A STUDY ON THE RELATIONSHIP BETWEEN THE EXTENT OF *RIGOR MORTIS* AND THE DIFFERENT POSSIBLE CAUSES OF DEATH IN RATS

by

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This is to certify that this undergraduate thesis, entitled "A Study on the Relationship between the Extent of Rigor Mortis and different possible causes of deaths in Rats" and submitted by Jonathan Emmanuel Arce Ignacio Sandejas to fulfill part of the requirements for the degree of Bachelor of Science in Biology was successfully approved on February 24, 1988.

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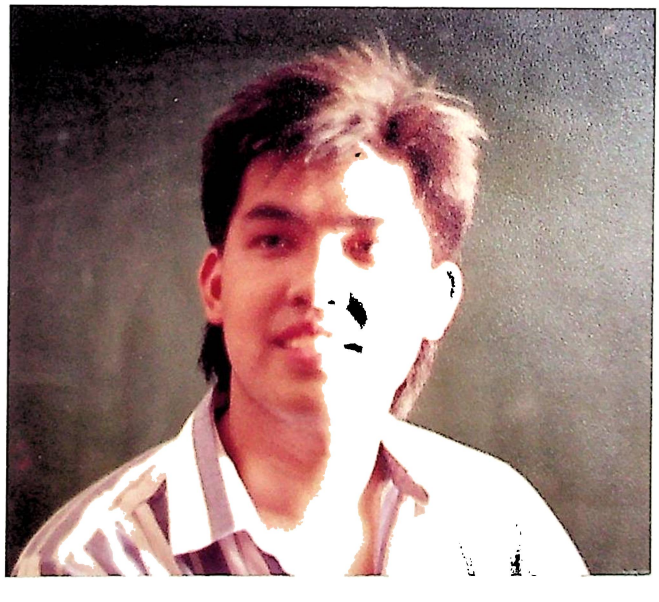
ABOUT THE WRITER

The writer, at present, is a graduating senior of the B.S. Biology program at the University of the Philippines (College of Arts and Science), Manila. He took his primary and secondary schooling at the La Salle Green Hills in Mandaluyong where he was a former varsity basketball player. His hobbies range from reading inspirational books, poetry writing, short story writing, collecting miniature objects and a host of other things. His interests vary from literature to the sciences.

After graduation, he will pursue a career in medicine. He hopes to specialize in a field which could benefit many individuals. He also dreams of establishing a clinic that can benefit the indigent and the needy .



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ABSTRACT

This research was conducted so as to determine whether a relationship does exist between the time needed for the onset of Rigor mortis and the different types of death that can be applied on to the rats. The rats were killed four different ways. They were killed through suffocation, strangulation, drowning, and laceration and it was discovered that the rate of Rigor Mortis was fastest for those rats that drowned. This was followed by strangulation, then suffocation and then by laceration. Since the loss of ATP can account for the manifestation of Rigor Mortis, it can be concluded that the activities of the rats prior to their death generally dictated how early this phenomenon will be exhibited. This is the only relationship that seems to be exhibited. Based on the statistical test used, it was established that there is a significant difference in the values that were taken. As to the existence of a relationship, there is no significant relationship that was established between the extent of Rigor Mortis and the different causes of death and this can be attributed to the very slight difference in the times for the manifestation of Rigor Mortis between those that died by strangulation and suffocation.

INTRODUCTION

The thesis of this writer deals with the property exhibited by the muscle after death which is referred to as Rigor Mortis. The purpose of this investigation is to determine the extent of Rigor Mortis and to determine

whether or not a relationship exists between the extent of Rigor Mortis and the different possible causes of death in rats. Based on muscle physiology studies conducted on some animals during a period of time after death, it was observed that this condition is manifested after 10 to 15 hours. The period of time often varies from species to species and this is often attributed to the differences in the muscles' structure and function which is a result of evolutionary adaptations. Though a lot of research work and several studies have been conducted by physiologists on the different aspects of Rigor Mortis, there is still so much to discover regarding the unexplained facts that characterize this condition. The fact that this condition occurs after death gives researchers the notion that it is not important and, instead, they choose to study the phenomenons associated with the living condition since their discoveries often find several immediate applications. Most of the research that is being conducted in the field of muscle physiology either deals with the effects of introducing pathological substances into the body and then studying its effects on the muscle as a whole or the probable effects that are dictated upon by the different hormones and/or steroids on the muscles. Therefore, it is the main objective of this paper to

analyze and determine whether a relationship really exists between the extent of Rigor Mortis in rats, as determined by the permanent contracture of the muscles which is recorded by the kymograph, with respect to the different causes that eventually lead to death in rats.

The purpose of this thesis may be considered as twofold. Firstly, the researcher hopes to find an explanation that can account for the differences in the manifestation of Rigor Mortis or in the post-mortem changes observed in muscle. This will be done with the use of valuable information that has already been published by other independent researchers. Secondly, the writer would like to determine whether other factors exists and how they may affect the test organisms, particularly the properties of their muscle prior to their death. This information will eventually be gathered during the course of the investigation and this will facilitate an easy analysis of the hypothetical relationship that is the focal point of this thesis. Aside from this, the writer chose to work on this particular thesis because of its inherent importance to the field of Forensic medicine. The layman's conception of this field is limited to the television series, "Quincy", which was featured in the late 70's. It is important to mention that the discovery of a relationship

could be used as a standard or basis for characterizing the muscles of different (dead) individuals and, at the same time, it can shed light to the possible causes of death. In this day and age where crimes are being committed every 15 seconds, the main preoccupation of murderers is the concealment of their crimes and oftentimes they even succeed in throwing off the experts. This thesis hopes to study other possible criterias or tests that may prove useful in correctly determining the cause of death. In so doing, we can be able to increase our knowledge regarding these post-mortem changes. The information that will be gathered from this thesis could also be used as a reference in studying other aspects pertaining to muscle physiology that may still remain a mystery to this day. There are several ways by which the information from this paper can be used. The study of the extent of Rigor Mortis, or of the pre-mortem condition of the muscle is helpful in determining the condition of the muscle prior to death as well as the cause of death which is often determined by doctors or practitioners of Forensic Medicine whose main preoccupation is to conduct autopsies.

REVIEW OF LITERATURE

In the introduction, the writer mentioned that although several advances are being made in the field of

muscle physiology, there are very few researchers who have made a serious effort to understand the phenomenon of Rigor Mortis as well as the various applications that can be made. In the course of studying muscle activity the writer was able to learn about the effects of certain stimulus on the rate and type of contraction. It is a fact that if a dissected gastrocnemius belonging to Rana is left overnight, it will not be suitable for studying the different types of muscle contraction on the next day. The fact the the muscle wouldn't be fresh would probably account for the malperformance of the muscle that was observed when it was subjected to the electrical stimulus that often produces a characteristic muscular contraction. It was later realized that the reason behind this was the effect of the permanent contracture of the muscle, a condition called Rigor Mortis. This was actually the first time that the writer ever experienced this phenomenon

In physiology experiments that deal with skeletal muscles of frogs, it was clearly shown how muscles contract depending on the strength and frequency of the electrical stimuli. After subjecting the muscle to repeated stimuli, the contractions reached a point called fatigue where the individual contractions appear to be fused. The behavior of the muscle was clearly

demonstrated after a short period of time after it was dissected. The writer was not able to subject the muscle to further stimulation prior to Rigor Mortis which should occur after an hour or so. It is imperative that the knowledge of the mechanism of contraction be known so as to enable us to fully comprehend the mechanism behind the post-mortem changes that are observed in the muscles after death.

In studying the mechanisms behind muscle contraction, it was discovered that the dissociation of myosin bridges from actin at the end of a bridge movement is achieved by the binding (not splitting) of a molecule of ATP to myosin. (Refer to Fig. 1.) Based on studies conducted by Vander, Sherman, and Luciano in 1975 they discovered that the process of binding ATP has an effect in breaking the linkage between the actin and myosin.

A . M + ATP ----------> A + M . ATP.

The reaction will return the bridge to its initial state so that it can now undergo binding to a new actin site and thus repeat the cycle of cross-bridge movement.

It was mentioned that the importance of ATP in dissociating actin and myosin at the end of a bridge cycle is illustrated by the phenomenon of Rigor Mortis (death rigor), in which the muscles of the body become very stiff and rigid shortly after death. They further

stated that this condition occurs directly from the loss of ATP in the dead muscle cells. In the absence of ATP, the myosin cross-bridges are able to combine with actin due to their natural affinity for each other. However, the bond between them is not broken causing the thick and thin filaments to become so cross-linked to each other that they cannot be passively pulled apart by stretching.

According to the research of Lippold (1981), there are certain properties of the muscle when they are subjected to certain extreme conditions. He discovered that in the resting muscle, the myofibrils offer very little permanent resistance to stretch because the thick and thin filaments can slide freely past one another . The lack of interaction between the actin ansd myosin under these circumstances is due to the "plasticizing" action of ATP when it is present but not being hydrolyzed because of the low Ca^{++} concentration. He added that if all the ATP is hydrolyzed, for example by stimulating an I.A.A. poisoned muscle to exhaustion, a powerful interaction between actin and myosin will occur, giving the myofibrils an almost crystalline structure. The myofibrils then become very inextensible and this state is referred to as rigor.

To this day, only a handful of works that specifically focuses on the analysis of the effects of

Rigor Mortis have been published. They are, nonetheless, important.

In August of 1985, a small group of physiologists set out to perform an experiment which would characterize certain post-mortem changes that are usually observed in the muscle secured from rats around 10 - 15 hours after death. They decided to kill rats in several different ways and the tension of the gastrocnemius was then measured to estimate Rigor Mortis as well as the ATP concentration in the femoral muscle. Based on the results, they learned that Rigor Mortis appeared and was established most rapidly in drowned rats killed by parathion poisoning, heat and finally by strangulation. The tension was greatest in rats killed by parathion poisoning, drowning, strangulation, followed by heat. The post-mortal concentration of ATP in the femoral muscle was seen to be very low in rats killed by drowning, parathion poisoning or heat than in control rats that were killed by extirpation of the heart. All in all, these results that were gathered can act as the groundwork for this research paper which is actually inspired by that investigation.

METHODOLOGY

1) Secure (15) 3-4 month old rats and place them in suitable cages.

2) Separate them by placing each groups of three rats into one of the four cages. (Get a cage with dividers so as to accomodate the rats). There should be five groups with 3 rats each.

3) Label group # 1 as the control group. This group will be allowed to live for a long period of time and it will be dissected while under the influence of chloroform.

4) For the succeeding (4) groups of mice, the same basic procedure will be used. This will be done after the rats have been killed in the different ways stated in the methodology. Prior to the experiment proper, it is advised that the researcher fixes his set-up so as to save time and to accomodate the length of time needed to secure the hind leg of the rat. He should already attach wax paper to the Kymograph drum and then proceed to smoke the paper so as to leave a sheet covered with black soot (see Figure 2). The drum should then be placed on the iron stand.

a) It is imperative that the researcher takes note of the time when the rat died and record it in the table provided above. Follow the instructions carefully and keep in mind that these things should be finished within 15 minutes. First, remove the fur covering the hindleg of the dead rat at the portion

connecting the leg to the body of the rat. After doing this, raise the fur using forceps or tweezers that have a very good grip.

b) Immediately after that, cut the fur while it is raised and continue cutting through the edges near the margin. After removing the fur, look for the delineation which is the point where the hindleg is connected to the lower body. Proceed by cutting the portion above the upper leg and then begin dissecting the whole leg.

c) Attach the whole leg to the clamps of the isometric recording system, which requires a kymograph that will measure the muscular contractions. See (Figure 3) for the technique in setting the muscle to the kymograph and then place the iron stand with the muscle and orient the pointer to the middle of the drum making sure that the pointer is in contact with the drum. All of these preliminaries should be done before 15 minutes has elapsed. This is one way of ensuring that the measurement will take place 15 minutes after death using 2 minute intervals after each electrical stimulation.

d) Wait for a period of 15 minutes after death before you begin with the actual measurement of

contraction because if you begin early, the results will hardly matter since the muscle hasn't reached the point of contracture. It is also important that they are measured at the same time so as to insure the accuracy of the measurements and to standardize the experiment proper.

e) Measure the contractions of the muscle for 120 second periods The kymograph should rotate slowly. During these 2 minute intervals, the muscle will be periodically stimulated by an electrode which will tap the muscle 10 times.

f) Observe whether the muscle will contract and observe the muscle will relax and return to its original state prior to contraction. After a while, the muscle will contract due to an electrical stimulus and it will not return to its original position before it was stimulated. This is the point of permanent contracture or Rigor Mortis. Take note of the time and record it in the table provided for. To be sure, wait for two minutes and then stimulate the muscle. If no change takes place, then it could be concluded that the muscle is in the state of Rigor Mortis. Take note of the kymograph reading. See the kymograph readings at the table of figures.

5) Prior to procedure 4, each pair of mice will be

killed 4 different ways that point to a violent death.

a) for group # 2, they will be subjected to a violent death by drowning. (See Figure 4).

i. Secure a large bottle (e.g. Nescafe) and then place the rat into the bottle facing the bottom of the jar then add water immediately till the water overflows.

ii. Cover the bottle with the lid then turn the bottle upside down.

iii. Then observe the rat and take note of the time when it expired.

iv. Remove the rat from the bottle then place it on a dissecting pan.

b) for group # 3, this group is to be killed by suffocation. Here, the absence of air is the method that will be used in killing the rats. (See Figure 5).

i. Place the rat inside a Nescafe bottle and then close the lid.

ii. Then observe the rat and take note of the time when it expired.

iii. Remove the rat from the bottle then place it on a dissecting pan.

c) for group # 4, this group will be killed by strangulation with the use of copper wire

(preferably) or a strand of rope that will be tied using a hangman's knot. (See Figure 6).

i. Tie one end of the rope to a long stick and then tie a loop and then insert the other portion of the rope into the loop.

ii. Place the rats neck into the loop and then, with your hand on the free portion of the rope, pull hard on it.

iii. When the rat has expired, place it on the dissecting pan.

d) for group # 5, the rat's life will be terminated by the laceration of the jugular vein of the rats.

i. Leave the rat inside the cage as it bleeds and make sure that the cut made was deep.

ii. When the rat has expired, place it on the dissecting pan.

6) After gathering all the results, place them in the table below and use the Analysis of Variance one way classification as the statistical test.

RESULTS AND OBSERVATIONS .

Based on the initial results gathered from the control group, it clearly shows that it took a much longer time for Rigor Mortis to set in. The 3 rats that

make up the control group took 44, 48 and 44 minutes, respectively, to manifest the phenomenon of Rigor Mortis which were the longest time(s) recorded for all of the rats. For the complete list of data that is being referred to, please refer to the tables concerned on pages.

Group 1 (Drowning)

It was observed that the rats that were subjected to drowning died immediately after it was submerged into the water with a mean dying time of 6.66 minutes. The recorded times for the occurence of Rigor Mortis were 36, 34, and 36 minutes respectively. Generally, the rats that were drowned struggled very much while it was submerged and it was noticed that this activity continued until the last minute prior to death. During this time, the rats were obviously engulfing large amounts of water and at the same time, it was loosing air from its lungs through his nose. The rat continously struggled to swim to the surface which is probably instinctive and the absence of air forced the rat to continously swim upward.

Group 2 (Suffocation)

It was observed that the rats that were subjected to suffocation did not die immediately. It took quite some time before the rats expired with the earliest time being 14 minutes while the latest was 20 minutes. This is

attributed to the presence of air inside the bottle at the beginning of the experiment. After a few minutes, the rat was slowly experiencing some difficulty breathing because of the dwindling air supply inside the bottle. It didn't struggle too much the way the drowning rat did but it did try to escape from the bottle. Externally, it was observed that the chest region was expanding enormously and this continued until death. After conducting the muscle experiments, it was learned that the respective times for the manifestation of Rigor Mortis was 30, 32, and 30 minutes respectively. This is much less than the results of the control and much faster than the other groups with the exception of drowning and strangulation.

Group 3 (Strangulation)

Next to drowning, this type of death is just as violent and it can be stated that the rate of Rigor Mortis supports this fact. The rats that were being strangled struggled intensely for a period ranging from 5 to 7 minutes as it tried to breath. The eyes were clearly dilated and after 4 minutes, became crystal clear as against its original red color. The muscles later became very tense as the rat began to succumb to the ill effects of the absence of air and blood circulation. Its tongue was purplish and bloated which suggests the entrapment of blood. The respective times observed for the

manifestation of Rigor Mortis was 30, 32 and 30 minutes respectively.

Group 4 (Laceration)

In this method, a relatively slow death was observed and this was similar in duration with suffocation. Here, the deep cut to the jugular vein of the rats resulted in the rapid loss of blood in the rats. Their activity was not violent but was quite restricted probably due to the effects of the injury to the rat's blood vessel.

DISCUSSION

Based on the results and observation gathered during the course of the experiment, it can be safely stated that the different causes of death can have an effect on the rate or the time needed for Rigor Mortis to be manifested. It was mentioned earlier that the loss of Adenosine TriPhosphate or (ATP) is responsible for the manifestation of this phenomenon primarily beacuse of the failure of the cross-bridges to separate from the actin filaments during the relaxation process.

In the control group, the rats were put to sleep with chloroform and their muscle was removed and tested. Based on the results that were gathered, it seems that the normal time for Rigor Mortis to be manifested falls in the range of 44 to 48 minutes. Using this as a standard for the other groups, it is clearly higher

indicating that the type of killing contributed significantly to the faster times for the manifestation of Rigor Mortis. It is important to remember that the rats of the control group were not killed and that they were only asleep. Their muscle can therefore be representative of pre-mortem muscle or the muscle prior to death.

The rats that were drowned exhibited Rigor Mortis at an early time ranging from 24 to 28 minutes. The best explanation that could be used for explaining the difference in the rates as compared to the normal times of the control group was the low concentration of ATP which, as an energy source, was depleted by the violent activity of the rat. The rat was swimming actively and it was utilizing both the upper and lower appendages. It was mentioned that this observation was the most significant among the different groups. Naturally, because of the decrease in the ATP concentration, the cross bridges and the actin filament fails to separate at an earlier time.

The rats that were killed by strangulation exhibited a much slower time for Rigor Mortis as compared to the rats that were drowned and this could be explained by the restriction in movement which resulted in a decrease in the activity of the muscle. However, the rat did struggle

but the movement of the rat was without purpose and unlike the drowned rats, their movement appeared to be restricted to the lower appendages only. This is probably the reason why the rat's lower limb began to tense at the last part of its life. It was observed that the eyes became crystal clear and this is indicative of the blockage of blood circulation from the heart to the brain resulting in brain death (caused by the absence of oxygen) and the subsequent death of the rat. In comparison with the time of Rigor Mortis of the rats of the control group, it was considerably faster with a range from 30 to 32 minutes. This simply means that the state of contracture took place at an early time with respect to the control.

For those rats that were killed by suffocation, it was observed that they exhibited Rigor Mortis at a later stage as compared to the drowned and strangled rats. The time it took for Rigor Mortis to set in was in the range of 34 to 36 minutes. Still, these figures are much faster than the actual time(s) of the control group. These rats did not die immediately and based on the results, it took between 14 to 20 minutes for them to expire. This can be attributed to the presence of a residual amount of air inside the bottle. It was further observed that the activity of the rats was not as highly charged as in the

case of the rats that were drowned and strangled. This probably is caused by the lack of Oxygen which restricted the rat's activity which was to breath slowly and deeply as indicated by the expanded thoracic cavity. Therefore, the decrease in the activity of the suffocated rat clearly accounts for the slower rate of Rigor mortis.

For the rats that were killed by the laceration of the jugular vein, it was observed that their behavior was initially active and they began to lose a lot of blood. Immediately after this, their activity was much slower and it could be a reaction to the loss of blood which would naturally decrease the amount of oxygen that is needed by the tissues. Nevertheless, their respective times for the onset of Rigor Mortis were still considerably faster than the control group. The recorded time was in the range of 34 to 40 minutes and this was the slowest recorded time(s) among the different experimental groups.

Based on these findings, it could be stated that the characteristic activity of the rats (during the killing process) generally dictates how early Rigor Mortis will take place. However, it must be mentioned that the closeness in the range of the recorded time(s) is indicative of the possibility that other factors may be responsible for the close values obtained.

CONCLUSION

There is clearly a relationship between the rates of Rigor Mortis and the different causes of death so long as the point of consideration is the activity of the rat which it is subjected to. However, based on the results, there are small differences in the ranges of these measured time(s) as in the case of the rats that were subjected to strangulation and suffocation whose range is between 30 to 32 minutes and 34 to 36 minutes. Had the range been much farther apart, then the relationship can easily hold true.

RECOMMENDATIONS

1) Before you begin working on the contractions, see to it that the electrodes you are using are functional and that they are indeed attached to the power supply. It is suggested that an adaptor be used in the event that the student would prefer to use varying voltages.

2) It is suggested that the rats be well fed before conducting experiments on them (unless starvation is required) so as to make these laboratory animals easy to manage since they bite. They have proven to be difficult when they are hungry and this can cause other problems.

3) Before killing the rats, it may be wise to let them inhale chloroform fumes so as to decrease their resistance and allow for better handling.

4) Make it a point to keep adding Ringer's solution because a solution of electrolytes would facilitate the flow of current from the electrodes to the sciatic nerve.

5) Test the electrodes by allowing the two points to come into contact with a metallic object (e.g. a blade), the presence of a spark is indicative of a current. In the event that the electrodes do not conduct a charge, it is suggested that the electrode be cleaned using a file or sand paper. Then test it again.

6) It is important to finish the dissection before fifteen minutes because there are some species of rats that undergo Rigor Mortis, 15 minutes after death. By doing so, the researcher can ensure that his rats will not undergo Rigor Mortis before subjecting the muscle to further electrical stimulation. At the same time, the researcher can standardize the experiment proper.

7) It is important to remove all other variables that are not involved in the experiment. By feeding them in exact proportions and by subjecting them to the same conditions, you decrease the risk of introducing a variable which may affect your results.

TABLES

Table 1

The Actual Recorded Times

Time ---->		killing was begun	of expiration	of measurement	Rigor Mortis occured
Type of Death	Date				
1 Drowning	1/14	1:30	1:37	1:52	2:20
	1/18	3:22	3:27	3:42	3:08
	1/18	4:20	4:28	4:43	4:07
2 Suffocation	1/21	12:56	1:16	1:31	2:07
	1/23	10:30	10:48	11:03	11:37
	2/04	2:33	2:47	3:02	3:38
3 Strangulation	2/04	3:40	3:45	4:00	4:30
	2/04	4:44	4:50	5:05	5:37
	2/04	5:52	5:59	6:14	6:44
4 Laceration	2/08	2:30	2:45	3:00	3:40
	2/08	3:56	4:17	4:32	5:10
	2/10	1:03	1:21	1:36	2:10
Time ---->					
Control	1/11	----	----	1:00	1:44
	1/11	----	----	1:52	2:40
	1/13	----	----	11:24	12:08

Table 2

Simplified Table for the Duration of Killing and the Time for Rigor Mortis to take place

Time --->	it took to die (in minutes)	for Rigor Mortis to manifest (im minutes)
1 Drowning	7	28
	5	26
	8	24
2 Suffocation	20	36
	18	34
	14	36
3 Strangulation	5	30
	5	32
	7	30
4 Laceration	15	40
	21	38
	18	34
Control	--	44
	--	48
	---	44

Table 3

Analysis of Variance of a One-Way Classification (time needed for Rigor Mortis to take place)

Treatment

	Control	Drowning	Suffocation	Strangulation	Laceration
1	44	28	36	30	40
2	48	26	34	32	38
3	44	24	36	30	34
Total	136	78	106	92	112
Mean	45.3	26.0	35.3	30.6	37.3

Computations

$$\text{Correction} = \frac{(524)^2}{15} = \frac{274{,}576}{15} = 18{,}305.066$$

$$\text{Total SS} = (44)^2 + (28)^2 + \ldots\ldots + (30)^2 + (34)^2 - C =$$

$$18{,}984 - 18{,}305.066 = 678.934$$

$$\text{Treatments SS} = \frac{(136)^2 + \ldots\ldots + (112)^2}{3} - C =$$

$$= \frac{56{,}824}{3} (-) \; 18{,}305.066 =$$

$$= 18{,}941.333 - 18{,}305.066 = 636.267$$

$$\text{Error SS} = \text{Total SS} - \text{Treatment SS}$$

$$= 678.934 - 636.267$$

$$= 42.667$$

Source of Variation	df	Sum of Squares	Mean Square	F
Treatments	4	636.267	159.066	37.287
Residuals (error)	10	42.667	4.266	
Total	14	678.934		

FIGURES

Figure 1. Splitting of the molecule of ATP to myosin.

Fe 2. Technique for attaching wax paper and for smoking it.

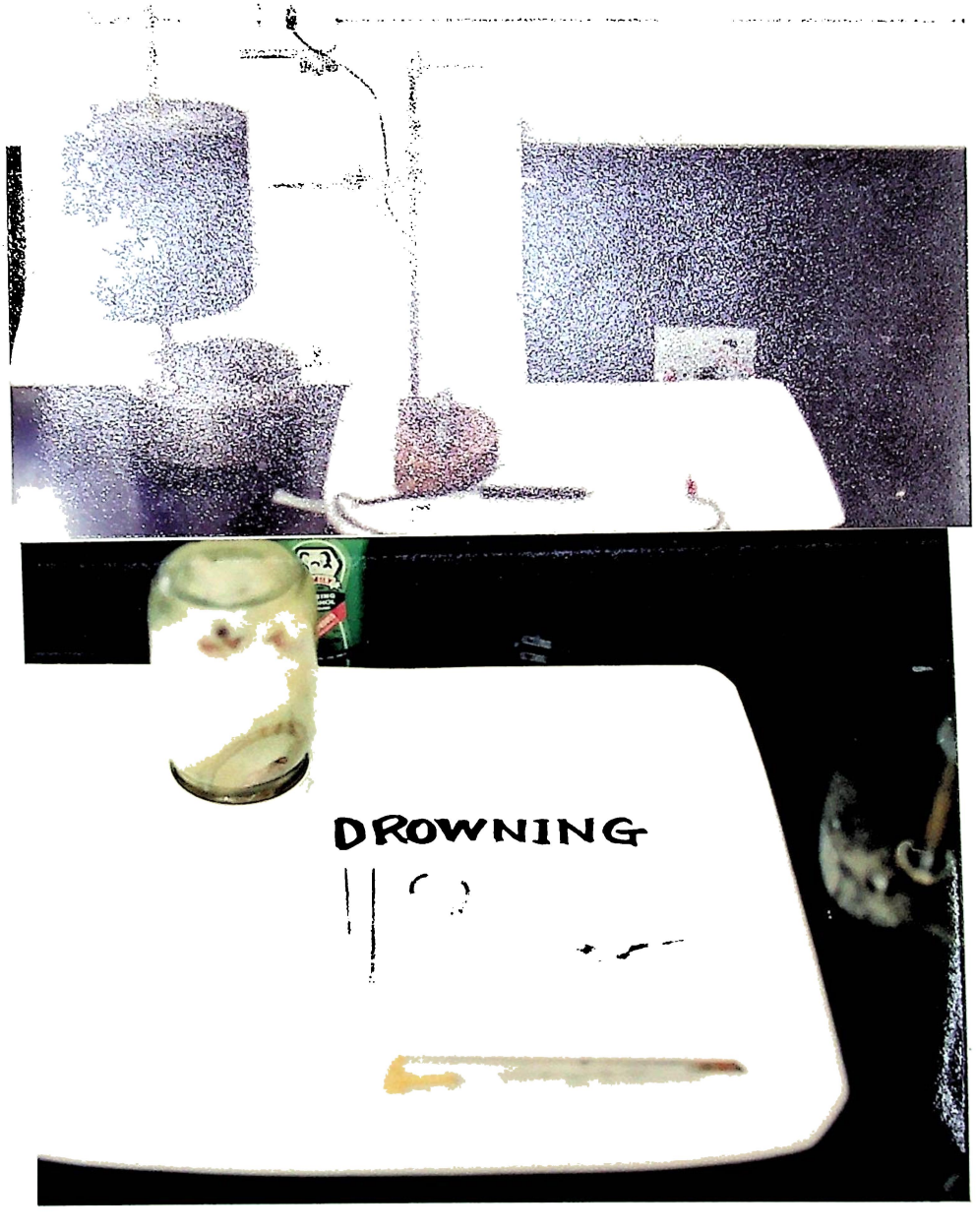

Figure 3. Technique for setting up the kymograph.

Figure 4. Procedure for drowning the rat.

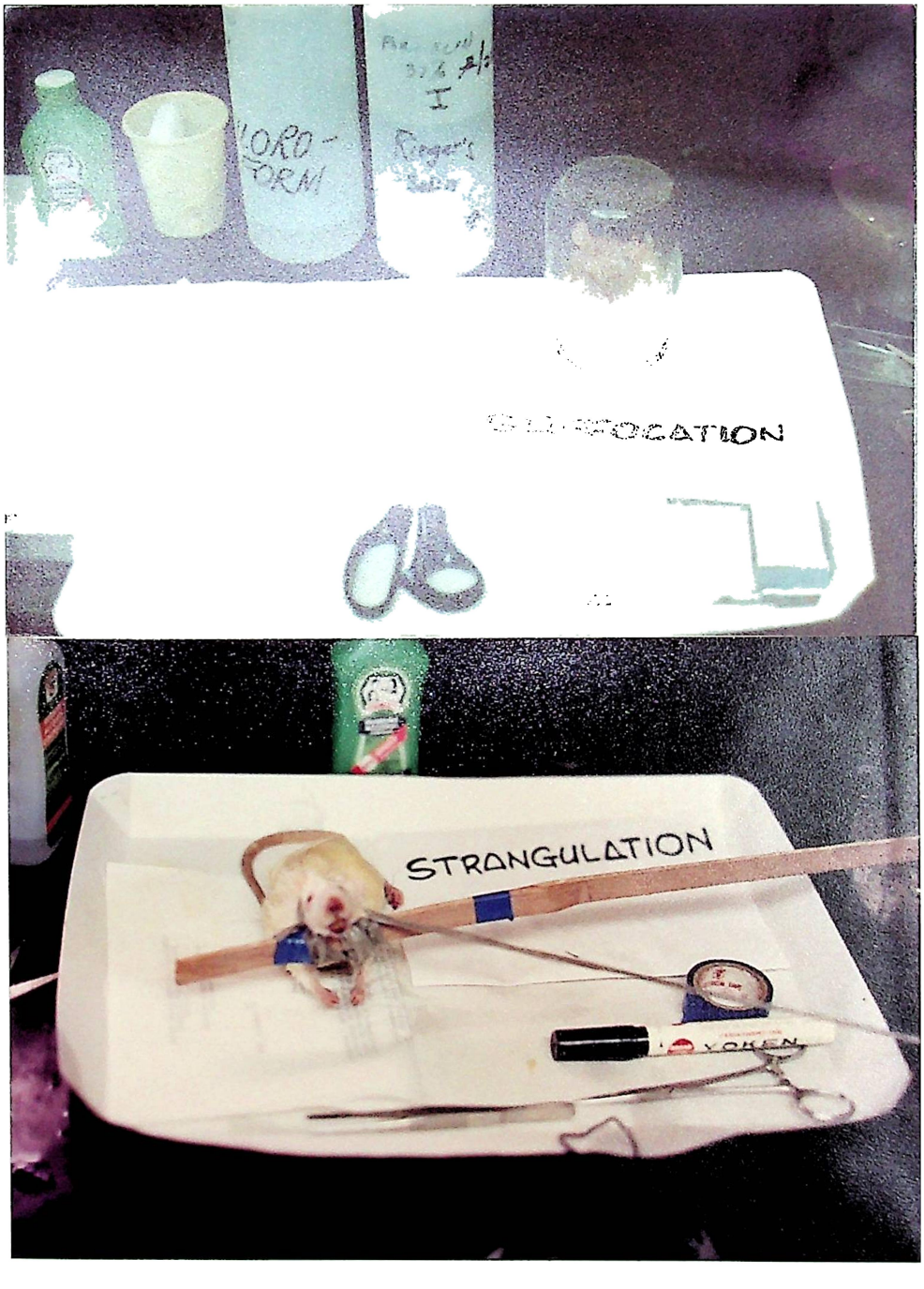

Figure 5. Procedure for suffocating the rat.

Figure 6. Procedure for strangling the rat

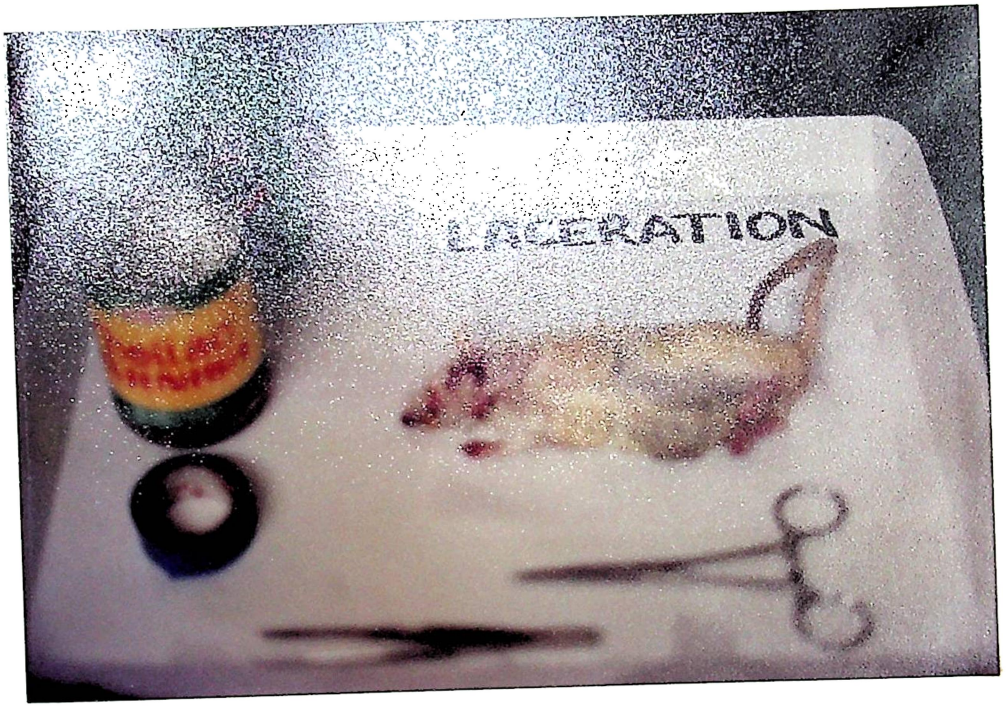

Figure 7. Procedure for lacerating the jugular vein of the rat.