

Acta Medica Okayama

Volume 4, Issue 4

1934

Article 7

DEZEMBER 1935

Action of Monoiodoacetic Acid upon Muscular Function. : Part 1. Action of Monoiodoacetic Acid upon Muscular Activity of the Skeletal Muscle of Frog.

Hisasi Kosaka*

*Okayama University,

Copyright ©1999 OKAYAMA UNIVERSITY MEDICAL SCHOOL. All rights reserved.

Action of Monoiodoacetic Acid upon Muscular Function. : Part 1. Action of Monoiodoacetic Acid upon Muscular Activity of the Skeletal Muscle of Frog.*

Hisasi Kosaka

Abstract

1. The threshold of a muscle poisoned with monoiodoacetic acid for galvanic and induction current increases as the poisoning proceeds, though it shows temporary decrease of the threshold at an earlier stage of the poisoning. 2. The height of the muscular contraction (isometric as well as isotonic) of a poisoned muscle decreases gradually as the poisoning proceeds. 3. A fully poisoned muscle has a longer chronaxie than that of a normal one. It is observed that there are two types of increasing chronaxie in the course of poisoning. The one is that the chronaxie remained practically unchanged though the poisoning progresses and suddenly increases at the moment when contracture sets in, while the other is that it increases gradually until at last the poisoned muscle goes into rigor. 4. The absolute refractory period of a poisoned muscle shows a marked increase as the poisoning proceeds. Sometimes, at an earlier stage of poisoning, a slight shortening of the absolute refractory period is observed. 5. The maximum work performed by a poisoned muscle shows a rapid diminution as the poisoning goes on. On the other hand, parallel with it, the total moment of inertia of the recording system must be increased in order to attain the maximum work. This fact suggests that the viscous property of a poisoned muscle increases and that the energy developed by activity is wasted in overcoming this resistance. 6. These characteristics displayed by a poisoned muscle may fairly be explained under the supposition that a poisoned muscle falls into some sort of fatigue. In conclusion I wish to thank Prof. S. Oinuma for his help and advice during this experiment.

From the Institute of Physiology, Okayama Medical College
(Director: Prof. Dr. S. Oinuma).

Action of Monoiodoacetic Acid upon Muscular Function.
Part 1. Action of Monoiodoacetic Acid upon Muscular
Activity of the Skeletal Muscle of Frog.

By

Hisasi Kosaka.

Received for the publication on the 27. May 1935.

Introduction.

Numerous researches by *Hill*¹⁾, *Meyerhof*²⁾ and their collaborators showed that the source of chemical energy of muscle is to be found in the reduction of glycogen to lactic acid and partial recovery of lactic acid to precursor. Down to most recent times, this view had been generally accepted and called "*Hill-Meyerhof's theory*" of muscular contraction.

However, *Lundsgaard*³⁾ (1930) showed independently of the previous paper by *Schwartz* and *Oschmann*⁴⁾, that muscle poisoned with monoiodoacetic acid may contract without lactic acid formation at all (alactacide muscle contraction), though after some 100 twitches it goes into characteristic contracture. Under this condition the phosphagen content of the poisoned muscle is nearly all exhausted. From the experimental results obtained, *Lundsgaard* offered a new working hypothesis that the energy supply for both normal and poisoned muscles lies in the heat of combustion when phosphagen resolves into creatine and phosphoric acid. In a normal muscle, the energy necessary for resynthesis of phosphagen is produced at the expense of the energy of the breakdown of glycogen to lactic acid. While in a poisoned muscle, this resynthesis cannot take place as there exists no lactic acid formation. According to his opinion, phosphagen is the material directly supplying energy for muscular contraction, while lactic acid formation in a normal muscle affords the energy for the decomposed phosphagen.

Judging from the fact that the energy supply for muscular contraction is chemical in nature, it is reasonable to suppose the difference in chemical processes in normal and poisoned muscles may indicate some differences in their mechanical performances.

It is the object of this investigation to test differences in the muscular functions of normal and poisoned muscles from the physiological point of view.

Experimental.

In all of the experiments, the isolated sartorius muscle of *R. nigromaculata* was used. A standard solution (1 p.c.) of monoiodoacetic acid was prepared and preserved in a colored bottle in a cool place to avoid its decomposition through the action of light and heat. Just before the use, this solution was exactly neutralised with sodium carbonate to prevent it from having any acid reaction on the muscle, and *Ringer's* fluid was added to make 0.01 – 0.005 p.c. solution. The muscles immersed in this solution, are fully poisoned after about 30 minutes.

The method, adopted by *Lundsgaard*, of injecting monoiodoacetic acid solution into the subcutaneous lymphsack of the frog, was rejected as the effect of the drug on the muscle is very variable between specimens.

Further details of experimental methods will be given later as they are used.

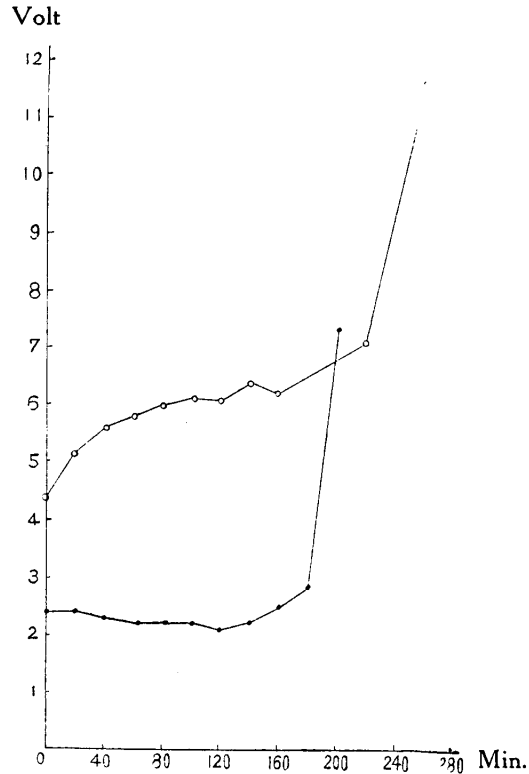
1. *Threshold for galvanic and induction current.*

After preparation, the sartorius muscle was immersed about 30 minutes in *Ringer's* solution, and the threshold of the normal muscle for galvanic or induction current was determined. Then the neutralised standard solution of monoiodoacetic acid was added to obtain a definite concentration (usually 0.01 p.c.). Thereafter, from this time, the course of the change of the threshold of the poisoned muscle was determined at a definite interval as poisoning proceeded.

As the process of the poisoning, as *Lundsgaard* noted, is an irreversible one, contrary to the action of fluoride⁵⁾, it is entirely impossible to determine the recovered state of muscle from poisoning. Therefore, as a control, many experiments were performed previously, to discover whether normal muscle alters its initial condition or not. It was fully confirmed that the initial condition of a normal muscle unaltered if care be taken not to raise the temperature of *Ringer's* fluid above 20°C.

Fig. 1 shows graphically the typical examples of the change of threshold for galvanic current. The similar results were obtained as to the threshold for induction current, but they are not represented in

Fig. 1. Threshold for galvanic stimulation of poisoned (monoiodoacetic acid) muscle (MIA).



Note: The voltage at 0 of abscissae represents the threshold of normal muscle.

the diagram because the real strengths of induction shocks were not determined.

The threshold of a poisoned muscle for galvanic as well as induction current gradually increases as the poisoning proceeds. Finally a poisoned muscle, sooner or later, goes into a peculiar type of rigor, which sets in spontaneously or as a result of stimulation. Sometimes, at an earlier stage of poisoning, the muscle shows a slight rise in its excitability. The time until rigor sets in, varies from one to another according to the condition of muscle, concentration of monoiodoacetic acid and frequency of stimulation.

2. Height of muscular contraction.

The maximum break induction shock for normal muscle was previously determined as usual and then the coil distance was

brought nearer by 1 cm to ensure further that the stimulating current was the maximum. The muscle was attached to an isometric lever which registered the height of contraction upon a smoked drum. From the height of contraction, the absolute value of the maximum tension developed was calculated and plotted as a function of time of poisoning, one of these is reproduced in Fig. 2.

Fig. 2. Maximum tension of MIA-muscle.

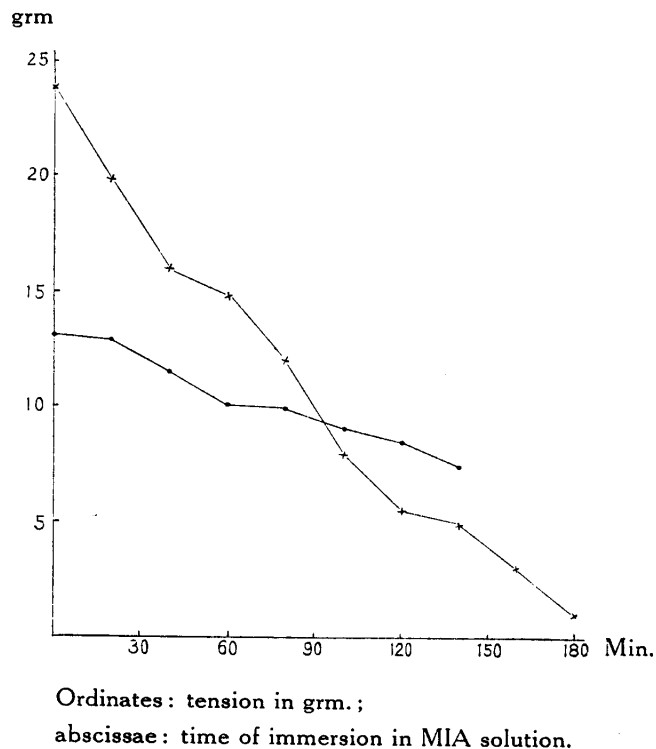


Fig. 2 shows clearly the maximum tension developed gradually diminishes as the time of immersion in the solution increased.

Also, the maximum isotonic contraction was determined which shows the same results as the isometric one. But in the former case, because of factors such as inertia of the recording system, speed of muscular contraction and change of muscle length during contraction, no conclusion can be reached from these results alone.

The rise of excitability at an earlier stage of poisoning does not affect the height of contraction. But at this stage, summated contractions by a single shock are apt to occur.

3. Chronaxie.

In connection with the threshold of a poisoned muscle above mentioned, what changes the chronaxie of a poisoned muscle may take, is a matter of interest.

Apparatus employed for determining the chronaxie is the well-known type of condenser discharge adopted by Lapicque. First of all, the rheobasic strength is determined by using a condenser 2 mF. capacity which will give a duration of discharge greater than effective duration for the tissue. Then the rheobasic voltage is doubled and the smallest capacity for excitement with this strength is determined. From the capacity of condenser and the resistance of the stimulating circuit, the chronaxie in absolute time units according to the following formulae is calculated;

$$\text{Chronaxie} = kRC$$

where

k = a constant proper to the instrument used,

R = resistance of the stimulating circuit in Ohms,

C = capacity of condenser in Farads.

All the precautions concerning the resistance of *Lapicque's* shunt and electrode were taken as *Lapicque* advised. Following the procedure previously cited, determinations of chronaxie were performed on normal as well as poisoned muscles.

They all show that the chronaxie of a poisoned muscle increases more and more in value as the poisoning proceeds, and finally a poisoned muscle goes into contracture with sudden increase of both its chronaxie and rheobase. Among them, two types of change are observed, i.e. the one is that both rheobase and chronaxie increase gradually to the last stage, while the other is that these are rather constant even if poisoning continues, until they suddenly increase at the moment when contracture sets in. The results are represented in Fig. 3.

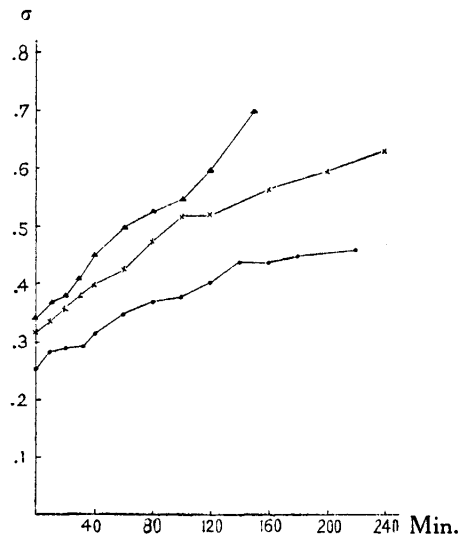
4. Absolute refractory period.

The absolute refractory period here referred to, is the least interval for muscular summation in the sense in which *Lucas* and *Adrian*⁶⁾ used the term. The apparatus and arrangement for determination are not here described as they were cited in my previous publication¹³⁾. The procedure of experiment for the normal as well as poisoned muscles is exactly the same as described above.

Fig. 4 shows the results obtained, which all show a marked prolongation in their absolute refractory period.

In accordance with the increase in excitability at an earlier stage

Fig. 3. Chronaxie of MIA-muscle.



Ordinates: chronaxie in σ ; abscissae: time of immersion in MIA solution.

of poisoning, shortening in absolute refractory period was sometimes observed. But this phenomenon does not always occur.

5. The realisable maximum work.

Hill⁽⁸⁾ constructed an inertia lever for frog's muscle and clearly showed by using this instrument, that the work performed by a muscular contraction varies with the moment of inertia of the recording system and the speed of contraction of a muscle at that moment. For instance, if the load on a muscle is too great, it shortens very slowly or not at all. So the potential energy converted into work is reduced and a larger part of the energy degraded into heat, while, on the contrary, if the load is too light, the muscle contracts rapidly and loses much of its energy in overcoming the viscosity of muscle. Therefore, the realisable maximum work may be attained by employing a recording system which has an adequate moment of inertia according to muscles contract more rapidly or slowly.

The changes may occur in the maximum work of a poisoned muscle was investigated as follows:

The muscles were poisoned as before. The realisable maximum work was determined at a definite interval (viz. 10-30 minutes) from the beginning of poisoning. The inertia lever employed was the same type as that of Hill. But the rod of the lever was made from

Fig. 4. Absolute refractory period of MIA-muscle.



Ordinates: distance of two successive stimuli;
 abscissae: time of immersion in MIA solution. (min.)

bamboo instead of steel in order that the weight of the lever might be as light as possible. If the weight of the lever is too heavy, the moment of inertia is not so finely changed by varying the distance of the mass from the axis of rotation. The heights of muscular contraction at different moments of inertia were registered on a kymographion, from which the maximum work performed was calculated.

The total moment of inertia of the recording system as well as the equivalent mass* at that time were calculated from the equation cited in *Hill's* paper. At the same time they were determined

* As shown in Fig. 5, a bamboo rod, B, resting on a knife edge, A, and carrying two balanced masses, M, is attached to a muscle at a distance (a) from the axis of rotation. The moment of inertia of the system about the axis when the muscle contracts, is equal to MK^2 , where M is the total balanced mass and K its radius of gyration. The movement is similar to that of a balanced mass, MK^2/a^2 , pulled directly by the muscle, which we term "equivalent mass".

experimentally. Both of them agree closely. Table I represents some of these experimental data.

It is obvious from the results that the total moment of inertia increases and the maximum work developed decreases as the poisoning proceeds. Consequently the equivalent mass increases gradually to a certain extent until at last it remains unchanged.

Potential energy produced by a poisoned muscle, as proved in the foregoing chapter, decreases, but the diminution of the realisable maximum work has a quicker rate.

From both the diminution of the maximum work and the increase of the equivalent mass of a poisoned muscle, we may consider that

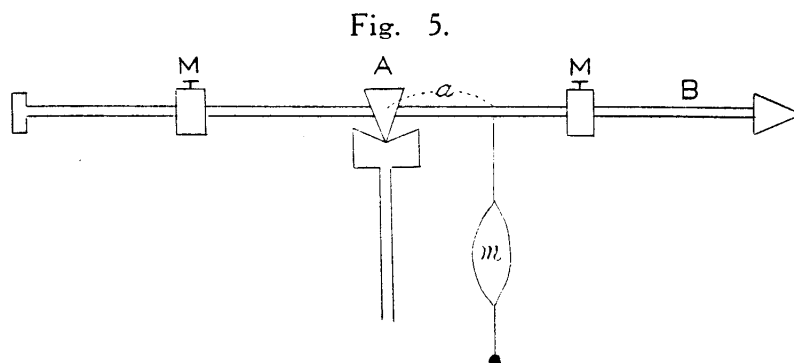


Table I.

No. of Experiments.	Norm. Muscle (in 0.6 % Ringer)	MIA-Muscle Time of Immersion in Monoiodoacetic Acid Solution (1/10000)							
		30 Min.	60 "	90 "	120 "	150 "	180 "	210 "	240 "
I	Max. Work	7.72	5.0	4.46	4.32	3.20	3.0	2.58	
	Equiv. Mass	265	265	313	313	313	313	313	
	Total Moment of Inertia	4240	4240	4990	4990	4990	4990	4990	
II	Max. Work	8.44	8.04	6.82	4.72	4.10	3.72	3.46	1.50
	Equiv. Mass	211	380	631	803	980	803	803	1080
	Total Moment of Inertia	3360	6080	10500	12800	15200	12800	12800	17220
III	Max. Work	6.56	6.34	5.04	4.20	3.74	2.70	2.04	
	Equiv. Mass	675	784	1020	1150	1150	784	1460	
	Total Moment of Inertia	10800	12500	16300	18400	20500	24300	20500	
IV	Max. Work	5.70	5.0	3.6	3.0	2.4	2.0	1.8	
	Equiv. Mass	662	999	1290	1290	1780	1780	2350	
	Total Moment of Inertia	10580	13940	18070	18070	22670	22700	33100	

Note: Equiv. Mass in gr.; Work in gr. cm. represented.

the potential energy developed and its conversion into mechanical work are largely affected by poisoning. The full importance of these results are discussed in the next chapter.

Discussion of results.

The experimental results as to a muscle poisoned with monoiodoacetic acid are not peculiar to poisoned muscles only, but general to fatigued muscles. The source of energy for activity in a poisoned muscle is solely phosphagen, the breakdown of which cannot be restored because there is no lactic acid formation. On the other hand, *Hukuda*⁹⁾ showed, in a poisoned muscle, that Tl/H is smaller than that of a normal one, and he considered that the spontaneous breakdown of phosphagen is the cause of this phenomenon. *Lundsgaard*¹¹⁾ observed the same phenomenon when a muscle is poisoned with monoiodoacetic acid of high concentration (above 1/5000). At the same time, a poisoned muscle, either stimulated or not, is sooner or later exhausted and goes into a peculiar type of rigor such may be seen as in the acid contracture in a fatigued muscle as a result of the accumulation of lactic acid.

According to *Nachmannsohn*¹⁰⁾, the phosphagen content of a muscle has a definite relation to chronaxie; i.e. the larger the content, the smaller the chronaxie. As poisoning proceeds, phosphagen breaks down continuously and diminishes its content, so the chronaxie of a poisoned muscle may be prolonged. This is the result of my own experiment. The results of *Lundsgaard* and others, which showed that the chronaxie of a poisoned muscle is not altered from that of normal ones, are to be considered due to the fact they performed their experiments on muscles which were in an earlier stage of poisoning. Also the decrease of excitability of a poisoned muscle for induction and galvanic current as well as the lengthening of the absolute refractory period indicates that the amount of phosphagen content has some relation to it, as in the case of chronaxie. In connection with these phenomena, the development of tension also diminishes. The essential cause is that the reservoir for energy supply is not filled because resynthesis does not occur. Here, again the results of *Lundsgaard* and others, contrary to my own, may be due to the fact that the degree of poisoning was at an earlier stage.

On the other hand, from the increase of the equivalent mass and the decrease of the maximum work of a poisoned muscle, it can be stated that the viscosity of a poisoned muscle increases and consequently the speed of the muscular contraction becomes slower as

well as the potential energy developed is wasted in overcoming the increased internal friction. And this increased viscosity is the essential cause that the time course of the diminution in the maximum work is faster than that of the potential energy.

All the results above described in connection with poisoned muscles are general characteristics displayed by fatigued muscles, and by this conception, all the phenomena displayed by a poisoned muscle may be explained satisfactorily. The "fatigue" here described is not an accumulation of the decomposed phosphagen as *Eggleton*¹²⁾ suggested.

Summary.

1. The threshold of a muscle poisoned with monoiodoacetic acid for galvanic and induction current increases as the poisoning proceeds, though it shows temporary decrease of the threshold at an earlier stage of the poisoning.

2. The height of the muscular contraction (isometric as well as isotonic) of a poisoned muscle decreases gradually as the poisoning proceeds.

3. A fully poisoned muscle has a longer chronaxie than that of a normal one. It is observed that there are two types of increasing chronaxie in the course of poisoning. The one is that the chronaxie remained practically unchanged though the poisoning progresses and suddenly increases at the moment when contracture sets in, while the other is that it increases gradually until at last the poisoned muscle goes into rigor.

4. The absolute refractory period of a poisoned muscle shows a marked increase as the poisoning proceeds. Sometimes, at an earlier stage of poisoning, a slight shortening of the absolute refractory period is observed.

5. The maximum work performed by a poisoned muscle shows a rapid diminution as the poisoning goes on. On the other hand, parallel with it, the total moment of inertia of the recording system must be increased in order to attain the maximum work. This fact suggests that the viscous property of a poisoned muscle increases and that the energy developed by activity is wasted in overcoming this resistance.

6. These characteristics displayed by a poisoned muscle may fairly be explained under the supposition that a poisoned muscle falls into some sort of fatigue.

In conclusion I wish to thank Prof. *S. Oinuma* for his help and advice during this experiment.

References.

- ¹ *Hill*, *Adventures in Biophysics* 1931. and *Muscular Activity* 1924. — ² *Meyerhof*, *Die chemische Vorgänge im Muskel*. Springer 1930. — ³ *Lundsgaard*, *Bioch. Z.* 217, p. 162, 1930; *ibid.* 227, p. 51, 1930. — ⁴ *Schwartz* and *Oschmann*, *C. R. Soc. Biol.* t. 91, p. 275 et t. 92, p. 169, 1925. — ⁵ *Lipmann*, *Biochem. Z.* 227, p. 110, 1930. — ⁶ *Lucas* and *Adrian*, *Jl. of Physiol.* 44, p. 68, 1912. — ⁷ *Kosaka*, now in press. — ⁸ *Hill*, *Jl. of Physiol.* 53, *Proc.* p. lxxxviii, 1919–20. — ⁹ *Hukuda*, *Jl. of Physiol.* 72, p. 439, 1931. — ¹⁰ *Nachmansohn*, *Biochem. Z.* 213, p. 262, 1929, and *Die chemische Vorgänge im Muskel*. p. 105. — ¹¹ *Lundsgaard*, *Biochem. Z.* 233, p. 322; 236, p. 219, 1931. — ¹² *Eggleston*, *Jl. of Physiol.* 63, p. 155 1927; *ibid.* 65, p. 15, 1928; *ibid.* 68, p. 193, 1929; *ibid.* 70, p. 294, 1930. — ¹³ *Kosaka*, *Okayama-Igakkai-Zasshi* 47 N. 3 G. p. 692.
-