable muscle	Same muscle after heating
1.	Equilibrium		8327.8	8327.9
	5 mins. after stimulation		8328.3	8328.2
	10	”	8328.9	8328.9
	15	”	8329.2	8329.1
	20	”	8329.2	8329.2
	25	”	8329.1	8329.2
	30	”	8329.0	8329.1
	35	”	8328.8	8328.8
	40	”	8328.3	8328.2
	45	”	8328.1	8328.2
	50	”	8327.9	8328.0
	55	”	8327.8	8327.7
	60	”	8327.9	8327.8
			Constant	Constant
2.	Equilibrium		8553.6	8553.4
	5 mins. after stimulation		8554.0	8553.9
	10	”	8554.7	8554.6
	15	”	8554.9	8550.0
	20	”	8554.9	8554.8
	25	”	8554.8	8554.8
	30	”	8554.6	8554.7
	35	”	8554.4	8554.5
	40	”	8554.1	8554.2
	45	”	8553.9	8554.0
	50	”	8553.7	8553.7
	55	”	8553.7	8553.6
	60	”	8553.7	8553.5
			Constant	Constant

In all the experiments that were made, it was found that the heat production upon stimulation was extremely small, and moreover that it was not

altered after the muscle had been heated at 100° for 10 minutes. The slight heat changes that did occur, therefore, were to be ascribed to the physical effects of the passage of the current through the electrodes and nerve, and not to any chemical processes taking place in the muscle as a result of stimulation. This finding, that stimulation of non-irritable muscle leads to no heat production, was not unexpected in view of the chemical experiments already reported.

Some typical figures for these thermal experiments are given in Table III.

It is seen that the changes in resistance, which in themselves are very small, run so closely parallel in the cases of the unheated and the heated non-irritable muscle that there can be no doubt that they are not to be attributed to any physiological heat production in the muscle.

C. THE RESTING HEAT-RATE OF NON-IRRITABLE MUSCLE.

A determination of the resting heat-rate of non-irritable muscle under aerobic and anaerobic conditions would be expected to give some indication as to whether the metabolic processes are quantitatively different from those in normal muscle, and as to whether the oxidative reactions are still going on or are suppressed. Several experiments were performed in order to obtain values for the resting heat production, using the differential resistance thermometer and employing the technique described in the previous paper.

The muscle was dissected as carefully as possible from a non-irritable limb, which was weighed before and after dissection. The known weight of muscle was placed in the tube V of the apparatus together with enough distilled water to bring the heat capacity to that of 3 cc. of water. The necessary balancing resistance was determined in the usual way, after which the air in the tube was replaced by nitrogen, this gas being bubbled through the water also for several minutes before re-closing the tube and inserting it in the apparatus. The shunt resistance at equilibrium was again determined. Finally, the tube containing the muscle was placed in a beaker of boiling water for 10 minutes in order to kill the muscle, was dried, cooled, and replaced. A current was passed through the heating coil of such magnitude as to necessitate the same alteration in the shunt resistance as was found necessary during the anaerobic experiment with the live muscle, after which the current was increased so as to obtain the value for the aerobic half of the experiment. The potentiometer readings were taken in both cases, and the heat production calculated from the formula given in the preceding paper.

The figures for the different experiments are set forth in Table IV.

It follows from these figures that the resting anaerobic heat-rate of non-irritable muscle is in the neighbourhood of 0.094 g. cals. per g. of tissue per hour. (This is the mean value of the seven recorded.) The observed variation between 0.080 and 0.117 is by no means larger than is usually encountered in determinations of this nature. The figure 0.094 is in remarkably good agreement with the currently accepted value for the resting anaerobic heat of

Table IV.

No. of experiment ...	1	2	3	4*	5*	6*	7
Weight of muscle (g.)	1.32	1.41	1.52	1.11	1.28	0.72	1.50
Potentiometer reading (aerobic)	0.088	0.102	0.122	0.094	0.097	0.083	0.100
Potentiometer reading (anaerobic)	0.064	0.072	0.081	0.062	0.065	0.051	0.067
Heat production in g. cal. per hour (aerobic)	0.208	0.278	0.398	0.237	0.252	0.185	0.268
Heat production in g. cal. per hour (anaerobic)	0.110	0.139	0.176	0.103	0.113	0.070	0.120
Aerobic heat rate (g. cal. per hour per g.)	0.158	0.197	0.262	0.214	0.197	0.257	0.179
Anaerobic heat rate (g. cal. per hour per g.)	0.083	0.099	0.117	0.093	0.088	0.097	0.080

* In these experiments the muscle was rendered anaerobic by the addition of cyanide instead of the substitution of nitrogen for air. This procedure is seen to make no significant difference to the results.

normal frog's muscle; Hill for instance gives for this the figure 0.092 g. cal. per g. per hour.

Under aerobic conditions, there is not so much constancy in the actual figures obtained, varying as they do between 0.158 and 0.262 g. cal. per hour, with a mean value of 0.211. But the fact that this is more than twice as great as the anaerobic figure, and that in every instance the imposition of anaerobic conditions leads to a substantial fall in the resting heat-rate, furnishes evidence that in non-irritable muscle the oxidative mechanisms are not impaired.

Before the construction of the micro-calorimeter was completed, preliminary experiments along the same lines had been performed, using the Dewar flask technique which is described by Blaschko [1928] and employing large quantities of non-irritable muscle of the order of 20-30 g. The results obtained by this method, which are not incorporated in the present paper, were entirely in agreement with those here reported, in that the anaerobic heat-rate of non-irritable muscle was found to be the same as that of normal muscle, and that the aerobic heat-rate was usually about twice as large as the anaerobic. It was usually found that when this procedure was employed, the actual figures were if anything slightly lower than those yielded by the differential resistance thermometer.

D. THE CALORIC QUOTIENT OF NON-IRRITABLE MUSCLE.

The term "caloric quotient" is defined as the heat production (in g. cal.) associated with the anaerobic appearance of 1 g. of lactic acid. It is usually assumed to have a fairly constant value, in the neighbourhood of 390, and recent determinations under conditions of resting anaerobiosis and slight anaerobic fatigue by Meyerhof, McCullagh and Schulz [1930] did not result in any material alteration of this figure. Nevertheless, in the course of several unpublished experiments by one of us (D. R. McC.) it became apparent that this constancy was not invariably to be met with, and a number of instances were encountered in which the value obtained for the caloric quotient was very definitely higher than the accepted figure, although so far as was known

the muscle was in a perfectly normal condition. The fact that from time to time these high caloric quotients were obtained is of great interest from a biochemical standpoint, since the supposed constancy of this ratio is a weighty factor in the evidence for the belief that the only exothermic reaction of quantitative importance taking place in muscle is the transformation of carbohydrate to lactic acid. In cases where an abnormally high caloric quotient is observed, it is clear that there must be occurring other processes which are productive of heat and which do not culminate in the appearance of lactic acid.

The first experiments which were made with the object of studying the caloric quotient of non-irritable muscle were carried out by means of the Dewar flask calorimetric method, and the high figure obtained led us to repeat the observations, using the resistance thermometer, with a view to ascertaining whether the values for the caloric quotient were consistently high when the muscle was non-irritable. As will appear, this was found to be the case, and in no instance was a value lower than 600 obtained.

Technique.

It is desirable in experiments of this kind to avoid, so far as is possible, any injury to the muscle, as such injury leads to exaggerated lactic acid formation. It was customary therefore to use in each experiment the gastrocnemius muscles of two frogs; these muscles can be rapidly removed without damage to any of their fibres. After weighing, the gastrocnemii of the two right legs were placed in the tube V, together with the appropriate amount of water, while those of the left legs were placed in a similar tube with the same amount of water and were treated in an identical manner until the heat measurements were begun. The tubes were made anaerobic by the addition of $M/500$ cyanide and filling with nitrogen. Two or three hours were usually allowed for the apparatus to come to equilibrium, as it was found by experience that for the first hour or two after immersing muscles in water the heat production sometimes tended to be high and irregular, perhaps as a consequence of osmotic processes or perhaps on account of the utilisation of remaining traces of oxygen. At the time of commencing the readings, the tube containing the control muscles, which had hitherto been immersed in the bath, was rapidly cooled in a freezing mixture and its contents (muscle + water) were ground with the addition of a little sand and enough ice-cold 20 % trichloroacetic acid to bring the total volume to 10 cc. Lactic acid estimations were performed upon this, and thus the value for the commencement of the "heat-period" was known. (The assumption made here, that the lactic acid content of corresponding muscles from opposite limbs is identical, is invariably found to be a justifiable one.)

The experiments were allowed to continue for 20 to 24 hours. Preliminary tests had shown that at the end of such a period the muscles were in the same condition as when they were put into the apparatus; that is to say, normal

muscles remained irritable, and non-irritable ones remained non-irritable, but could be restored to irritability in oxygenated Ringer. Bacterial contamination was never encountered under these conditions.

At the expiration of the period, the contents of the calorimeter tube were treated exactly as the control muscles had been, and lactic acid estimations were performed. The apparatus was then calibrated in the usual manner, using 3 cc. of water in the tube V and passing a current through the heating coil.

Table V gives the results of a number of determinations made with normal *gastrocnemii*; a mean value of 375 is obtained for the caloric quotient. In Table VI are recorded similar experiments in which non-irritable muscle was substituted for normal muscle, and it will be seen that, while the values are considerably more variable, the average figure is much higher, *viz.* 740.

Table V. *Normal muscle.*

No. of experiment	...	1	2	3	4	5
Weight of muscle (g.)		1.20	1.38	1.09	1.23	1.06
Duration of experiment (hrs.)		24	20	20	24	20
Lactic acid before experiment (mg.)		3.9	3.5	4.3	4.5	3.2
Lactic acid after experiment (mg.)		10.9	10.3	9.6	12.4	8.5
Increase in lactic acid—A (mg.)		7.0	6.8	5.3	7.9	5.3
Potentiometer reading (volts)		0.063	0.070	0.059	0.071	0.060
Heat produced per hour (g. cal.)		0.107	0.131	0.093	0.135	0.096
Total heat produced—B (g. cal.)		2.57	2.62	1.86	3.24	1.92
Caloric quotient ($B/A \times 1000$)		367	385	351	410	362

Mean value of caloric quotient—375.

Table VI. *Non-irritable muscle.*

No. of experiment	...	1	2	3	4	5	6
Weight of muscle (g.)		1.31	1.02	1.22	1.05	1.28	1.17
Duration of experiment (hrs.)		20	22	22	20	20	24
Lactic acid before experiment (mg.)		4.4	3.0	3.7	3.9	4.8	3.0
Lactic acid after experiment (mg.)		7.5	5.1	8.2	6.5	8.8	6.2
Increase in lactic acid—A (mg.)		3.1	2.1	4.5	2.6	4.0	3.2
Potentiometer reading (volts)		0.067	0.056	0.068	0.060	0.069	0.059
Heat produced per hour (g. cal.)		0.120	0.084	0.124	0.096	0.128	0.093
Total heat produced—B (g. cal.)		2.40	1.85	2.73	1.92	2.56	2.23
Caloric quotient ($B/A \times 1000$)		774	881	607	739	640	697

Mean value of caloric quotient—740.

Since the resting anaerobic heat-rate is the same as in normal muscle, one is compelled to adopt the supposition that in non-irritable muscle the observed heat production is a result of changes other than lactic acid formation; changes which, owing to the suppression of the latter, assume an enhanced quantitative importance. It is not our intention at this stage to put forward any suggestions as to the nature of these processes. In view of these results it appears however that the value is considerably lessened of the supposed “constancy” of the caloric quotient and of the resting heat-rate as bases for the argument that lactic acid production is the main exothermic reaction taking place in muscle. Since the appearance of the work of Lundsgaard [1930] this argument has indeed become increasingly untenable, and there seems to be no escape

from the conclusion that the explanations, hitherto accepted as adequate, for the various thermal relations in muscular tissue will have to be considerably revised. Hartree, for instance [1931], has shown that poisoning by iodoacetic acid, while preventing lactic acid formation, has nevertheless no effect upon the heat distribution during contraction, and the same result has also been arrived at by Fischer [1931]. This may be compared with the fact reported in the present paper, that the resting heat-rate of non-irritable muscle is normal although less lactic acid is being formed. At the same time, the interference can scarcely be of the same, or even similar nature in the two cases, since stimulation of non-irritable muscle does not lead even to carbohydrate breakdown.

SUMMARY.

1. Upon electrical stimulation of "non-irritable" frog muscle, no lactic acid is formed and no carbohydrate disappears.
2. There is no heat production upon stimulation of non-irritable muscle.
3. The resting anaerobic heat-rate of non-irritable muscle is normal.
4. The oxidative mechanisms are intact.
5. The caloric quotient of non-irritable muscle is much higher than that of normal muscle, being of the order of 740 instead of 375.

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