The Effects of Electric Shock and Fatigue on Post-mortem Changes in Muscle*

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This experiment was made with use of albino-rats, as a preliminary test for learning the effects of electrocuting method upon post-mortem freshness of whale carcass, which Japanese whalers are now making trial of. The electrocuting method is a method which gives electrical shock to a whale through a harpoon shot into its body in order to kill it in a short time. When this method is used, there is no longer struggle before death such as seen in the hitherto used method. It is an original purpose to learn the effect of this struggle and electric shock upon the post-mortem freshness of whale carcass. In order to get some key authors made an experiment with use of albino-rats instead of whale body.

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Method

1. *Method to give rats fatigue.*

Rats were forced to swim for one hour in an enamelled cylindrical tank about 37 cm in diameter and in depth, at about 37°C.

2. *How to kill them.*

To electrocute rats, alternating current of 50 cycles was sent for about one minute into the tank filled with water with copper plates as electrodes. As mechanical shock, rats were heavily struck on their heads with a wooden hammer.

3. *Sampling.*

After a certain hours' storage of five albino-rats carcasses, each about 120 g. in body weight, in an incubator at 37°C, the same quantity of muscle was sampled out of hind leg of each rat for the following observations.

* Chemical studies on freshness of whale meat. V.

4. Measurement of pH.

Sample was brayed with a small amount of sea sand in the mortar and mixed with distilled water four times as much as the sample and its pH was electrically measured with antimony electrode.

5. Measurement of the amount of amino-nitrogen and volatile basic nitrogen.

10 g. of sample with some sea sand was well brayed in the mortar and mixed with 30 cc of distilled water. After 15 minutes' heating in the steam-kettle, supernatant was separated by a centrifuge and the residue was washed again and again with warm distilled water, of which the above supernatant was made to 100 cc. 1 cc of phenolphthalein solution, 20 cc of 10 % $BaCl_2$ and by 5 cc excess of saturated $Ba(OH)_2$ solution enough to neutralize the mixture were added to 50 cc of the above solution to precipitate phosphate and carbonate, and then through vacuum distillation at 45°C volatile base was collected in 0.02 n–H_2SO_4. Excess of the acid was titrated back with 0.02 n–NaOH to determine volatile basic nitrogen. The distilled water was added so as to make the remnants 100 cc. Amino-nitrogen was determined by Sörensen's formol titration with 50 cc of the filtrate.

6. Measurement of number of bacteria.

All treatments were aseptically done. With some emery powder the sample was well brayed. Then the physiological salt solution diluted it and the number of bacteria was counted in the ordinary way.

7. Lactic acid determination.

The sample was treated by Tanaka and Endo's method[1] to get a testing solution and lactic acid in it was determined by Friedemann and co-workers' method.[2]

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Results obtained

I. Comparative study of post-mortem changes in muscle between normal and fatigued rats electrocuted.

As see in Table I, both groups showed the lowest pH from 12th to 18th hour after death. Normal group showed always more acid than fatigued one.

Although small till 12th hour, the amount of volatile basic nitrogen was suddenly increased after that time. Till 12th hour there was

Toble I. Elecıocution.

Lapse of time after death (hrs.)	Normal*				Fatigued*			
	pH**	Volatile basic N. (mg/100 g)	Amino N. (mg/100 g)	Number of bacteria (in 1 g)	pH**	Volatile basic N. (mg/100 g)	Amino N. (mg/100 g)	Number of bacteria (in 1 g)
0	6.4	12.6	51.9	0	6.7	12.4	53.0	0
6	5.9	13.0	53.6	0	6.5	13.2	61.8	0
12	5.8	15.9	68.4	170	6.1	16.3	71.9	130
18	5.7	28.9	86.8	560	6.0	21.6	103.8	2720
〃	5.7	52.8	114.4	740	6.2	40.0	110.8	4320
24	6.2	107.9	186.2	166.4×10^3	6.3	132.0	193.4	27695×10^3
〃	6.4	143.6	245.9	countless***	5.6	114.8	184.0	countless***

Intensity of electric current through the water-tank:—— Normal group-96～100 V, 77～79 mA ; Fatigued group-96～100 V, about 160 mA (because water in the tank was soiled with excrement). Carcasses were kept at 37°C.

* Every time 5 rats of each group were used for measuring.

** About an hour passed from their dissection to measurment.

*** *It was macroscopically obvious that there were more bacteria in the fatigued group than in the normal group, though impossible to account for them.*

little difference between two groups, while at 18th hour, normal group showed larger amount of volatile basic nitrogen than fatigued one as seen in two instances. At 24th hour, there was no clear trend.

As for amino-nitrogen, till 12th hour a little larger amount of it was found in fatigued group and then it became irregular.

Bacteria were remarkably numerous in number in fatigued group.

No special feature was found by macroscopic observation of rat carcass till 6th hour. At 12th hour rather with offensive smell, gas generated in its intestine and swelled the abdomen. The difference between two groups was difficult to find. At 18th hour, both groups gave out a putrid smell and their muscle increased its viscosity and degree of grey colour and lost its elasticity. In both instances, however, fatigued group was seemingly better than normal group and kept still slight reddish colour and exudation on the side of carcass on the floor was never found or a little less than the normal group. At 24th hour, in the first experiment, the apparent difference between two groups was difficult to find. While, in the second one the fatigued group was distinctly better.

In short, in comparison between two groups on post-mortem changes, the results of comparison of macroscopic state of putrefaction

agreed quite well with that of the amount of volatile basic nitrogen but not with that of the number of bacteria. From pH and the number of bacteria, it was naturally expected that macroscopic putridity appeared stronger in fatigued group than normal one but the truth was not so. It is, however, too premature to draw the conclusion, for only a few experiments were made.

Rigor mortis observed in all experiments will be mentioned later.

II. Comparative study between normal and fatigued rats killed by mechanical shock.

Table II. Ceating to death.

Lapse of time after death (hrs.)	Normal*			Fatigued*		
	pH**	Volatile basic N. (mg/100 g)	Amino N. (mg/100 g)	pH**	Volatile basic N. (mg/100 g)	Amino N. (mg/100 g)
0	6.9	13.8	57.2	6.8	12.3	60.1
12	5.9	19.6	80.2	6.1	19.4	82.5
18	6.0	75.0	156.7	6.5	34.8	138.5
〃	6.2	110.0	166.0	6.8	132.8	188.5

* Same as in Table I. ** 30~40 minutes passed from their dissection to measurment. Carcasses were kept at 37°C.

As for pH, in both groups, at 18th hour already the turn towards alkalinity was found. Initially in the normal group, it was nearer neutral than the fatigued group and then it changed to more acid side. The increasing ratio of pH was rather smaller in the normal group than the fatigued group. This was probably due to the fact that the normal group contained larger amount of glycogen, so lactic acid was made more abundant in post-mortem glycolysis than the fatigued one. The amounts of volatile basic nitrogen and amino-nitrogen suddenly increased at 18th hour as seen in Exp. I. No definite difference could be, however, found between these two groups. As stated in I, comparison of the amount of volatile basic nitrogen agreed always with that of degree of rottenness in macroscopic observation.

III. Comparison between electric and mechanical shocks as method to kill normal rats.

As seen in Table III, the initial pH was in far more acid side in electrically shocked group than in mechanically shocked one. At 12th hour and 18th hour, their pH were approximately equal.

Table III.

Lapse of time after death (hrs.)	Electrocution *			Beating to death *		
	pH **	Volatile basic N. (mg/100 g)	Amino N. (mg/100 g)	pH **	Volatile basic N. (mg/100 g)	Amino N. (mg/100 g)
0	6.3	12.3	54.9	6.9	12.3	57.2
12	5.9	19.5	76.1	5.9	24.3	81.9
18	6.0	55.1	123.3	6.1	134.1	238.2

* and ** same as in Table II. Carcasses were kept at 37°C.

The volatile basic nitrogen and amino-nitrogen increased their amounts faster in mechanically shocked group than in electrocuted group. The comparative results between I and II can make it clear. This agreed with the result of macroscopic observation. Namely the mechanically shocked group was decomposed earlier than the electrocuted one.

This is probably due to heavy bruise on the head and to the change of pH in muscle to acid side through electric shock (*Confer* ***IV***).

IV. pH change of muscle through electric shock.

(*a*) *Comparison between normal and fatigued conditions.*

In the above experiments, normal rats electrocuted gave smaller pH in muscle than fatigued, electrocuted rats and normal, mechanically shocked ones. It is in general, however, that fatigued muscle showes more acid than not fatigued one. So the above result was probably due to the electric shock. This experiment was carried on for the purpose of assurance of it.

In order to measure pH as soon as possible after death, the sample, without braying, was closely attached to the electrodes in the shape of chop. About 5 minutes passed from death to measurement. Sample was obtained from the same part of hind leg as in the previous experiments.

Forced to swim in the above mentioned tank, rats were thrown into a small tank full of clean water and charged with alternating current. They were fatigued rats. Normal rats were directly thrown into the small tank. The current intensity was, therefore, different from that of the above experiments.

Probably due to the chop used, sensibility of the pH-meter was so bad and unconstant that it was difficult to measure pH with it. It could be affirmed, however, that electric shock changed pH in muscle

Table IV.

Normal, Electrocuted			Fatigued, Electrocuted			Normal, Beaten		
Sex	Weight (g)	pH	Sex	Weight (g)	pH	Sex	Weight (g)	pH
m	160	6.4	m	175	6.9	f	120	7.1
m	185	6.2	m	215	7.0	f	220	6.9
f	90	6.6	f	90	7.0	f	80	7.5
f	75	6.7	f	75	7.5	f	80	7.4
f	75	6.7	f	70	7.5	f	80	7.3
m	120	6.7	m	195	6.9	f	80	6.8

to acid side and normal rats showed smaller value in pH than fatigued one.

(*b*) *Effect of electric current upon pH of muscle cut off.*

After the normal rats were killed by mechanical shock, muscle of one side of their hind legs was cut off and it was hung in the water charged with the alternating current of 95 V and 45 mA for 30 or 60 minutes. Then brayed swiftly and fully with some quantity of sand. Distilled water four times as much as the sample was added to it and its pH was measured. For comparison, it was made with the use of the same part of muscle of another leg with no electric current flown. This result showed that the electric current gave no effect upon pH of muscle which was cut off carcass. So the change in pH to acid side by electric shock might take place only in the living body.

Table V.

Lapse of time after death (hrs.)	Electrifying time (seconds)	pH	pH (control)
0	30	7.1	7.3
0	60	7.0	7.0
0	60	7.0	6.8
1	60	6.4	6.4
2	60	6.1	6.1

Carcasses were kept at 37°.

(*c*) Any constant relation between the *current intensity*, *sex*, *body weight* and pH change was not found.

(*d*) *Cause for pH change.*

As the cause for pH change in electrically shocking, the authors first expected lactic acid and measured the amount of lactic acid of male rats normal and fatigued which were killed by electric or mechanical shock, instantly after death.

Table VI.

	Electrocution			Beating to death	
	Weight (g)	pH	Lactic acid (mg/100 g)	Weight (g)	Lactic acid (mg/100 g)
Normal	175, 200	6.2	84.5	190, 210	52.1
	110, 110	6.3	125.5		
Fatigued	175, 200	6.5	77.8	190, 210	75.9
	125, 135	6.9	95.0		

As shown in the above table, the amount of lactic acid ranks as follows.

normal rats electrically shocked > fatigued rats electrically shocked > fatigued rats mechanically shocked > normal rats mechanically shocked

Consequently the cause for pH change is nearly sure to lie in the growth of lactic acid.

Electric stimulus inspires the muscle and is followed by fatigue. So the increase of the amount of lactic acid in muscle is naturally conjectured. Rats are gradually fatigued through swimming and muscle glycogen is consumed and lactic acid accumulates. Grown lactic acid is however not all accumulated but a part of it is gradually excreted even in the process of fatigue. Now, when electric impulse stimulates very intensely the muscle of normal and fatigued rats, the former rats produce the considerable amount of lactic acid in a very short time and accumulate all of it without excreting. We can be ready to imagine that the amount is larger than the total amount of lactic acid accumulated before the electric shock and lactic acid newly produced through it in the latter. So pH is to be smaller in the former than in the latter. This is the authors' interpretation for the phenomenon that pH of the muscle of normal rats is smaller than that of the fatigued rats in electrically shocking.

V. Rigor mortis.

Rigor mortis observed in the above experiments is summerized as follows. Carcasses were kept at 37°C.

Table VII.

	Electrocution		Beating to death	
	Normal	Fatigued	Normal	Fatigued
Appearance (hrs. after death)	1~1.5	0.5~1	1.5~2	1~2
Beginning of Loose (hrs. after death)	4~4.5	3.5~4	3.5~4	

Rigor mortis began earlier and stronger and later got loose in electrically shocked rats than in the mechanically shocked ones.

Rigor mortis began earlier and got loose earlier in fatigued rats than in the normal ones.

Résumé

1. Either through electric shock or through mechanical shock the condition of rats instantly before death (normal or fatigued) did not give any definite effect on the post-mortem change of the amounts of volatile basic nitrogen and amino-nitrogen. The increasing speed of them is however larger in rats mechanically shocked than in the rats electrocuted. Namely the former rotted faster than the latter. From this result only, putrefaction seems to depend more on the killing method than on the condition instantly before death.

2. Number of bacteria on the electrocuted rats was always larger in fatigued group than in the normal group. The result of the comparison of number of bacteria, however, did not agree with that of macroscopic observation. On the contrary, the result of the comparison of the amount of volatile basic nitrogen agreed quite well with that of macroscopic observation.

3. In all experiments pH changed swiftly to acid side immediately after death. When rats were killed by mechanical shock, it began to turn towards alkalinity around 12th hour after death and when electrocuted, it began at 12th to 18th hour. About that time rot increased its degree. pH moved in more alkaline side in the fatigued group than the normal group.

4. The electric shock changed pH of muscle to acid side. In this case, pH of muscle of normal group was smaller than that of fatigued

group. This is probably due to the sudden rise of the amount of lactic acid caused by sudden and intense stimulus by electric shock.

5. *Rigor mortis* lasted longer and stronger in the electrocuted group than in the mechanically shocked group.

Literatures

1) Biochem. Z., **210**, 120 (1929).
2) J. Biol. Chem. **73**, 335 (1929).

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