63-3-1

U. S. ARMY MEDICAL RESEARCH & NUTRITION LABORATORY

PHYSIOLOGICAL AND BIOCHEMICAL EVALUATION OF POTENTIAL ANTI-FATIGUE DRUGS II THE EFFECTS OF ASPARTIC ACID SALTS (MG AND K) ON THE PERFORMANCE OF RATS AND DOGS

REPORT 274
7 MARCH 1963

UNITED STATES ARMY
MEDICAL RESEARCH AND DEVELOPMENT COMMAND

US ARMY MEDICAL RESEARCH AND NUTRITION LABORATORY
FITZSIMONS GENERAL HOSPITAL
DENVER 30, COLORADO

Report No. 274

7 March 1963

Report on

PHYSIOLOGICAL AND BIOCHEMICAL EVALUATION OF POTENTIAL ANTI-FATIGUE DRUGS. II. THE EFFECTS OF ASPARTIC ACID SALTS (Mg and K) ON THE PERFORMANCE OF RATS AND DOGS

By

Le Roy O. Matoush
C. Frank Consolazio
Richard A. Nelson
Gerhard J. Isaac
SSGT Juan B. Torres

Report No. 274
Project No. 3A012501A803 - Military Internal Medicine
Task No. 04: Pharmacology of the Combat Soldier
USAMRNL Sub-Task No. 1 - 9

7 March 1963

PHYSIOLOGICAL AND BIOCHEMICAL EVALUATION OF POTENTIAL ANTI-FATIGUE DRUGS. II. THE EFFECTS OF ASPARTIC ACID SALTS (Mg and K) ON THE PERFORMANCE OF RATS AND DOGS

OBJECTIVE:

The objective of these experiments was to determine the effect of Mg and K salts of aspartic acid (Spartase) on the swimming times of laboratory animals performing single or double swims to exhaustion.

SUMMARY:

Five separate experiments were performed to evaluate the effects, if any, of "Spartase" on the swimming times to exhaustion of rats and dogs. Experiments 1 and 2 were with rats doing a single swim test only, with the water temperature maintained at 17°C and 25°C. In experiment 3 dogs were utilized swimming at 17°C and also performing the single swim test. During the first three experiments each animal underwent two treatments; a no-treatment control, and a "Spartase" therapy. Experiment 4 was a long-term single swim test. The rats were divided into two groups: one group receiving a placebo and the other group receiving "Spartase". Each group swam to exhaustion in 25°C water twice a week for 6 weeks. Experiment 5 was of the same experimental design as experiment 4 with the exception that a double swim test was used to measure performance.

Under the conditions of these studies, the swimming times of rats and dogs receiving "Spartase" therapy did not differ significantly from the control animals whether using a single or a double swim-to-exhaustion.

APPROVED:

Marion E. McDowell

MARION E. McDOWELL
Lt. Colonel, MC
Director

PHYSIOLOGICAL AND BIOCHEMICAL EVALUATION OF POTENTIAL ANTI-FATIGUE DRUGS. II. THE EFFECTS OF ASPARTIC ACID SALTS (Mg and K) ON THE PERFORMANCE OF RATS AND DOGS

Introduction

This study is the second of a series to investigate the possible use of aspartic acid salts, (Spartase, containing equal quantities of potassium and magnesium salts of aspartic acid) as a means of increasing endurance in animals. A previously reported study was conducted in humans (1). Laborit and associates (2) have reviewed the work on aspartic acid salts and feel that they may be beneficial in prolonging (or delaying) the onset of fatigue. Rosen, et al. (3) have also observed that the onset of exhaustion was significantly and consistently delayed by the administration of aspartic acid salts. Swimming time to exhaustion has been used as a criteria of endurance in many animal studies. Recent work by Wilber and Hunn (4) has shown that the swimming time of mice could be reduced or prolonged by decreasing or increasing water temperature. Similar work has been reported for rats and guinea pigs (5, 6) and for rats with a load fastened to their necks (7, 8). Consideration also was given to the possibility of using running time of dogs on a motor driven treadmill. But in recent work with dogs at this Laboratory it was found that motivation was an extremely important variable which could not be effectively controlled. On the basis of these factors it was decided that swim-to-exhaustion tests would best serve to measure performance in the present study.

Experimental Design

In the 4 experiments using Sprague-Dawley rats, the swimming was done in large glass jars, 12 inches in diameter and 24 inches deep. During the swim only one rat swam per jar. The water level was maintained at 18 inches so that the rats could not get support from their tails touching the bottom of the jar and so the margin at the top was enough that the rats could not climb out. To keep the water mixed so that the temperature was uniform and to provide turbulence that served to keep the rats from floating, compressed air at the rate of 2 liters/minute was forced through a $2\frac{1}{4}$" O.D. glass tube placed down one side of the jar.

In all 5 experiments the animals were forced to swim to exhaustion and the swimming time noted to the nearest 10 seconds. Timing was started when the animals were placed in the water and ended when they appeared to be unable to continue the test. The ability to recognize and achieve the proper termination of each swim was tested by preliminary experimentation. However, during the course of these experiments, 5 rats (three in experiment 1, one in experiment 2 and one in experiment 5) expired during their swimming tests.

The first experiment was a single-swim test with the water temperature maintained at 25°C. The 40 male rats used were randomly assigned to two groups of 20 each. All animals of each group were then randomly scheduled to perform two swims, one swim with no treatment (control) and the other swim 30 minutes after a single ingestion of Spartase (0.5 gm). In order to rule out the effects of any possible training or adjustment to swimming, half the animals performed their control swim first, and the other half their Spartase swim first. The two treatments per animal allowed each animal to serve as his own control.

The second experiment was the same design using 40 male rats but with one exception, the water temperature was maintained at 17°C. Here again the Spartase animals swam 30 minutes after a single ingestion of the drug.

In the third experiment 4 dogs were tested using a single-swim test with the water temperature maintained at 17°C. The dogs swam in a 5 foot square, 5 foot deep tank with smooth stainless steel sides to prevent paw damage. Each dog was subjected to two treatments: 1) control, no treatment, and 2) Spartase ingestion, 2 tablets/dog 0.5 gm Spartase/tablet. The Spartase treated dogs swam 30 minutes after the single ingestion of the drug. Two of the dogs performed their control swim first and the other two had their Spartase swim first. A loose harness was attached to each dog to facilitate removal from the tank at the end of the swim. Rectal temperatures were recorded before, during, and after each swim.

Experiment 4 was a long-term, single-swim test in water kept at 25°C. Forty male rats were divided into two groups of 20 each. One group received a placebo and the second group received 500 mg of Spartase. The animals received Spartase therapy on 5 days of each week by stomach tube. The administration of therapy on a scheduled swim day was always accomplished 30 minutes prior to swimming. The experiment was carried out for 6 weeks, 5 weeks of therapy and one week of recovery (post therapy). All animals swam twice each week, with a 9 gm weight about their neck to reduce their swim time.

The 5th experiment was a double-swim test in water at 25°C. In the double-swim test, a second swim was initiated 2½ hours after completion of the first swim. All animals in this experiment were tested for 6 weeks, the first a control week, followed by 4 weeks of therapy, with a final control or recovery week with no therapy. All animals swam on two days of each week with an added weight equal to approximately 3% of their body weight. (The males carried 10 gm and the females 6.5 gm.) The 24 rats, 12 males and 12 females, used in this experiment were randomly assigned to two equal groups. One group received placebos and the second group received Spartase tablets (0.5 gm/day). Therapy was administered during the afternoon, 7 days a week. On swim days therapy was always administered after completion of both swims.

Results

In the first experiment at 25°C, the swimming times of the rats, ranged from 12.5 to 146.5 minutes for the control swim and 12.8 to 96.3 minutes for the Spartase swim. The means and standard deviations for the swimming times were 37.0 ± 25.2 minutes for the control swim, and 32.3 ± 15.6 for the Spartase swim (Table I). The mean for the Spartase swims was not significantly different from the control mean ($P > 0.05$). The standard deviations indicate there was extreme variation in the swimming times of the rats regardless of the treatment they received.

In the second experiment with the temperature lowered to 17°C, the swimming times ranged from 7.0 to 17.3 minutes for the control swim and 7.4 to 18.6 minutes for the Spartase swim. The mean swimming times and standard deviations were 12.6 ± 2.4 minutes for the control swim, and 12.5 ± 2.5 minutes for the Spartase swim (Table I). The mean for the Spartase swims was not significantly different from the mean for the control swims ($P > 0.05$).

In the third experiment with dogs swimming at 17°C, the swimming times ranged from 61 to 219 minutes for the control swim and 68 to 173 minutes for the Spartase swim (Table II). The mean swimming times and standard deviations were 117 ± 70 minutes and 115 ± 45 minutes for the control and Spartase swims, respectively. Again the mean for the Spartase swims was not significantly different from the mean for the control swims ($P > 0.05$). As shown in Table II, at the end of each swim, there was a significant decrease in body temperature which ranged from 5.0 to 9.5°C.

The mean swimming times of the Spartase and control groups in experiment 4 are presented in Table III. During the course of this experiment each rat of both groups performed a total of 12 swims, 2 swims each week for 6 weeks. Statistical analyses of swims 1, 7 and 11 (post therapy) showed that the means for the Spartase group were not significantly different from the means for the control group ($P > 0.05$).

The mean swimming times for the double-swim tests of experiment 5 are presented in Tables IV and V. Statistical analyses of the mean swimming times for the Spartase and control groups for both male and female rats showed no significant difference between groups in swimming times for swims 1 or 2 on any of the days tested. The comparison of swimming times for swims 1 or 2 within the same group also showed no significant difference. Table VI shows the number of times that either swim 1 or swim 2 was the longer on a given day. Analysis of the data again showed no significant difference between therapy groups.

Table VII presents the mean weekly body weights of the rats for both groups. The body weight changes either within a group or between groups were not significantly different.

Discussion

The results of these experiments are in disagreement with the work of Laborit, et al. (2) and Rosen, et al. (3), but in agreement with the recent studies on humans (exercising on a treadmill) by Consolazio, et al. (1).

The swimming times of rats at 17°C were considerably shorter than those at 25°C. This difference was apparently related to the water temperature as all the rats exhibited much greater activity in the colder water. The lowered body temperatures of the dogs in both groups in experiment 3 was also attributed to the 17°C water.

Under the conditions of these experiments the ingestion of Spartase appeared to have no significant effect on the swimming times of rats and dogs swimming to exhaustion.

Summary

1. Five experiments were conducted on rats and dogs to determine the effects of Mg and K aspartic acid salts (Spartase) on physical endurance.

2. Statistical analysis of the mean swimming times for both rats and dogs in the 5 experiments showed that there was no significant difference between the control and Spartase swims at the 5% level of probability.

3. Under the conditions of the experiments conducted at this Laboratory no significant effect on the swimming times of rats or dogs could be attributed to the ingestion of Spartase.

4. It was observed that a decrease in water temperature from 25°C to 17°C greatly reduces the swimming time of rats and dogs.

ACKNOWLEDGEMENTS

We especially wish to thank Mr. Billy J. James, SP4 Duane A. Reuer, SP5 Oktawian Tarnowieckyi, SP5 Gary L. Beal, SP5 Lynn J. Maland, SP4 Warren E. Hendricks, SP4 Lester E. Jones, SP4 Ronald R. DeMeritt, and SP4 Samuel D. Wilkins for their technical assistance during these studies.

LITERATURE CITED

1. Consolazio, C. F., Nelson, R. A., Matoush, L. O. and Isaac, G. J.: Physiological and biochemical evaluation of potential anti-fatigue drugs. I. The effects of aspartic acid salts (Mg and K) on the performance of men. USAMRNL Report No. 273, 4 March 1963.

2. Laborit, H., Moynier, R., Coirault, R. Thiebault, J., Guiot, G. Niaussat, P., Weber, B., Jouany, J. M. and Baron, C.: The importance of certain d,l-Aspartic acid salts in the mechanisms of the maintenance of reactional activity to the environment. Presse Med. 66: 1307-09, 1958.

3. Rosen, H., Blumenthal, A. and Agersborg, H. P. K.: The effects of the potassium and magnesium salts of aspartic acid on metabolic exhaustion. J. Pharm. Sci. (In press.)

4. Wilber, C. G. and Hunn, J. B.: Swimming of albino mice. J. Appl. Physiol., 15: 704-705, 1960.

5. Tan, E. M., Hanson, M. E. and Richter, C. P.: Swimming time of rats with relation to water temperature. Fed. Proc., 13: 150, 1954.

6. Wilber, C. G.: Influence of temperature on performance in guinea pigs. Am. J. Physiol., 190: 457-458, 1957.

7. Kimeldorf, D. J. and Jones, D. C.: The relationship of radiation dose to lethality among exercised animals exposed to roentgen rays. Am. J. Physiol., 167: 626-632, 1951.

8. Kimeldorf, D. J., Jones, D. C. and Fishler, M. C.: The effects of exercise upon the lethality of roentgen rays for rats. Science 112: 175-176, 1950.

TABLE I

Experiments 1 and 2: Mean Swimming Times of Rats*

(In Minutes)

Treatment Group	No. of Animals	Swimming Time Mean ± S.D.	
Experiment 1 (Water 25°C)			
Control	37	37.0	25.2
Spartase	37	32.3	15.6
Experiment 2 (Water 17°C)			
Control	39	12.6	2.4
Spartase	39	12.5	2.5

* Single swim for each treatment at 17°C and 25°C.

TABLE II

Experiment 3: Swimming Times and Body Temperatures of Dogs

		Control			Spartase		
		Rectal Temp. °C		Swimming Time	Rectal Temp. °C		Swimming Time
No.	Sex	Before	After	Minutes	Before	After	Minutes
1	F	39.5	30.0	99	39.0	30.0	68
2	F	39.0	30.0	219	39.0	33.0	173
3	F	39.3	30.0	88	39.0	31.5	97
4	M	37.0	32.0	61	39.0	30.1	123
No.				4			4
Mean				117			115
S.D.				70			45

TABLE III

Experiment 4: Swimming Time of Rats

By Treatment and Swim Day*

(In Minutes)

	Treatment			
	Control		Spartase	
Swim Day	Mean	± S.D.	Mean	± S.D.
1	15.6	9.8	18.5	12.2
2	17.8	8.4	17.3	8.1
3	25.7	19.9	22.2	15.1
4	30.3	24.4	22.7	15.0
5	33.8	21.9	43.4	55.4
6	23.0	10.3	27.7	21.5
7	24.9	12.1	48.2	47.8
8	32.9	29.2	27.5	23.9
9	30.7	17.2	27.6	21.1
10	28.8	16.1	27.5	11.2
Post-Therapy				
11	38.9	27.9	34.4	29.9
12	23.4	7.4	22.6	9.8
Body weight change, 6 weeks, gm	+10		+24	

* Single swim, two times a week.

TABLE IV

Experiment 5: Swimming Time of Male Rats
By Treatment and Swim Day*
(In Minutes)

	Swim Day 1		Swim Day 2			
Weeks of Treatment	Swim 1	Swim 2	Swim 1		Swim 2	
Control Group	Mean	Mean	Mean	± S.D.	Mean	± S.D.
1	11.1	15.1	19.0	11.8	13.1	9.0
2	12.7	11.6	14.7	8.3	11.4	5.8
3	11.9	11.7	15.3	8.2	30.1	39.4
4	14.8	13.5	11.9	3.5	17.9	10.3
5	19.8	29.2	48.8	57.7	27.5	25.0
6	14.8	45.5	12.9	2.2	45.6	50.6
Spartase Group						
1	17.5	17.4	22.4	18.0	12.8	5.6
2	19.9	15.1	15.6	2.6	23.2	25.9
3	11.4	16.8	10.8	4.2	23.7	21.1
4	14.4	21.1	11.1	2.1	14.3	4.1
5	11.7	16.3	18.5	7.3	17.3	8.3
6	19.8	18.5	22.3	14.7	55.9	90.4

* Male rats had a double swim on Monday and Thursday each week. The second swim was always 2½ hours after the first swim-to-exhaustion.

TABLE V

Experiment 5: Swimming Time of Female Rats

By Treatment and Swim Day*

(In Minutes)

	Swim Day 1		Swim Day 2			
Weeks of Treatment	Swim 1	Swim 2	Swim 1		Swim 2	
Control Group	Mean	Mean	Mean	± S.D.	Mean	± S.D.
1	15.1	9.6	14.3	3.4	15.3	6.2
2	12.5	12.3	22.5	24.3	10.1	2.2
3	18.4	29.6	11.0	2.4	11.4	5.0
4	13.5	12.7	12.0	2.1	10.6	6.2
5	11.3	10.4	15.0	9.5	21.8	32.8
6	10.4	8.9	14.7	4.8	29.7	43.8
Spartase Group						
1	16.1	13.9	11.5	6.8	9.9	5.2
2	14.1	11.2	23.6	36.8	11.5	7.7
3	14.9	11.2	11.7	1.8	13.3	5.5
4	15.2	26.7	10.3	3.3	12.8	6.1
5	14.3	26.7	15.8	5.2	18.0	13.5
6	14.4	36.6	15.4	4.7	18.2	12.9

* Female rats had a double swim on Tuesdays and Fridays each week. The second swim was always 2½ hours after the first swim-to-exhaustion.

TABLE VI

Experiment 5: Number of Times Swim 1 or Swim 2 of A Given Day was the Longer, By Treatment and Sex

Group		Treatment Group: Control	Spartase
Males:	Swim 1	33	30
	Swim 2	36	41
Females:	Swim 1	44	37
	Swim 2	27	35
Combined:	Swim 1	77	67
	Swim 2	63	76

TABLE VII

Experiment 5: Mean Body Weights By Sex,
By Treatment and Week
(In grams)

	Males		Females	
Weeks of Study	Spartase	Placebo	Spartase	Placebo
1	349	353	226	220
2	338	354	225	224
3	341	367	232	231
4	337	358	233	228
5	337	346	238	233
6	330	327	235	239

USA Medical Research & Nutrition Laboratory Proj #3A012501A803 Report #274

Physiological and Biochemical Evaluation of Potential Anti-Fatigue Drugs. II. The Effects of Aspartic Acid Salts (Mg and K) On the Performance of Rats and Dogs

Le Roy O. Matoush, C. Frank Consolazio, Richard A. Nelson, Gerhard J. Isaac and SSGT Juan B. Torres

12 pps, 7 Tables 7 March 1963 UNCLASSIFIED

Five separate swim-to-exhaustion experiments were performed to evaluate the effects of administering Mg and K salts of aspartic acid (Spartase) as a possible means of increasing endurance in animals. In all experiments, whether using a single or double swim-to-exhaustion, the swimming times of rats and dogs receiving therapy did not differ significantly from those for control animals.

1. Performance
2. Anti-Fatigue Drugs
3. Swimming Tests
4. Mg and K salts of Aspartic Acid

USA Medical Research & Nutrition Laboratory Proj #3A012501A803 Report #274

Physiological and Biochemical Evaluation of Potential Anti-Fatigue Drugs. II. The Effects of Aspartic Acid Salts (Mg and K) On the Performance of Rats and Dogs

Le Roy O. Matoush, C. Frank Consolazio, Richard A. Nelson, Gerhard J. Isaac and SSGT Juan B. Torres

12 pps, 7 Tables 7 March 1963 UNCLASSIFIED

Five separate swim-to-exhaustion experiments were performed to evaluate the effects of administering Mg and K salts of aspartic acid (Spartase) as a possible means of increasing endurance in animals. In all experiments, whether using a single or double swim-to-exhaustion, the swimming times of rats and dogs receiving therapy did not differ significantly from those for control animals.

1. Performance
2. Anti-Fatigue Drugs
3. Swimming Tests
4. Mg and K salts of Aspartic Acid

USA Medical Research & Nutrition Laboratory Proj #3A012501A803 Report #274

Physiological and Biochemical Evaluation of Potential Anti-Fatigue Drugs. II. The Effects of Aspartic Acid Salts (Mg and K) On the Performance of Rats and Dogs

Le Roy O. Matoush, C. Frank Consolazio, Richard A. Nelson, Gerhard J. Isaac and SSGT Juan B. Torres

12 pps, 7 Tables 7 March 1963 UNCLASSIFIED

Five separate swim-to-exhaustion experiments were performed to evaluate the effects of administering Mg and K salts of aspartic acid (Spartase) as a possible means of increasing endurance in animals. In all experiments, whether using a single or double swim-to-exhaustion, the swimming times of rats and dogs receiving therapy did not differ significantly from those for control animals.

1. Performance
2. Anti-Fatigue Drugs
3. Swimming Tests
4. Mg and K salts of Aspartic Acid

USA Medical Research & Nutrition Laboratory Proj #3A012501A803 Report #274

Physiological and Biochemical Evaluation of Potential Anti-Fatigue Drugs. II. The Effects of Aspartic Acid Salts (Mg and K) On the Performance of Rats and Dogs

Le Roy O. Matoush, C. Frank Consolazio, Richard A. Nelson, Gerhard J. Isaac and SSGT Juan B. Torres

12 pps, 7 Tables 7 March 1963 UNCLASSIFIED

Five separate swim-to-exhaustion experiments were performed to evaluate the effects of administering Mg and K salts of aspartic acid (Spartase) as a possible means of increasing endurance in animals. In all experiments, whether using a single or double swim-to-exhaustion, the swimming times of rats and dogs receiving therapy did not differ significantly from those for control animals.

1. Performance
2. Anti-Fatigue Drugs
3. Swimming Tests
4. Mg and K salts of Aspartic Acid