

THE EFFECTS OF PRETREATMENT WITH EXERCISE ON ADULT MALE ALBINO RATS IN ACQUIRING CROSS-RESISTANCE TO COLD WATER IMMERSION

> Thesis for the Degree of M. A. MICHIGAN STATE UNIVERSITY Donald Peter Frank 1963

THESIS



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THE EFFECTS OF PRETREATMENT WITH EXERCISE ON ADULT MALE ALBINO RATS IN ACQUIRING CROSS-RESISTANCE TO COLD WATER IMMERSION

Ву

Donald Peter Frank

AN ABSTRACT OF A THESIS

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

MASTER OF ARTS

Department of Health, Physical Education and Recreation

1963

Approved:

ABSTRACT

THE EFFECTS OF PRETREATMENT WITH EXERCISE ON ADULT MALE ALBINO RATS IN ACQUIRING CROSS-RESISTANCE TO COLD WATER IMMERSION

by Donald Peter Frank

The purpose of the problem was to determine whether adult male albino rats can acquire cross-resistance to cold water immersion by pretreatment with exercise.

Thirty-three adult male albino rats of the Carlworth Farm's CFE strain of specific pathogen-free rats were used in this experiment. The animals in group I B were forced to swim daily carrying an additional five per cent of their weight attached to their tails until they reached the crucial point. The animals in group I A were forced to swim carrying a weight that would permit them to swim for a half hour. Group V and the control group were forced to swim only during the all out swims.

To determine whether the exercised animals had acquired cross-resistance to cold water the following procedure was used. All animals were loosely taped around the thorax, and a wire inserted into the tape. The wire was used to adjust the animal's depth in the water during the cold water immersion test. Survival time was recorded from the time the animal was placed in the water until drowning occurred.

Donald Peter Frank

The results indicate that adult male albino rats pretreated with exercise did not survive significantly longer, during the cold water immersion test, than the control animals. The weight of the control animals was significantly higher than the animals that were exercised to the crucial point daily. A correlation between body weight and survival time was .339, which is statistically significant at the .05 level. This indicates the heavier animals survived longer than the lighter animals.

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Dedication is made to my wife, Marilyn, whose patience and love has given me added purpose and encouragement to complete the task.

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The author wishes to acknowledge Wayne Van Huss whose professional assistance has given me confidence in the undertaking and completion of this task. Acknowledgment is also made to Wynn Updyke for his assistance while doing my research.

D.P.F.

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CHAPTER I

THE PROBLEM

Introduction

The problem of stress, since its formal presentation by Hans Selye (12) in 1936, has been an interesting and thought provoking area of experimentation. This can readily be confirmed by the numerous articles written on the subject of stress.

We, in physical education in general, have failed to realize the real implications of the stress theory in the area of physical education, since strenuous exercise is a stressor which produces physiological changes. We, in physical education, state that exercise is good for the normal individual, implying that the physiological changes are beneficial. Many authors (4, 8, 9, 10, 13) have stated the benefits of exercise upon animals or humans. However, few studies have shown whether pretreating an organism with a stressor will increase the organism's resistance to another stressor.

The experimental animals in this study were stressed using exercise. When the treatment phases were completed the experimental animals, along with the controls, were exposed to the cold water immersion. The data obtained determined whether pre-treating the adult male albino rats with exercise had elicited cross-resistance to cold water immersion.

Statement of the Problem

The purpose of the problem was to determine whether adult male albino rats can acquire cross-resistance to cold water immersion by means of pretreatment with exercise.

Importance of the Study

If it could be proven that exercised animals are able to withstand the stressor cold water significantly better than sedentary animals, then further research, using exercise as the means of adaptation to other stressors, might be performed. If animals which have been pre-treated with exercise are able to acquire cross-resistance to the effects of cold water immersion then one should consider the implications of exercise as a means of withstanding other stresses in humans.

Limitations of the Study

1. No attempt was made to control the amount of food or water consumed by the animals.

2. No attempt was made to control the humidity in the test rooms.

3. The exercise level might not be effective to bring about cross-resistance to cold water in the experimental animals.

4. The results of animals studied are not directly applicable to humans.

Definition of Terms

<u>Cross-resistance</u>. Cross-resistance is increased tolerance to a stressor other than that to which the body has adapted.

<u>Stress</u>. Stress is the state manifested by the specific syndrome which consists of all the non-specifically induced changes within a biological system.

Stressor. A stressor is that which produces stress.

<u>Control group</u>. Control group is used to denote that group of animals whose activity was confined to cages, except for the all out swims in phase three (see Appendix for all out swim time).

<u>Crucial point</u>. The crucial point is that time during the animal's swim when drowning will take place, according to the observer, if the animal is not rescued.

CHAPTER II

REVIEW OF THE LITERATURE

Selye (12) has stated that bodily changes take place as an organism reacts to stress. "Stress causes certain changes in the structure and chemical composition of the body. Some of these changes are merely signs of damage. 'Others are manifestations of the body's adaptive reactions, its mechanism of defense against stress."(12) The general adaptation syndrome is the total of all the changes that take place as an organism faces stress. This general adaptation syndrome has three stages: (1) the alarm reaction, (2) the stage of resistance, and (3) the stage of exhaustion.

When the alarm reaction stage is entered the organism's level of resistance is lowered, due to the effect of the stressor. This stage is manifested by enlarged adrenals, loss of weight, shrinkage of the thymus, stomach ulcers, and changes in the body's chemistry. During this stage one of two alternatives may happen. Either the organism adapts to the stressor and enters the stage of resistance or the stress is so intense that the organism dies.

The manifestations of the stage of resistance are quite different from, and in many instances the exact opposite,

of those that characterized the alarm reaction stage. The organism's adaptation to the stress during the second stage raises its resistance above normal. Although the organism can live in the stage of resistance, in time, the acquired resistance is lost and the organism enters the stage of exhaustion.

The symptoms of the stage of exhaustion are similar to those of the alarm reaction stage. During the alarm reaction stage the organism is able to adapt to the stress and lives. In contrast in the stage of exhaustion the organism can not adapt to the stress and dies.

Such stressors as cold, exercise, irradiation, and drugs can produce the general adaptation syndrome with its three stages. Selye (12) states that any one agent is a stressor in proportion to the degree of its ability to produce stress. In this experiment the stress was produced by the exercise.

Numerous articles have been written stating the effects of stress on animals and humans. The review of literature in this study was limited to those articles which dealt with the effects of cross-resistance.

According to the definition of cross-resistance it is feasible for an organism to increase its tolerance to stress by pre-treatment with any stressor.

The review of the literature dealt with the following: (1) pre-treatment with stressors other than exercise, and (2) pre-treatment with exercise.

Pre-treatment with Stressors Other than Exercise

Heroux and Hart (5) have demonstrated that adaptation to the stressor cold provided a degree of resistance to restraint hypothermia.

Bajusz and Selye (2) found that cardiac necrosis produced under experimental conditions can be prevented by pre-treatment with cold bath, noradrenaline, and restraint.

Egolinskii and Bogorad (15) established that training animals by regular cooling of the body increases the resistance of rats to hypoxia and irradiation.

Korobkov, Golavacheva, and Shkurdoda (7) state that increased resistance to irradiation can be acquired by injections of "dibazol."

Most studies found that cross-resistance can be acquired by pre-treating the animals with stressors other than exercise, however, some authors found that pre-treating animals with certain stressors would not produce crossresistance.

Egolinskii and Bogorad (15) stated that cooling did not increase rats' cross-resistance to heat.

Selye (11) found that pre-treatment of animals with corn oil prevented the cardiotoxic action of subsequent treatment with the same substance, but pre-treatment with corn oil did not prevent other stressors from eliciting cardiac necrosis.

Pre-treatment with Exercise

Bartlett,(3) using a group of twenty control animals and a group of twenty forced exercised animals, found there was a highly significant difference in the response of the two groups disclosing that the daily exercise produced a degree of inhibition to restraint hypothermia.

Bajusz and Selye (2) found that animals which were gradually pre-treated with exercise had developed crossresistance to cardiac necrosis when exposed to the full pathogenic dose of other stressors.

Korobkov, Golovacheva, and Shkurdoda (7) state that rats trained to climb a pole 1.5 cm. in diameter, in which small circular steps were cut every 5 cm., exhibited a greater tolerance to hypoxaemia at a height of 13,000 meters than control animals.

Zimnitskay (15) found that rats trained by swimming are more capable of withstanding the effects of cooling and heating than control animals.

Zimkin and Korobkov (15) found that rats' resistance to the action of trichlorethylamine was increased by muscular training. Eighty-six per cent of fifteen trained rats survived the poison, whereas only fifty-eight per cent of the twelve untrained rats survived. They further state that of the animals which died the trained animals survived longer.

Shernyakov,(15) studying whether the body can increase its resistance to certain infective illness through exercise, found that medical assistance was sought by fifty-eight per cent of individuals not doing any exercise, thirty-eight per cent by individuals who did exercise irregularly, and twentyone per cent by individuals doing regular exercise.

Trifonov (15), experimenting with rats, indicates that moderate physical impositions (swimming or maintaining position on a pole for 50-60 minutes daily over a period of six to eight weeks), increased cross-resistance to subsequent irradiation much more than an increase of three hours daily swimming,

Kimeldorf and Jones (6) found that exercised animals did not survive any longer than controls animals when faced with the stressor, irradiation.

Kudryashov and Nikolayeva (15) found no difference between trained and untrained animals when faced with the stressors strychnine and carbon monoxide. The same authors found that trained rats were less resistant to cyanide poisoning than untrained animals.

CHAPTER III

EXPERIMENTAL METHOD

The purpose of this experiment was to determine whether forced exercise could produce cross-resistance to cold water immersion in adult male albino rats.

Subjects

The thirty-three animals in this experiment were part of a group of 120 male albino rats which were used in another study. The Carlworth Farm's CFE strain of specific pathogen-free rats were used in this experiment. All animals were born on October 24, 1961. These animals were 59 days old when they were received in the laboratory, 61 days old at the beginning of phase one, 67 days old at the beginning of phase two, 151 days old at the beginning of phase three, and 227 days old when thirty-three of the animals were used in the cold water immersion test.

The thirty-three animals used in the cold water immersion test were previously treated according to the treatment of animal data sheets (see Appendix). After the treatment phase the animals used in the cold water immersion test were 227 days old. The animals used in the cold water immersion test were loosely taped around the thorax and a wire was inserted in the tape. The wire was used to adjust the animals' depth in the cold water and to prevent the animal from submerging. Survival time was recorded from the time the animals were placed into the water until drowning occurred.

Treatment of Animals

The diet of the animals consisted of Dietrich and Gambrill pathogen-free rat and mouse biscuits which were manufactured by Dietrich and Gambrill, Inc., Frederick, Maryland. Food was placed on the bottom of the animal's cage. Water was supplied to the animals by means of an inverted bottle which was fastened to the outside of the cage. A metal tube extended from the bottle into the cage from which the animals received water by placing their mouths at the end of the tube. Food and water were permitted ad libitum.

The animals were housed in individual rectangular cages which measured ten inches long, eight inches wide, and seven inches high. These cages were made of four by four hardware cloth and were fitted into a large mobile rack which provided housing for sixty animals.

Because the animals were used in another study the experimenter feels that an understanding of the animals' treatment prior to their cold water immersion test is imperative. The treatment the animals received was divided into three phases. Each phase is summarized on the animal data sheets (see Appendix). Along with the summary there is a statement of the objectives of each phase.

The rats which were forced to exercise were swum according to the exercise schedule (see Appendix). When the animals were forced to exercise a weight was attached to their tails by means of waterproof tape. The weight consisted of lead sinkers attached to wires. The dates when the animals were forced to swim are listed in the treatment of animal data sheets (see Appendix).

The rats swam in a galvanized tank that measured three feet wide, eight feet long, and thirty inches deep. Inside the tank were twenty-four plastic swimming sections each twelve inches wide, twelve inches long, and thirty inches deep. These sections prevented the animals from interfering with one another while swimming. The water temperature was kept between 35 and 37 degrees centigrade.

After completing the forced exercise the animals were placed in various cages to dry. The twenty-four drying cages were placed thirty inches beneath four 250 watt Ken-Rad infra-red heat lamps. The animals, after thirty minutes in the drying cages, were placed into their regular cages.

Cold Water Immersion Procedures

On June fifth, when the animals were 227 days old, the cold water immersion experiment was conducted. Group IA, IB, V, and a random selection of ten control animals were used. At this time there were six animals in group IA, nine animals in group IB, and eight animals in group V. The experimental procedures were conducted in the following way: A wooden frame was hung from the ceiling, thirty inches above the eight by four foot immersion tank. After weighing an animal a ten by two inch strip of waterproof adhesive tape was loosely placed under the animal and brought up on both sides of the thorax to the nap of the animal's neck (see Appendix for weight of animals). The two inches of excess tape were taped together above the animal's neck.

The ends of a ten inch piece of wire were bent into the shape of hooks. One hook was inserted into the excess tape of the animal. The other hook was attached to the hardware cloth. The hardware cloth was connected to a wire which was suspended from the wooden frame. The animal's depth in cold water was adjusted by placing the wire at different positions on the hardware cloth.

The animals were attached to wires and then placed in water which was kept between fourteen and sixteen degrees centigrade. (See Appendix, Figure 1, for the cold water temperature chart.) In order to keep the animal's body below the surface of the cold water a twenty-eight gram weight was attached to the animal's tail. After placing an animal in the cold water the wire was adjusted periodically so that the animal's head was kept above the cold water. No animal had to swim to keep its head above the cold water; however, every animal swam quite rapidly for about twenty minutes after being placed in the water. While the animals had control of their physiological functions they could keep their heads out of the water. When the animals began to lose control of these functions, because of the anesthetic effect of the cold water, their noses would drop into the water. The animal's nose dipped into the water five to seven times before the rat could no longer lift its nose out of the water for the purpose of breathing. When an animal's nose would dip into the water, the time was recorded. If the animal's nose remained below the surface of the water for five minutes the animal was considered unable to withstand the stressor cold water.

The survival time was measured from the time the rat was placed into the cold water until the animal could no longer raise its head to inhale. The survival time is recorded in minutes (see Appendix). It should be noted that the length of the animal's survival time was related to its ability to withstand the cold water stressor; however, actual death occurred because of drowning.

CHAPTER IV

RESULTS

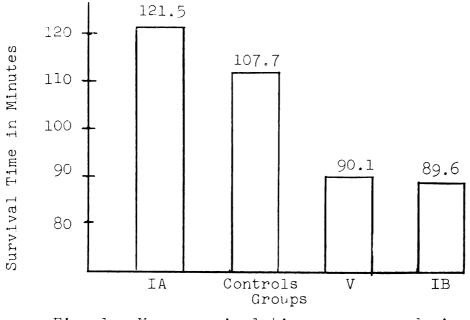
The following groups were used in this experiment to determine whether pre-treatment with exercise will elicit cross-resistance to the stressor cold water immersion: Group IA, six animals; Group IB, nine animals; Group V, eight animals; and ten control animals.

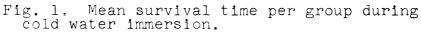
Survival Time

Survival time was measured according to the procedure discussed earlier. The null hypothesis was tested on the basis that no difference existed between the groups. The variance, due to the treatment, was not significant at the five per cent level (F=2.20), therefore, the null hypothesis was accepted. Data are presented in Figure 1, and the analysis of variance in Table I.

Weight of Animals

All animals were weighed, on June sixth, prior to the cold water immersion test (see Appendix). The null hypothesis was tested on the basis that no difference exists between groups. The variance due to the treatment was highly significant at the .001 level (F-7.64), therefore, the null hypothesis was rejected. The data is presented in Figure 2, and the analysis of variance in Table II. These results





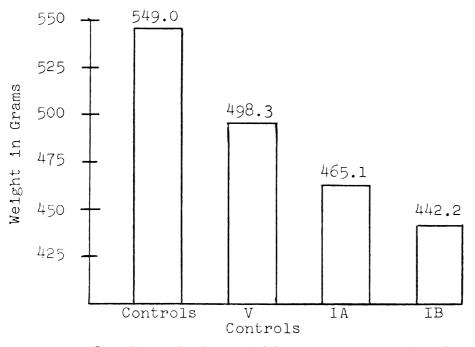


Fig. 2. Mean body weight per group prior to cold water immersion test.

confirm previous work indicating the greater the exercise stress the lower the weight of the animal.

TABLE I

ANALYSIS OF VARIANCE DATA FOR SURVIVAL TIME DURING THE COLD WATER IMMERSION TEST

Source of Variance	Sum of Squares	Degree of Freedom	Variance Estimate	F	F.05
Total	27382,	32			
Between Means	5072.49	3	1690.83		
Within Groups	22311.	29	769.3	2.20	2.93

TABLE II

ANALYSIS OF VARIANCE DATA FOR WEIGHT OF ANIMALS

Source of Variance	Sum of Squares	Degree of Freedom	Variance Estimate	F	F.001
Total	134,598	32			
Between Means	59 , 419	3	19,806.33	7.64	7.12
Within Groups	75 , 179	29	2,592.37		

Survival Time and Body Weight

In order to determine if a correlation between survival time and body weight exists the Pearson "r" statistical method was used. The correlation between body weight and survival time was .339, which is statistically significant at the .05 level. The heavier animals survived longer than the lighter animals.

Discussion

The results indicate that no difference exists between pre-treated exercised rats and control adult male albino rats in their ability to withstand the effects of cold water immersion.

At the time of the cold water immersion test the control rats were significantly heavier than the experimental rats which were trained to the crucial point daily. Their weight was not significantly different from the other groups.

When the weights and immersion test survival times for the animals in all groups were pooled, a significant relationship was found between weight and survival time. Considering the insulating properties of fat this was an expected finding.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The purpose of this experiment was to determine whether exercised adult male albino rats can acquire cross-resistance to cold water immersion. The animals used in this experiment were the Carlworth Farm's CFE strain of specific pathogen-free rats. The animals were treated in another study prior to the cold water immersion test. The rats in Group IA were forced to swim for thirty minutes, carrying an overload which was gradually increased daily. The rats in Group IB were forced to swim, carrying an additional five per cent of their body weight, until they reached the crucial point. The rats in Group V were exercised during phase two, but during phase three their activity was confined to cages except for the three all out swim tests. The rats in the control group were confined to cages during phase two and three except for the three all out swim tests.

The cold water immersion test was conducted on June sixth using thirty-three adult male albino rats. The groups used in the cold water immersion test were: group IA, six animals; group IB, nine animals; group V, eight animals; and ten control animals. In conducting the experiment a wooden frame was hung from the ceiling, thirty inches above the immersion tank. Wires were suspended from the wooden frame. At the end of each wire was a strip of hardware cloth enabling the experimenter to adjust the animals' depth in the water.

Each rat was weighed prior to the cold water immersion test. After weighing the animals, a ten inch strip of waterproof tape was placed around the rats' bodies to the nape of their necks. A wire was hooked through the excess tape, the opposite end of the wire being hooked to the hardware cloth. The wire was adjusted periodically so that the animals' bodies were submerged except for their heads. The animals had a twenty-eight gram lead sinker attached to their tails to keep their bodies from floating. The water temperature was kept between fourteen and sixteen degrees centigrade.

The survival time was measured from the time the rat was placed into the cold water, until the rat could no longer raise its head to inhale.

The results indicate that no significant difference exists between exercised rats and sedentary control rats in their ability to survive during cold water immersion.

Conclusion

Adult male albino rats pre-treated with the stressor exercise did not survive significantly longer during the cold water immersion test than control animals.

Recommendations

Research should be done to find out if there is an optimum exercise level which will produce cross-resistance in adult male albino rats.

Research should be done to determine whether other forms of exercise will bring about cross-resistance in adult male albino rats.

Research should be done using larger numbers of animals in each group when using swimming as a means of exercise.

Animal studies should be conducted in sanitary areas where heat and humidity can be controlled.

Visitors must be prohibited in animals quarters while an experiment is being conducted.

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APPENDIX A

DATA SHEETS -- TREATMENT OF ANIMALS

DATA SHEET--TREATMENT OF ANIMALS¹

Summary of Phase I

Date	All Animals N-120
12/23/61	Handled
12/24/61	Handled
12/25/61	No Treatment
12/26/61	Five minute swim
12/27/61	Ten minute swim, two per cent of body weight was attached (a lead sinker).
12/28/61	Two fifteen minute swims (A.M.), (P.M.), with two per cent of body weight attached.

Objective of Phase I

To accustom the animals with the experimental procedures.

¹The treatment in phases I, II, and III were part of another study. The actual treatment the animals received is summarized.

DATA SHEET--TREATMENT OF ANIMALS

Summary of Phase II

Dates	Experimental Animals N-100	Controls N-20
12/29/61 to 1/17/62	Experimental animals were forced to exercise by attaching a lead sinker which was four per cent of their body weight to their tails. Time was to be increased until animals could carry four per cent for thirty minutes.	No Treatment
1/18/62 to 2/25/62	Animals are losing weight, coughing and have bloody noses, therefore, a rest period was necessary.	No Treatment
2/26/62 to 3/20/62	Resumed exercising animals. Animals were forced to swin with one per cent of body weight attached. Weight was increased if animals could swim for thirty minutes. The purpose of this phase was to have the animals reach the objective of Phase II. Because the objective of this phase was not reached the main- tenance study was cancelled.	No Treatment

Objective of Phase II

To train the animals to swim for thirty minutes carrying four per cent of their body weight attached to their tail.

	Controls	N-19 animals carried 3.5 g the all out swim.	N-19 No treatment, except for all out swims.				
Phase III	Animals	experimental and control cent of body weight durin	5 study. The experimental o nine groups on the basis n. The mean swim time of s nearly as possible.	Schedule	Group B*	<pre>I Every day (N-9 II Every 2.days (N-10) III Every 5 days (N-9) IV Every 10 days (N-9) V Same treatment as controls (N-9)</pre>	Bgroups exercise ac- cording to exercise schedule with four to five per cent of body weight attached until they reach the crucial point.
	Experimental	N-95 First All Out Swim: The oper oper oper oper oper oper oper ope	N-95 New design is a training study. animals were divided into nine of the first all out swim. The the groups was equated as nearl	Ixercise	Group A [*]	I Every day (N-10) II Every 2 days (N-10) III Every 5 days (N-10) IV Every 10 days (N-9) V Same treatment as controls (N-9)	L C C S
	Date	3/21/62	3/22/62 to 3/23/62				

DATA SHEET--TREATMENT CF ANIMALS

Phase III	Phase III (continued)	
Date	Experimental Animal s Controls	
3/24/62 to 4/26/62	N-19 Training according to exercise schedule. No treatment	
4/27/62 to 4/28/62	Animals rested on $4/27/62$. Second all out swim on $4/28/62$. Experimentals and controls carried 3.5 per cent of body weight attached to their tails.	s. s.
4/29/62 to 6/ 1/62	N-19 Training according to exercise schedule.	
6/2/62 to 6/3/62	N-74 N-18 Animals rested on $6/2/62$. Third all out swim on $6/3/62$. Experimentals and controls carried 3.5 per cent of body weight attached to their tails.	ω.
Objective	of Phase III	

Objective of Phase III

To study two methods of To discover reasons for decline in swimming ability. improving poor swimming ability. 27

DATA SHEET--TREATMENT OF ANIMALS

Phase IV

Date	Exŗ	perimental Animals
6/5/62	Group IA (N-6) Group IB (N-9) Group V (N-8) Controls (N-10)*	The animals in Phase IV are the same animals that received exercise under the exercise schedule which is listed under Phase III. All animals were placed in water which was 15° centigrade on 6/5/62. Survival time in cold water was recorded.

*The ten controls were a random selection from the control animals in Phase III.

Objective of Phase IV

To determine whether adult male albino rats can acquire cross-resistance to cold water through exercise.

APPENDIX B

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WEIGHT OF ANIMALS

<u> </u>	Dates						
Group	12/30/61	1/3/62	1/13/62	1/17/62	1/20/62		
IA 2 3 4 7 8 9	261 348.5 288 253 316 264	314.5 354 296.5 288.5 334.5 279	363.5 370.5 293.5 287.5 343 280	360 358 369.5 306.5 344 291.5	378.5 363 385 320.5 356.5 299		
IB 1 2 3 4 56 7 8 9	253.5 273 320.5 329 276 320.5 290 310.5 294.5	284 259.5 334 363.5 301.5 352.5 291.5 317.5 317.5	268.5 275 335 378.5 307 374 292 343.5 332.5	300 287.5 278 399 302.5 397 313 344 292	317.5 297.5 287 414 312 415 324.5 347 305		
V 1 2 3 4 6 7 8 9	307.5 272.5 334.5 268 273.5 304 279 247.5	346 291.5 311 294 289 273.5 306.5 272	378 326 333 310.5 287.5 304.5 322 283	371.5 324 340 321 263.5 316.5 334 281	382 327 355 321 260 324.5 340 285		
Controls 1 2 3 4 5 6 7 8 9 10	257 313 284 249.5 302.5 288.5 339 316.5 332 270	286 342.5 309.5 293.5 327 320 379 344 375.5 296.5	336.5 258 350.5 328.5 373.5 350 422 382 411 335	357 342.5 372.5 347 400 378.5 432.5 403.5 434.5 346	345 355.5 372.5 332.5 408.5 379.5 441 412 437 330		

WEIGHT OF ANIMALS BY DATES

WEIGHT OF ANIMALS BY DATES (continued)

	Dates					
Group —	1/24/62	1/27/62	1/31/62	2/3/62	2/10/62	
IA 2 3 4 7 8 9	405 349.5 410 290 373.5 309.5	412 336.5 416.5 310 386.5 323.5	427.5 328 430.5 322 414.5 336	437 332 455 340 429.5 347.5	465.5 355 481 375 461 373	
IB 1 2 3 4 56 7 8 9	338.5 319 304.5 435 321 441 337.5 367 341	355.5 334.5 315.5 444 327.5 446 343.5 366.5 351	363 357.5 311.5 454 341 445 355 381.5 365	375 371 333 469 354 484.5 382 389 387.5	393.5 377.5 355 496 382 489.5 403.5 415 400	
V 1 2 3 4 6 7 8 9	394.5 338 380 310.5 266 345 363 300	402.5 327 380.5 316 278 351 371 307.5	406.5 332 394 316.5 284.5 374 390 314.5	417 346.5 396 333.5 340.5 396.5 405 329.5	430 368 420 361 332 414 430 351	
Controls 1 2 3 4 5 6 7 8 9 10	368.5 382 384.5 356.5 404 388 452.5 4156.5 348.5	370.5 389 382 385.5 417 366 420 406 458 328.5	387.5 413.5 399.5 363 409.5 333 388.5 403.5 403.5 315.5	390.5 416 405.5 360 404 318 380 403.5 487.5	405.5 432 421.5 361 377.5 353 350 399 505	

<u></u>			Det	<u></u>	
- Group	2/17/62	2/24/62	Dates 2/28/62	3/3/62	3/7/62
IA 2 3 4 7 8 9	483.5 395 408 400.5 468 396	513 410.5 510.5 433 485 405.5	496 422 525 437.5 483 410	477 414.5 502.5 433 482 403	481 432 507 444 477.5 410
IB 12 34 56 78 9	420 415 370 506 412 543 428 438.5 405	437 433 384.5 517 424 574.5 451 461 425	437 441 392.5 526.5 431 575.5 452 465 435	430.5 437.5 385.5 508 420 554.5 446.5 423.5	438 436 391.5 510.5 455 557.5 455.5 460 445
V 12 34 6 78 9	465 392 435 359 351 426 446.5 374	469 406 378.5 377 440 456.5 389.5	466 418.5 463 378 385 450 462.5 393.5	460 408 451 382 375 433.5 458 384.5	479 420 448 393 391 451.5 440 397.5
Controls 1 2 3 4 5 6 7 8 9 10	411.5 449 386 355 368 400 370 529	421 460 452 401 381.5 395 441 406 537.5		432 477.5 469 408 422 416 463 450 542.5	444 484 477 417 444 437 497.5 471 565.5 455.5

WEIGHT OF ANIMALS BY DATES (continued)

.

			Dates		
Group	3/10/62	3/12/62	3/17/62	3/21/62	3/27/62
IA 2 3 4 7 8 9	480 426 499.5 437 460 401	491.5 428.5 518 447.5 466.5 416	491 464 507 448.5 460 402.5	492 411 502 448.5 462 398	499.5 428.5 501 454.5 462.5 395.5
IB 1 2 3 4 56 7 8 9	434.5 419.5 389.5 507.5 457.5 437.5 447.5 445	442 431 398 503.5 456 554.5 443 453.5 457	443.5 432 387.5 503 461 662 436.5 454.5 456	444.5 433.5 398.5 507 464.5 553.5 442 454.5 461	446.5 441 400 520 464.5 550 449 465 455
V 1 2 3 4 6 7 8 9	471 410 442 394 391 437.5 435 400	480 421.5 457 410 397 449 438 409.5	484 420.5 457.5 421 394 447 442.5 420	421.5 421 464 410 397 445 443.5 419	415.5 436 475 428 414 463 449 446
Controls 1 2 3 4 5 6 7 8 9 10	449 494 426 455 449 505 567 468	452.5 497.5 491.5 428.5 465 458.5 519 499.5 572 470	460 508 499 441.5 473 464 528 507.5 577.5 482.5	467 500 493.5 438.5 475.5 460.5 506.5 584 465.5	466 475 503.5 447 492.5 514.5 514.5 585 480

WEIGHT OF ANIMALS BY DATES (continued)

	Dates						
Group	3/31/62	4/4/62	4/7/62	4/11/62	4/14/62		
IA 2 3 4 7 8 9	480 423.5 487 446.5 458 394	466 436.5 487.5 453.5 463.5 401.5	457.5 425.5 469 456 459 400	447.5 425.5 467 449 460 398	448 420 461.5 454 468.5 397.5		
IB 1 2 3 4 56 7 8 9	437.5 436 388 513 454 529 442 453 459.5	447 438 389.5 522 464.5 518.5 448 456 456	442 432 385 520.5 461 507.5 440 449.5 456	444.5 427 376 515 462 509 432.5 447.5 460	446 431 382 517 462 507 436 449 466		
V 12 34 6 78 9	409 448 470 435 417 467.5 457 448	412.5 458 473.5 441.5 432 480 470 461	414 465.5 463.5 441 433.5 485 471 466	413.5 472 467.5 449 441 493.5 476 470	417 481.5 468.5 454.5 450 504.5 483 474		
Controls 1 2 3 4 5 6 7 8 9 10	479 475 506 457.5 518.5 430.5 584.5 584.5 484	481 482.5 520 473 506.5 524.5 599.5 599.5 494.5	490 486.5 517 475.5 510.5 532 448.5 556.5 600 497.5	491.5 494.5 521.5 485.5 510.5 532 454 562.5 608.5 511	491.5 495 524 491 513 537 462 577 611 515.5		

WEIGHT OF ANIMALS BY DATES (continued)

			Dates		
Group	4/18/62	4/21/62	4/25/62	4/28/62	5/3/62
IA 2 3 4 7 8 9	460 413.5 473 452.5 475 405.5	458.5 404.5 466.5 451.5 476 400	413.5 400 464.5 451 482.5 396.5	457.5 387 474 461 487 403.5	460 378 474.5 458 483 409
IB 12 34 56 78 9	453 436 387 518 462.5 434 450 469	447 426 385.5 515.5 460 490 433.5 445 466.5	446 424 387.5 504 465 486 432 446.5 468	445.5 430 388.5 504.5 458 492 439 453.5 474.5	451.5 423.5 394.5 494.5 456.5 493 452 452 475
V 1 2 3 4 6 7 8 9	413.5 493.5 480 465 457.5 513.5 495.5 489	419 493.5 480 465 457.5 513.5 495.5 489	418.5 498.5 467 475.5 465 520 496 499	422.5 505 452 477 466.5 533 499.5 507	418 510 437 475.5 473.5 528 498 503
Controls 1 2 3 4 5 6 7 8 9 10	598.5 506.5 533.5 49.8.5 520 548 475 588 622 523	501 512.5 540 502.5 524 545.5 476 588 623.5 523.5	501.5 520 551 505 527 549.5 481 598 619 534.5	506.5 525 550 510 522 559 479 600 610.5 538.5	507 522 559.5 506 509.5 555.5 481 598 618 537.5

WEIGHTS OF ANIMALS BY DATES (continued)

<u></u>			Dates		
Group	5/5/62	5/9/62	5/12/62	5/16/62	5/19/62
IA 2 3 4 7 8 9	461 380 477 455.5 486 403	458 388 490 460 484 413	461.5 386.5 478.5 454.5 486 413.5	469 388 474 462.5 494 410	475 393 495 460 497 417
IB 1 2 3 4 5 6 7 8 9	449 425.5 389.5 488.5 457 500 428.5 446 476	450 428.5 389.5 478 460 509 427 446 481	448.5 425.5 386 470 457 506.5 426 449 475.5	453 426 390 469 511.5 428 452 452 480	458 428.5 391 466 467 513 455 478.5
V 1 2 3 4 6 7 8 9	414.5 511.5 441.5 483.5 472 534 505.5 511	430 516 444 497 482 538 509.5 517	424.5 514 434 494.5 474 539 504 514.5	431.5 521 430 484 481 552 509 523	428 520 424 507 480 561.5 513 528
Controls 1 2 3 4 5 6 7 8 9 10	509.5 524.5 561.5 507 508 561 480 601.5 613.5 537.5	514 535 573.5 500 507 474.5 492 624 626.5 534.5	508 534.5 572 510 494.5 566 492 613 604.5 551	516.5 534 576 508 490 574 496 616 610 554.5	525.5 541 581 511.5 470 575.5 501 627 593 560

WEIGHTS OF ANIMALS BY DATES (continued)

	Dates				
Group	5/23/62	5/26/62	5/30/62	6/2/62	6/5/62
IA 2 3 4 7 8 9	467 401 430 457 494 415	470 401 460 459 494.5 421.5	472 404.5 495 463 493 420	473.5 407 501.5 458 492.5 419	481 408.5 501.5 473 501 427.5
IB 1 2 3 4 5 6 7 8 9	459 428 393 486.5 460 511 433.5 449.5 485.5	454.5 429.5 394.5 427.5 469 510 435.5 487.5	457 433 396.5 410 469 510 439 449 492	451.5 430 392.5 397.5 473.5 505 439.5 450 490	455.5 439.5 401 392 380 508 443 460 502
V 1 2 3 4 6 7 8 9	429 524.5 411.5 508 472.5 560 518 529.5	434 529 414 520 482 565.5 523.5 540	421 531.5 404 517.5 457 566.5 525.5 547	416.5 533 403 521 484 568.5 523.5 550	414 531 396.5 510.5 478.5 596 519.5 542
Controls 1 2 3 4 5 6 7 8 9 10	521 539.5 580 512.5 471 575 504 627.5 595 559	529 542.5 585 517.5 473.5 578 510 627 584 563	532 544.5 590.5 514 460 584.5 510 629 594 569	538.5 549 587 520 458 585.5 516 532 587	538.5 545 589 519 455 582.5 586 589 555

WEIGHTS OF ANIMALS BY DATES (continued)

APPENDIX C

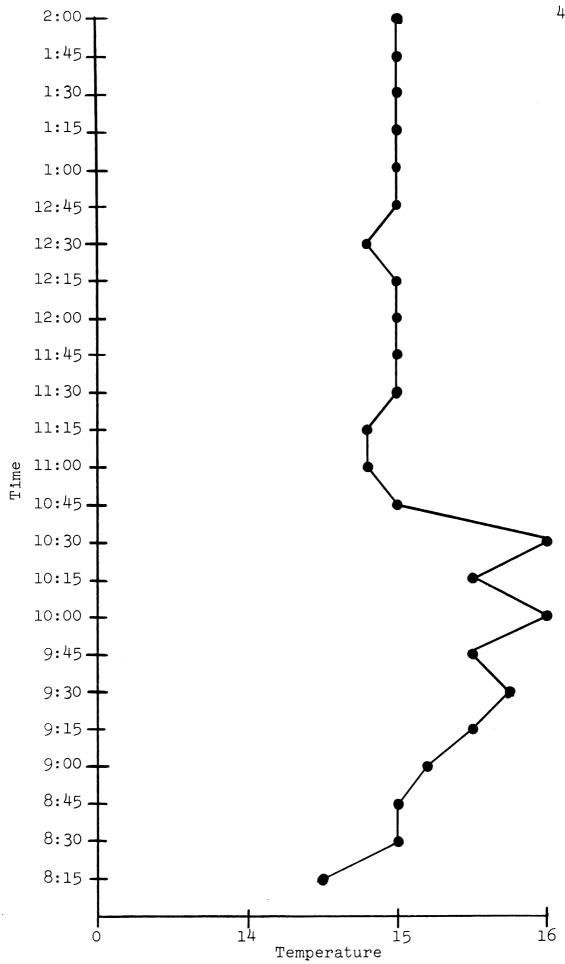
COLD WATER SURVIVAL TIME

Group	Animal	Minutes
IA	2 3 4 7 8 9	125 73 126 134 117 154
IB	1 2 3 4 5 6 7 8 9	88 86 65 57 97 59 147 125 85
V	1 2 3 4 6 7 8 9	66 88 55 84 117 100 95 116
Controls	1 2 3 4 5 6 7 8 9 10	133 105 113 133 71 143 157 137 84 101

DATA SHEET--COLD WATER SURVIVAL TIME

APPENDIX D

WATER TEMPERATURE



APPENDIX E

ALL OUT SWIM TIME

			Time		
Group	Animal	3/22/62	4/28/62	6/3/62	
IA	2 34 7 8 9	8:00 6:15 4:40 4:05 4:00 3:20	4:30 9:00 5:15 5:30 4:00 3:45	3:30 31:55 5:00 5:30 21:15 3:45	
IB	1 2 3 4 56 7 8 9	6:00 9:50 4:45 4:50 4:00 3:30 4:10 4:30 2:30	4:45 4:00 8:00 7:45 3:45 9:00 7:00 6:00 4:50	8:45 5:15 6:45 67:45 3:50 63:30 5:30 5:30 4:00	
V	1 2 3 4 6 7 8 9	69:45 9:25 5:15 3:45 4:05 2:45 3:50 3:10	4:45 6:00 5:15 6:00 4:45 3:45 1:45 4:00	51:00 13:30 32:40 17:00 5:10 3:25 2:35 4:30	
Controls	1 2 34 56 7 8 9 10	Pilot 205:30 Pilot 3:45 114:45 163:20 Pilot 94:25 3:05 5:50	4:00 62:30 2:30 11:00 63:00 7:00 6:30 3:15 2:30 3:15	7:20 72:30 2:25 63:40 41:10 5:25 56:35 5:40 4:15 62:30	

DATA SHEET--ALL OUT SWIM TIME¹

 $^{\rm l}$ All animals carried 3.5% of their body weight during all out swims.

