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Physical Conditioning and Ammonium Carbonate Affecting Blood Ammonium and Swimming Performance of Dogs

Larry Alan Bjurstrom

PHYSICAL CONDITIONING AND AMMONIUM CARBONATE AFFECTING BLOOD AMMONIA AND SWIMMING PERFORMANCE OF DOGS

BY

LARRY ALAN BJURSTROM

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser Date

Head, Entomology-Zoology Department Date

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INTRODUCTION

Ammonia is produced by the working muscle of exercising mammals and cellular metabolic processes of resting mammals. Normally relatively minute blood ammonium-nitrogen (BAN) levels are present because mammals have efficient systems for excreting ammonia or converting it to non-toxic end products. Impaired or inadequate detoxification processes allow elevated BAN levels with excessive ammonia production. Above normal BAN levels produce deleterious effects which are well know to many research fields. When impaired or inadequate detoxification processes occur in an exercising mammal, deterioration of performance is manifested in physical exhaustion and fatigue.

Mobilization and enhancement of the Krebs-Henseleit urea cycle by addition of cycle components have been attempted to facilitate detoxification.

The purpose of this study was to investigate daily ammonium carbonate administration and physical training in relation to increased ammonia removal and lowered circulating blood ammonia levels.

LITERATURE REVIEW

Scientific literature of the past half century related blood ammonia to various clinical syndromes from mental disorders to hepatic encephalopathies. The relationship of blood ammonia to onset of metabolic exhaustion and physical fatigue in an exercising animal has been explored by a number of investigators (Laborit, et al., 1956-57-58, Barnes, et al., 1964, Rosen, et al., 1962, Matoush, et al., 1964, and Consolazio, et al., 1964).

Hahn, et al. (1893) indicated a possible relationship between central nervous system disorders and elevated blood ammonia levels in dogs with portacaval anastomosis (Eck-fistula). Marfori (1894) investigated the toxicity of ammonium salts and reported ammonium carbonate to be more toxic to rabbits and dogs than ammonium lactate or tartrate. Monguio and Krause (1934) suggested that elevated blood ammonia levels might be responsible for the symptoms characteristic of intoxicated Eck-fistula dogs.

Tashiro (1922) reported ammonia production at the myoneural junction. Elevated venous blood ammonia, draining from an exercising arm, indicated ammonia formation during skeletal muscle contraction (Parnas, 1932). Work with isolated muscle indicated that ammonia formation was more prominent following a period of muscle fatigue than during the active period of contraction (Mozolowskl, et al., 1931). Schwartz, et al. (1958) demonstrated that human peripheral blood ammonia increased from a normal value of 1.0 ug./ml. to values ranging between 2.1 and 3.1 ug./ml. following voluntary muscular

exercise, and that increased circulating blood ammonia could be a factor in hyperpnea following muscular exercise. A marked rise in peripheral blood ammonia and convulsions followed Metrazo(R) administration in dogs.

Feinberg and Alma (1961) observed that there was a correlation between ammonia production and cardiac effort (heart rate x blood pressure) in an isolated working rabbit heart. The ratio of ammonia production to oxygen consumption, when both were expressed as micromoles per minute per gram of heart weight, averaged 0.064 ± 0.006. Continuously infused L-epinephrine, 0.1-1.0 ug./ml. of perfusate, caused increased cardiac effort, oxygen consumption, ammonia production, and ratio of ammonia production to oxygen consumption.

Perry (1961) proposed that in the working muscle, adenylic acid was deaminated to inosinic acid with the release of ammonia, this being the source of blood ammonia in exercise. This blood ammonia, in contrast with the ammonia coming from the gastrointestinal tract, had access to brain tissue where toxic manifestations may develop.

McDonald (1948) found that a portion of the ammonia produced by ruminal microorganisms was absorbed via the portal system and transported to the liver where a large percentage was converted to urea by hepatic tissue. Lewis, et al. (1957) confirmed the above and found a close relationship between rumen ammonia concentration and portal blood concentration.

Rosado, et al. (1962) studied ammonia loads in normal rats to appraise the relative capacity of liver and other tissue to dispose of

increased ammonia loads. Most of the load had been removed from the circulation two minutes after injection (500 micromoles of ammonium chloride into portal vein). Rosado and his co-workers (1962) concluded that the sequence of events following the injection of an ammonia load appeared to be the following: initially, ammonia was rapidly removed from the circulation by muscle, then glutamine synthesis took over, utilizing the ammonia gradually from muscle and from enzymatic hydrolysis of glutamine.

The normally functioning liver was capable of metabolizing large quantities of ammonia. This capacity was increased by feeding aspartic acid which was an obligatory substrate for the formation of argininosuccinic acid, an intermediate in the conversion of citrulline to arginine (Barnes et al., 1964). Greenstein, et al. (1956) and Fahey (1957) reported Krebs-Henseleit enhancement following L-arginine administration which increased blood urea levels.

Laborit, et al. (1957-58) proposed that exhaustion in the swimming rat was related to blood ammonia levels. This was based primarily on the observation that the rise of blood ammonia following a short period of exercise was partially prevented by a prior oral administration of either aspartic acid or the mixed potassium and magnesium salt of aspartic acid. These compounds would prolong the time required for the rats to reach physical exhaustion due to forced swimming. Laborit postulated that "aspartic acid, which functions in an intermediate position in the Krebs cycle, should provide a suitable

substrate for increased metabolic efficiency or delayed metabolic exhaustion." Since this original investigation, conflicting and inconsistent reports have characterized the related research.

Tamaki, et al. (1961) investigated aspartic acid salts affecting swimming performance of mice, and Rosen, et al. (1962) and Barnes, et al. (1964) investigated aspartic acid salts affecting swimming performance of rats. Their findings were consistent with those of Laborit.

Laborit, et al. (1956) investigated 144 athletes in training and living under carefully controlled environmental conditions in the Joinville Battalion of the French Army. Athletes were treated with potassium and magnesium salts of aspartic acid, a variety of other substances previously studied in animal research, and with placebos. A complete medical survey was performed on each subject before and after physical exertion. There was no doubt that aspartic acid salts produced definite therapeutic benefit. Fatigue was appreciably lessened and training expedited in 89% of treated athletes.

Agersberg and Shaw (1961) studied aspartate effects in more than 2,000 patients and observed that 80% of the subjects believed they had experienced subjective signs of relief from fatigue. Kruse (1961) and Taylor (1961), in similar subjective investigations, also reported moderate beneficial effects when utilizing magnesium and potassium salts of aspartic acid administered to patients complaining of chronic fatigue.

Consolazio, et al. (1964) investigated the effects of aspartic acid salts on physical performance of men and found no convincing evidence that aspartate therapy was beneficial to humans in delaying the onset of exhaustion.

Matoush, et al. (1964) studied the effects of aspartic acid salts (Mg and K) on swimming performance of rats and dogs. They reported the mean swimming times for both rats and dogs in five experimental groups in which data suggested that average swimming times under control and aspartate therapy were not significantly different.

Flores, et al. (1962) proposed that a depression in liver enzyme activity was associated with the ornithine cycle when rats were given ammonium chloride in their drinking water.

Barnes, et al. (1964) reported that daily feeding of ammonium carbonate over a four-week period prevented blood ammonia rise due to exercise, and that it prolonged the exhaustion time of rats forced to swim. These same changes have been observed in rats that were exercised daily by short periods of forced swimming. After three weeks of this type of physical training rats were tested for blood ammonia response to exercise and time required for exhaustion. Barnes concluded that daily ammonium carbonate feeding and training resulted in adaption of ornithine cycle enzymes so as to increase the rate of blood ammonia removal.

Swimming time to exhaustion has been used as a criterion of endurance in many animal studies, assuming that an animal faced with

a swim or drown situation would perform to his utmost ability. Wilbur and Hunn (1960) and Richter (1957) disclosed that swimming times of mice were reduced or prolonged by decreasing or increasing water temperature respectively. Similar results have been reported for rats and guinea pigs by Tan, et al. (1964) and Wilbur (1957) and for rats with a load fastened to their neck (Kimeldorf, et al., 1950). Birren and Kay (1958) induced fatigue and eventual exhaustion in rats with a swim test similar to that used by a number of authors (Harris and Ingle, 1940; Miller and Darrow, 1941; Ershoff, 1954; and Scheer and Dorst, 1947).

Motivation of dogs on a motor driven treadmill was found to be an extremely important variable which could not be effectively controlled. On this basis, Matoush, et al. (1964) decided swim to exhaustion tests would better serve to measure performance.

EXPERIMENTAL METHODS

Experimental Animals

Fifteen mongrel dogs, nine female and six male, weighing 6.39 kg. to 16.36 kg. were selected for this investigation. All dogs were examined before experimentation and found alert and in apparent good health. Fecal examinations prior to investigation showed negligible parasitic infection. Experimental animals were individually kenneled and maintained on commercial dog food.[1] Food was provided once daily following completion of experimental procedures and water provided *ad libitum*.

Experimental Groups

Three experimental groups with five animals per group were utilized for investigation. Moderate weight and phenotype fluctuation within groups were present. Weight, maturity, hair length, and swimming ability were used to partially equate animals prior to experimentation. The latter proved very subjective in nature since each dog exhibited a seemingly inherent ability to swim. Random allocation of animals within three groups followed initial stratification. Group A was designated a swimming control, Group B an ammonium carbonate control, and Group C a training and ammonium carbonate experimental.

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[1] Supersweet Dog Food. Division of International Milling Company. Minneapolis, Minnesota.

Experimental Equipment

A swimming tank measuring 2.4 m. x 1.2 m. x 1.7 m. and lined with smooth galvanized sheet metal to prevent claw damage was employed. Water depth was maintained at 107 cm. and water temperature was 20.3 ± 1° C. during all reported tests. The tank was drained and refilled with fresh water every three days. Water depth was such that dogs could not get support from their tails or legs touching bottom, nor could they reach the top edge for support. No attempt was made to aid or antagonize swimming efforts. A device to provide turbulence was decided against because sufficient turbulence to prevent floating was provided by the dog's initial swimming efforts.

All swimming performances were timed to the nearest second. Timing began when dogs were placed in the water and stopped after completion of a specified time or, in maximal-time swim tests, when animals were unable to continue. Ability to recognize termination of performance and inability to continue was tested by preliminary experimentation. Although individual idiosyncrasies existed when dogs neared exhaustion, a constant sign which was always observed was the sinking of the mouth and nose below the surface of the water. This reproducible and constant sign was selected as the end point.

Experimental Procedures

All experimental animals participated in a nine-day adjustment period prior to treatment. Blood samples were taken from each dog on the first, second, and third days of the adjustment period and blood

ammonia concentrations determined for each sample. The term "blood ammonium-nitrogen" (BAN) in this paper represents ammonium ions present in venous blood and is used to report analytical data. Mean BAN values were established for each dog and these values were designated normal resting BAN concentrations.

On the fourth, fifth, and sixth days of the adjustment period, all dogs participated in a daily two-minute adjustment swim, followed by a mandatory five-minute recovery period during which movement was restricted. Mandatory post-exercise recovery periods afforded dogs the opportunity to shake and relax sufficiently to minimize physical restraint during blood sampling procedures. All blood samples were taken five minutes after exercise in accordance with the findings of Schwartz et al., (1958). They demonstrated maximal BAN levels five minutes after completion of physical exercise. The seventh and ninth days were designated rest days. The eighth day all dogs participated in a timed maximal swim.

A fourteen-day treatment period followed the initial nine-day adjustment. On completion of the treatment, a six-day post-treatment ensued. A timed swim, corresponding in duration to the pretreatment maximal swim, was administered on day one of the post-treatment period. Blood samples were taken and BAN concentrations recorded. Following a one-day rest, a timed post-treatment maximal swim was performed and BAN concentrations again determined. Normal resting BAN concentrations

were determined on the fourth, fifth, and sixth post-treatment days and a mean level established. This value was designated the post-treatment normal resting BAN concentration.

Group A: Physical Training

Two successive six-day physical training weeks constituted the fourteen-day treatment for Group A dogs: the seventh day of each week designated a rest day. The first week, dogs were subjected to daily swims equal in duration to 50% of their previous timed maximal swim. The second week, daily swims equal in duration to 75% of the maximal were employed. Blood samples were taken on the second, fourth, and sixth days of each week following exercise periods and BAN concentrations determined. A cellulose[2] placebo in number 01 capsules was administered four hours prior to daily exercise periods.

Group B: Ammonium Carbonate

Daily administration of capsules containing ammonium carbonate[3] (30mg./kg. of body weight) and packed with cellulose, for two successive six-day weeks constituted treatment for Group B experimental animals: the seventh day of each week designated a rest day. Blood samples were drawn seventy-five minutes after administration of ammonium carbonate. Group B dogs were not subjected to physical training during the investigation.

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[2]Cellulose. Mann Research Laboratories. 136 Liberty Street, New York, New York, 10006.

[3]J. T. Baker Chemical Company. Phillipsburg, New Jersey.

Group C: Physical Training and Ammonium Carbonate

Combination of the physical training program of Group A with the ammonium carbonate regimen of Group B constituted the fourteen-day treatment for Group C dogs. Daily oral administration of ammonium carbonate, 30mg./kg., preceded daily physical training periods. All capsules were administered four hours prior to exercise and were individually weighed and packed before administration.

The physical training program was consistent with that initiated in Group A. The first week, daily training was equal in duration to 50% of pretreatment maximal swims and the second week daily training was equal in duration to 75% of pretreatment maximal swims. Blood samples were taken after completion of exercise periods on the second, fourth, and sixth days of both weeks and BAN concentrations determined.

Collection of Data

Before collection of experimental data began several dogs participated in a preliminary feasibility study to test experimental equipment utilized and refine techniques and procedures employed during the investigation. Dosage level of ammonium carbonate was selected in accordance with that recommended by Milks (1946). This dosage elevated resting BAN concentrations yet was not sufficient to produce emetic effects.

BAN concentration was determined 75, 100, 125, and 150 minutes post administration. Highest BAN concentrations were demonstrated 75 minutes following administration. Near resting normal levels were

demonstrated 150 minutes post administration. The 75-minute time interval was selected most suitable and all consequent blood samples were taken 75 minutes following administration for Group B dogs. BAN concentrations for Group A and Group C dogs were determined from blood samples taken five minutes following exercise.

Blood Collection Technique

Blood samples were drawn with ammonia free one ml. tuberculin syringes. Blood volume was adjusted to 1.0 ml. and 0.5 ml. was immediately transferred to each of two previously prepared centrifuge tubes. All samples were taken from the median cephalic vein of the left or right foreleg. Hair was clipped from the median surface of both forelegs of all dogs before initial experimentation. Aqueous 17% Zephiran Chloride[4] diluted 1 : 750 was applied topically before sampling. All physical exercise periods and blood collection procedures were performed within laboratory facilities adjacent to the kennel.[5]

Blood Ammonium-Nitrogen

A modification of Hutchinson and Labby's (1962) ion-exchange method for blood ammonia was utilized rather than methods involving distillation, aeration, and microdiffusion. Ammonium ions exchange for sodium and potassium ions on cation exchange resins. Dowex 50W-X8,

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[4]Winthrop Laboratories. New York, New York.

[5]Animal Physiology Research Farm. South Dakota State University, Brookings, South Dakota.

50-100 mesh, hydrogen ion form resin[6] was employed. Resin was made ammonia free and converted to the sodium-potassium form before use. Stock resin suspension was stored in a tightly capped polyethylene storage bottle. Periodic washing with deionized-distilled water prevented bacterial growth in stock resin suspension.

One-half ml. aliquots of freshly drawn blood were transferred to each of two previously prepared centrifuge tubes containing 1 ml. of resin suspension in water. Tubes were stoppered and shaken for three minutes to lyse erythrocytes and complete resin adsorption of ammonium ions.

Washing procedures involved careful decantation of laked blood to prevent resin loss. Deionized-distilled water was then added and tubes shaken for 30 seconds. This process was repeated a total of four times to remove all blood protein. Washing began immediately after collecting and shaking blood samples. Tubes were labeled and stored for analysis.

Contamination errors in preparation of materials, collection of samples, and analysis were essentially eliminated by subtracting reagent blank optical density from that of blood samples and standard before calculating final BAN concentration.

Standard ammonium sulfate stock solution (1 mg. NH_4^+-N/ml.) contained 4.7166 Gm. of dried ammonium sulfate analytic reagent. This reagent was added to a liter volumetric flask containing 1,000 ml. of.

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[6] J. T. Baker Chemical Company. Phillipsburg, New Jersey.

deionized-distilled water. Stock solution was diluted 1 to 200 to yield a working standard of 5 ug. NH_4^+-N/ml. One-half ml. of working standard was added to duplicate resin tubes to serve as a standard. This standard was chosen because it represented median blood ammonia values expected.

One and one-half ml. of 1 to 5 dilution of Nessler's reagent, formula of Bock and Benedict,[7] were added to each resin tube. Tubes were shaken for three minutes to elute ammonia and promote color development. Ammonia-Nessler's complex was decanted into a 10 mm. cell and read against a water blank in a spectrophotometer[8] operated at 420 mu.

Ammonia levels in ug. NH_4^+-N/ml. were calculated after subtracting optical density of the blank, according to the following formula: where 1 ml. working standard equals 5 ug. NH_4^+-N.

$$\text{ug. } NH_4^+\text{-N/ml.} = \frac{5 \times \text{O.D. Unknown}}{\text{O.D. Standard}}$$

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[7] Hawk, P., Oser, B. and Summerson, W. 1954. Practical Physiological Chemistry. New York: The Blakiston Company, Inc. 13th ed. p. 1329.

[8] Beckman Instruments, Inc., Beckman Model DB-G, Fullerton, California.

RESULTS

The various treatments employed in this investigation lowered blood ammonia resulting from physical exercise and prolonged exhaustion time of swimming dogs. Increased duration of physical exercise was demonstrated by a statistically significant difference between the groups on pre- and post-treatment maximal swims. Dogs in each group swam longer during post-treatment maximal swims than pretreatment maximal swims. Statistical analysis showed a highly significant difference between BAN concentration following pretreatment maximal swims and post-treatment swims equal in duration to pretreatment maximal swims. Highly significant differences in BAN concentration were also demonstrated between post-treatment resting normals following the treatments employed in this investigation. No significant differences in BAN concentration were determined, however, following post-treatment swims and post-treatment maximal swims.

Mean BAN values following a pretreatment maximal swim for Groups A, B, and C were 15.2, 47.5, and 37.7 ug. NH_4^+-N/ml., respectively. Mean post-treatment values for the same groups following post-treatment swims (equal in duration to the pretreatment maximal swims) were considerably lower (7.2, 4.2, and 5.7 ug. NH_4^+-N/ml.).

Statistically significant differences were demonstrated between experimental groups for duration of pre- and post-treatment maximal swims. Mean pretreatment maximal swim durations for the three

groups considered were 20:55, 40:15, and 28:15 minutes. Mean post-treatment maximal swim durations for the same groups were 31:57, 44:38, and 54:18 minutes.

Highly significant differences in BAN values were obtained between post-treatment resting normals. Mean post-treatment resting normal BAN values for Groups A, B, and C were 2.8, 5.1, and 4.5 ug. NH_4^+-N/ml., respectively. Statistical analyses were not applied to pretreatment resting BAN values because Group A values were higher than Groups B and C due to sample storage prior to laboratory determinations.

No significant differences in BAN were found between post-treatment swims and post-treatment maximal swims.

Analysis of variance was performed on all raw data. Raw data obtained from measured parameters are listed in the appendix, Table 1. Overall group means for each parameter determined are summarized in the appendix, Table 2. Analysis of variance results are listed in the appendix, Table 3.

Fig. 1 BLOOD AMMONIA

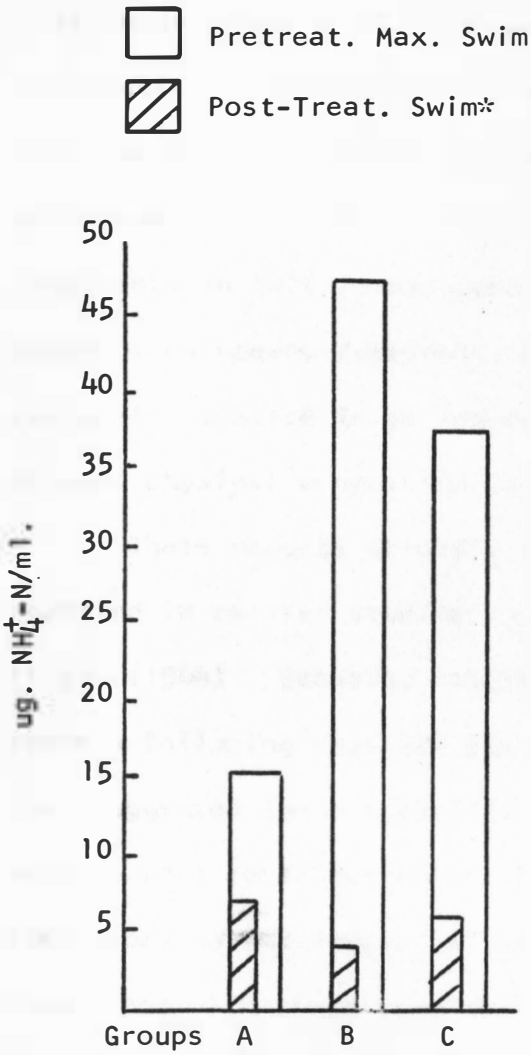

Fig. 2 SWIMMING TIME

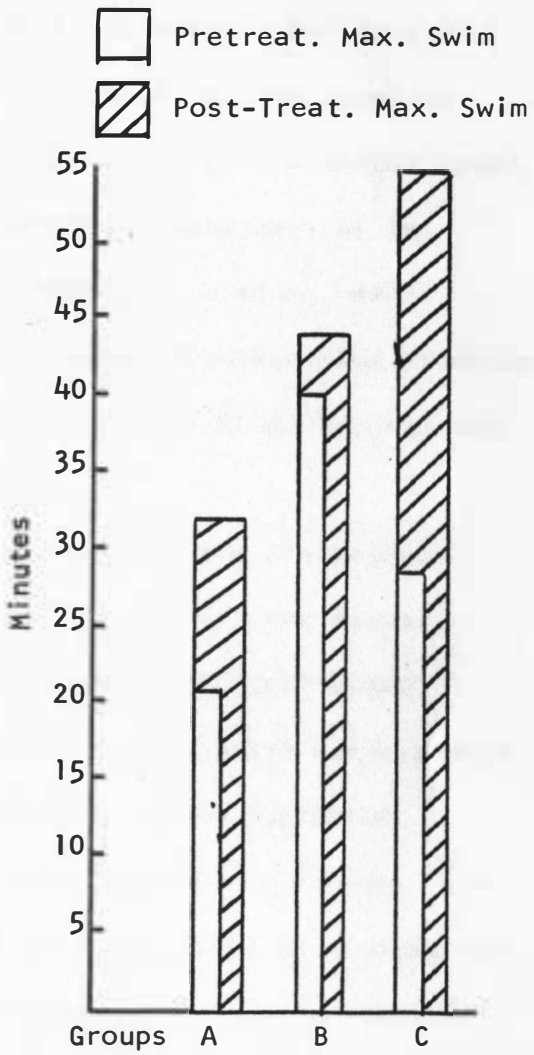

Fig. 1. Graphic comparison of mean blood ammonium-nitrogen (ug./ml.) following pretreatment maximal swim and post-treatment swim* by the 5 dogs in each group: Group A (swimming control); Group B (ammonium carbonate control); and Group C (swimming and ammonium carbonate experimental).

Fig. 2. Graphic comparison of mean pre- and post-treatment maximal swim times of the 5 dogs in each group: Group A (swimming control); Group B (ammonium carbonate control); and Group C (swimming and ammonium carbonate experimental).

*Equal in duration to pretreatment maximal swim.

DISCUSSION

The results of this study indicate that a cause and effect relationship may exist between peripheral blood ammonia and fatigue. A reduction in blood ammonia concentration resulting from physical exercise and an increase in duration of physical exercise before onset of exhaustion in swimming dogs were attained by employment of the treatments in this investigation. Daily physical exercise, daily ammonium carbonate treatment, and daily physical exercise plus ammonium carbonate resulted in an improved ability to remove blood ammonia and delayed physical exhaustion in swimming dogs.

These results strongly support and substantiate the results reported in earlier studies by Schwartz, et al. (1958) and Barnes, et al. (1964). Schwartz reported elevations of peripheral blood ammonia following physical exercise. He also established correlations that suggested the possibility that increased circulating blood ammonia were contributory to the hyperpnea of muscular exercise. The elevations in BAN levels following physical exercise in this study are consistent with these results. Barnes suggested that an increase in peripheral blood ammonia contributed to fatigue and physical exhaustion in the swimming rat and that the prevention of an increase in blood ammonia with exercise that was observed when ammonium carbonate was fed over a period of time appeared to be an example of enzyme adaptation.

Flores, et al. (1962) found a depression in the activity of liver enzymes associated with the ornithine cycle when rats were given NH_4Cl in their drinking water. They found no increase in the rate of removal of ammonia from the blood in NH_4Cl treated rats. A major difference in their study and that reported here was that a very large dose of NH_4Cl was administered directly into a portal vein and blood samples for ammonia analysis were taken from the hepatic vein.

Regardless of the exact mechanism for disposal, the present study supports the concept of an adaptation to dietary ammonia with the net result that blood ammonia is more rapidly removed. The effect of daily exercise provided similar results with respect to ammonia metabolism. A priori reasoning leads to the conclusion that training would result in an improved ability to swim, with a consequent prolongation of exhaustion time. The reduction of blood ammonia following a period of exercise in the trained dog might logically be explained by a decreased production of ammonia by the trained muscles. This could be part of the mechanism, but it is quite clear that the trained dogs had a greatly increased ability to remove ammonia after an oral dose of ammonium carbonate. This directly implicates a disposal mechanism and even though an ornithine cycle adaptation might be suggested, direct proof has not been given. Since adaptation to chronic ammonia administration has been shown, it appears most likely that the training effect is another manifestation of this phenomenon, for daily exercise means daily elevations in blood ammonia.

Marshalling all evidence, there is a strong implication of cause and effect between ammonia levels in peripheral blood and exhaustion time or fatigue. Inference to the existence of a similar cause-effect relationship in other species, including humans, must be guarded as in all such cases where data from one species are related to that of another. Until such data are discredited or proven species definitive, the possibility of the existence of the relationship in other species cannot be disregarded.

SUMMARY

Effects of physical conditioning and orally administered ammonium carbonate upon blood ammonia and swimming performance of dogs were investigated. Fifteen mongrel dogs were individually kenneled and maintained on a commercial dog food with water ad libitum. Following initial stratification, dogs were randomly allocated to one of three groups containing five animals per group.

Group A dogs participated in a fourteen-day physical training program, Group B dogs in a fourteen-day ammonium carbonate regimen consisting of daily ammonium carbonate administration (30 mg./kg. of body weight), and Group C dogs in a fourteen-day treatment combining the physical training program of Group A with the ammonium carbonate regimen of Group B.

An ion exchange method for blood ammonia was utilized for all blood ammonium-nitrogen (BAN) determinations in this investigation. Analysis of variance was employed for analysis of raw data.

BAN values increased following physical exercise. Statistical analysis showed a highly significant difference between BAN levels following pretreatment maximal swims and post-treatment swims (equal in duration to pretreatment maximal swims). Highly significant differences in BAN concentrations were also demonstrated between post-treatment resting BAN levels.

Increased duration of physical exercise following treatment was demonstrated by a statistically significant difference between the

groups with pre- and post-treatment maximal swims. No significant differences in BAN values were shown between post-treatment swims and post-treatment maximal swims.

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APPENDIX

TABLE 1: Individual pre- and post-treatment BAN concentrations (ug. NH_4^+-N/ml.) and swimming time (min.) for Groups A (swimming control), B (ammonium carbonate control), and C (swimming and ammonium carbonate experimental).

Animal number	Mean pre-treat. rest (BAN)[a]	Pretreat. max. swim (Time)[b]	Pretreat. max. swim (BAN)	Post-treat.[c] swim (BAN)	Post-treat. max. swim (Time)	Post-treat. max. swim (BAN)	Mean post-treat. rest (BAN)
Group A1	12.73	13:00	21.89	9.81	16:32	5.53	3.15
A2	8.52	18:30	14.04	5.16	38:55	5.83	2.92
A3	16.14	44:00	15.91	8.19	67:30	8.31	2.11
A4	14.52	9:50	11.70	8.44	14:04	8.64	3.19
A5	12.92	19:16	13.21	4.22	22:42	2.78	2.52
Group B1	3.90	30:38	49.44	4.60	38:23	5.33	5.67
B2	4.56	48:47	62.22	4.40	50:45	3.61	5.83
B3	2.77	62:39	46.67	4.72	72:14	7.89	3.37
B4	3.70	29:14	35.28	3.53	28:08	6.36	5.24
B5	3.99	29:59	43.89	3.64	33:19	3.72	5.33
Group C1	2.46	14:54	46.11	3.11	35:18	9.81	4.06
C2	1.58	14:39	28.61	4.42	30:17	4.56	5.36
C3	3.07	35:48	42.36	14.03	69:35	8.83	4.33
C4	2.39	23:13	38.47	3.50	38:12	5.47	4.54
C5	1.69	54:08	33.05	3.47	98:10	9.89	4.47

[a]Blood Ammonium-Nitrogen in ug./ml.
[b]Time in Minutes
[c]Duration Equal to Pretreatment Maximal Swim

TABLE 2: Means of pre- and post-treatment BAN concentrations (ug. NH_4^+-N/ml.) and swimming times (min.) for Groups A (swimming control), B (ammonium carbonate control), and C (swimming and ammonium carbonate experimental).

Source	Group A	Group B	Group C
Pretreat. Resting Normal (BAN)	12.97	3.78	2.24
Pretreat. Max. Swim (BAN)	15.23	47.50	37.72
Post-Treat. Swim (BAN)[1]	7.16	4.18	5.71
Post-Treat. Max. Swim (BAN)	6.22	5.38	7.71
Post-Treat. Resting Normal (BAN)	2.78	5.09	4.45
Pretreat. Max. Swim Time	20:55	40:15	28:32
Post-Treat. Max. Swim Time	31:57	44:38	54:18

[1] Duration equal to pretreatment maximal swim

TABLE 3: Analyses of Variance for Groups A (swimming control), B (ammonium carbonate control), and C (swimming and ammonium carbonate experimental).

Source	d.f.	Sum of squares	Mean squares	"F"
BAN Concentration; pre- and post-treatment timed maximal swim				
Total	14	3,825.6139		
Treatment	2	3,216.9716	1,608.4858	31.713**
Error	12	608.6423	50.7202	
BAN Concentration; post-treatment resting normal				
Total	14	20.3321		
Treatment	2	14.6175	7.3088	14.35 **
Error	12	5.7146	.4762	
BAN Concentration; post-treatment maximal swim				
Total	14	148.9630		
Treatment	2	22.9586	11.4793	-11.08^{NS}
Error	12	126.0044	10.5004	
Swimming Time; Pre- and post-treatment maximal swim				
Total	14	2,332.1185		
Treatment	2	1,201.2387	600.6193	6.37*
Error	12	1,130.8798	94.2400	

* $P \leq 0.05$
** $P \leq 0.01$
NS Not significant