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OBSERVATIONS ON THE EFFECT OF EXERCISE ON BLOOD AMMONIA CONCENTRATION IN MAN†

In 1925 Luck, Thacker and Marrack reported elevated blood ammonia levels in epileptic patients after grand mal seizures.[19] Although they suspected that muscular exercise might be responsible for this rise, they apparently excluded this possibility by their inability to produce increased venous ammonia levels by vigorous muscular exercise. Several years later Parnas and his co-workers noted that the blood ammonia concentration was elevated in the veins which drained the exercising forearms of normal subjects.[28] These investigators demonstrated that ammonia liberated by the muscle during fatiguing exercise was responsible for this rise. These observations were confirmed by Kalk and Bonis.[17] Recently, Schwartz, Lawrence, and Roberts re-investigated some aspects of this problem.[24] They induced convulsions in dogs and observed an increase in the arterial ammonia concentration which could be prevented by suppressing the motor aspects of the convulsions with *Anectine*℞. In addition, they were able to produce increased systemic blood ammonia levels in normal men by vigorous generalized exercise.

The recognition by Bessman and his co-workers of the importance of the peripheral tissues in ammonia metabolism has given fresh significance to these earlier observations. These investigators showed that as the arterial ammonia concentration rises in patients with hepatic coma, the peripheral tissues extract correspondingly large amounts of ammonia.[3, 4] These findings which were based on simultaneous arterial and venous ammonia determinations have been confirmed[29, 30, 34] and extended to show that in the terminal phases the peripheral tissues may sometimes liberate ammonia into the systemic circulation.[29, 34] Similar observations have been made following the administration of ammonium salts to normal subjects and animals.[1, 12, 31] The significance of the uptake of ammonia by the peripheral tissues, presumably muscle, is not clear. It has been suggested that this extraction of am-

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† The material for this paper is taken from a thesis submitted in 1959 by Dr. Allen to the Faculty of the Yale University School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Medicine.

Received for publication 18 April 1960.

monia protects more vulnerable sites from high ammonia concentrations. Furthermore, acetazoleamide (Diamox ℞), which may induce impending hepatic coma in cirrhotic patients, has been shown to decrease the arterial-venous ammonia difference and may thus interfere with such a protective function.[11]

This investigation was undertaken to evaluate the effects of muscular exercise on the release of ammonia from peripheral tissues, to compare these phenomena in normal subjects and in cirrhotic patients, and to further our understanding of these phenomena.

MATERIALS AND METHODS

The subjects of these investigations comprised three groups: 10 normal male volunteers, 31 male ambulatory patients with a variety of benign, nonhepatic disorders, and 17 male ambulatory patients with histologically proven cirrhosis.

The blood ammonia* values were determined in triplicate according to the method of Seligson and Hirahara[25] within ten minutes of collecting the blood. Blood was drawn without stasis into 20 ml. syringes which had been coated with heparin. Venous blood was withdrawn from an antecubital vein through an indwelling needle after a small specimen of blood had been discarded. Arterial blood was obtained from either the brachial or femoral artery.

The normal venous blood ammonia concentration, which was determined on ten normal subjects, was 161 ± 21 μg. NH_3 N per 100 ml. This normal range, which was obtained with Upjohn Co. heparin (1000 units per ml.), is much higher than our previous normal range (102± 23)[2] which was established with Organon, Inc. heparin as anticoagulant. It was demonstrated, in retrospect, that these higher normal values were caused by the greater ammonia content of the Upjohn heparin solution. These observations were the subject of a separate communication on the effect of different heparin solutions on the blood ammonia determination.[9] A single lot of Upjohn heparin (1000 units per ml.) was used throughout this study.†

Blood oxygen saturation was determined by the method of Van Slyke and Neill.[32] The hydrogen ion concentration of blood was measured with a Cambridge Model R pH meter. Serum carbon dioxide, sodium, chloride, potassium, magnesium, and glutamic oxalacetic transaminase (SGOT) were determined according to standard methods previously employed in this laboratory.[7,8]

All subjects were studied in the sitting or supine position after resting for 15 minutes. None had had any vigorous muscular exertion prior to examination and none was allowed to squeeze or clench his fist prior to or during the blood collection. Mild exercise consisted of clenching the fist vigorously one hundred times at a rate of one per second. A spring-type hand grip exerciser ("Power Gripper") was em-

*Although it is recognized that ammonia exists almost entirely as ammonium ion at the pH of blood, the term blood ammonia is used throughout this paper to represent the total ammonia-ammonium content of blood.

† There were small variations in the amount of heparin required to wet the barrels of individual syringes (0.14 to 0.18 ml.). Such variations could cause small fluctuations in blood ammonia levels. This study, however, is concerned primarily with relative, rather than absolute, blood ammonia values, and the random distribution of this artifact among the experimental determinations permits valid statistical analysis.

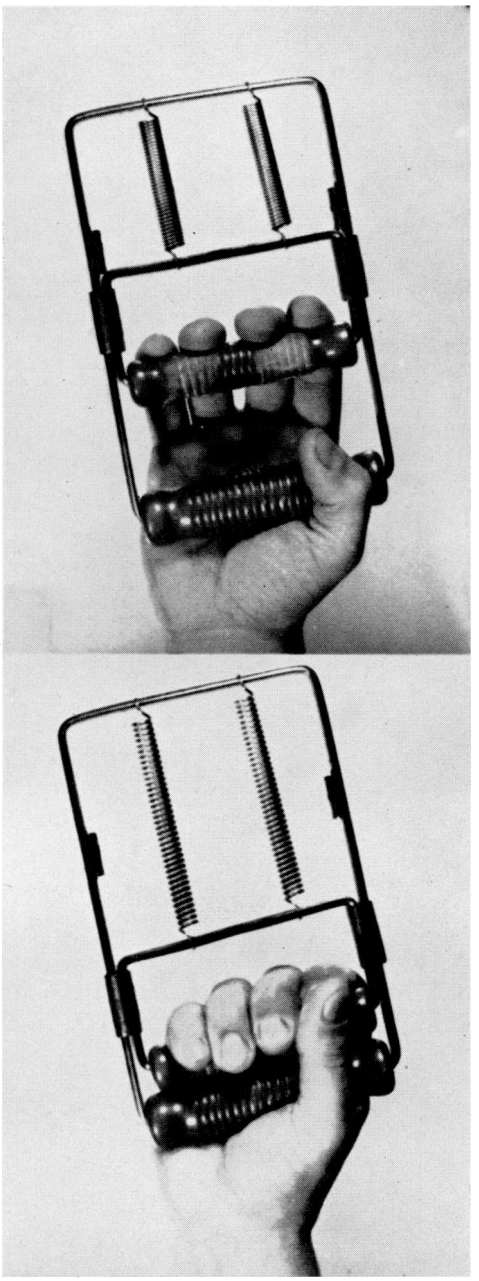

FIG. 1. "Power Gripper" used to exercise the hand and forearm muscles.

ployed to exercise the subjects more strenuously (Fig. 1). Fifty "power grips" at the rate of one per second were considered moderate exercise. One hundred such "power grips" (defined as severe exercise) were sufficient to fatigue the forearm muscles of all normal subjects.

Acetazoleamide (250 mg.) was given orally 13 hours and 1 hour prior to studying the effect of muscular exercise on the blood ammonia concentration in a group of cirrhotic patients in whom baseline exercise studies had previously been made.

RESULTS

Mild exercise. In 10 control patients and 7 cirrhotic patients venous ammonia samples were drawn from the exercising arm before and immediately after 100 fist clenches. The results are shown in Table 1 and Figure 2.

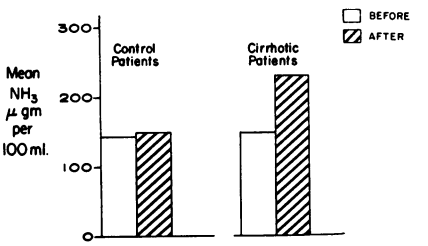

FIG. 2. A comparison of the effect of mild exercise (100 fist clenches) on the mean venous ammonia levels in the exercising arm in cirrhotic and non-cirrhotic patients.

Five cirrhotic patients showed elevations in excess of 40 μg. per 100 ml. and 2 showed no appreciable change. Only 1 patient in the control group showed a large rise after exercise and 1 showed a moderate fall. The difference between the two groups is highly significant ($p < 0.01$). Although the resting blood ammonia level was higher in the cirrhotic patients, there was no correlation between the initial blood ammonia concentration and the absolute or percentage rise observed.

Moderate exercise. In 6 control patients and 7 cirrhotic patients venous ammonia concentrations were determined before and immediately after 50 "power grips." The results are shown in Table 1 and Figure 3.

There was a mean rise of 5 μg. per 100 ml. in the control group and of 80 μg. per 100 ml. in the cirrhotic group. Although 2 of the control patients showed increases of 35 and 36 μg. per 100 ml., respectively, all 7 cirrhotics exhibited increments greater than 45 μg. per 100 ml. The difference between the two groups is highly significant ($p < 0.001$).

Severe exercise. The effect of 100 "power grips" was compared in 10 normal subjects, 10 control patients, and 10 cirrhotic patients.

The mean venous blood ammonia values before exercise were not significantly different in the three groups. All 30 subjects showed large increments in the blood ammonia concentration after exercise. The difference in

response among the three groups is not statistically significant (Table 1, Figure 4).

In addition to these measurements blood ammonia determinations were made 15 minutes after the termination of exercise in some of the subjects in each group. The mean venous ammonia level was still significantly elevated 15 minutes after exercise although several patients in each of the three categories had returned to the resting level (Table 2).

Simultaneous venous blood specimens were also obtained from the resting arm before, immediately after, and 15 minutes after exercise in some of the

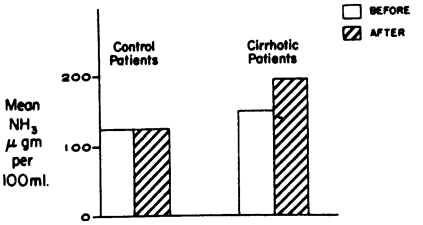

FIG. 3. A comparison of the effect of moderate exercise (50 "Power Grips") on the mean venous ammonia levels in the exercising arm in cirrhotic and non-cirrhotic patients.

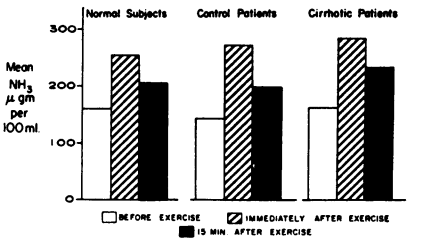

FIG. 4. A comparison of the effect of fatiguing exercise (100 "Power Grips") on the venous ammonia concentration in normal subjects, cirrhotic patients and non-cirrhotic patients.

subjects in each group. These ammonia levels did not change significantly from the resting levels (Table 2).

In 3 control patients and 2 cirrhotic patients both arterial and venous ammonia concentrations were measured before and after 100 "power grips." In each of the 5 patients large increments in the venous ammonia levels were observed after exercise with a mean increase of 121 μg. per 100 ml. However, the mean increment in the arterial ammonia concentration was only 11 μg. per 100 ml. This rise was not statistically significant. In each instance the venous ammonia value was much higher than the arterial concentration. This negative arteriovenous difference, which ranged from -66 to -154 μg. per 100 ml., was not related to either the pre-exercise arterial or venous ammonia concentrations.

Nine cirrhotic patients in whom the effect of severe muscular exercise on blood ammonia had been studied were re-studied within one week to evaluate the effect of acetazoleamide on this phenomenon. Conditions were otherwise unchanged. There was no significant difference in response after acetazoleamide from the results obtained previously. Each patient, both

before and after this drug, showed a sharp rise in venous ammonia concentration. It is interesting to note the similarity of response of the individual patients on the two occasions (Table 3).

TABLE 1. VENOUS BLOOD AMMONIA BEFORE AND AFTER EXERCISE

	Mean and standard deviation Venous NH_3 N in μg. per 100 ml.		
Mild Exercise	*Resting*	*After exercise*	*Difference*
Control patients (10)	127 ± 21	127 ± 24	0 ± 20
Cirrhotic patients (7)	151 ± 26	195 ± 47	43 ± 22
Moderate exercise			
Control patients (6)	146 ± 6	151 ± 30	5 ± 24
Cirrhotic patients (7)	148 ± 15	229 ± 21	80 ± 35
Severe exercise			
Normal subjects (10)	161 ± 12	254 ± 64	94 ± 69
Control patients (10)	144 ± 51	272 ± 82	128 ± 87
Cirrhotic patients (10)	163 ± 44	286 ± 54	122 ± 49

TABLE 2. VENOUS BLOOD AMMONIA BEFORE, IMMEDIATELY AFTER AND 15 MINUTES AFTER SEVERE EXERCISE

	Mean and standard deviation Venous NH_3 N in μg. per 100 ml.		
Exercising arm	*Resting*	*Immediately after exercise*	*15 Minutes after exercise*
Normal subjects (8)	157 ± 13	259 ± 70	202 ± 65
Control patients (8)	143 ± 24	230 ± 86	200 ± 51
Cirrhotic patients (6)	190 ± 58	300 ± 58	236 ± 70
Resting arm			
Normal subjects (7)	164 ± 29	167 ± 28	156 ± 26
Control subjects (5)	163 ± 15	159 ± 20	158 ± 18
Cirrhotic subjects (5)	170 ± 53	179 ± 45	175 ± 32

In a group of 5 control patients and 4 cirrhotic patients venous blood samples were obtained for blood pH, oxygen saturation, serum carbon dioxide content, sodium, chloride, potassium, and magnesium before and immediately after 100 "power grips." These determinations were performed in order to evaluate the relationship of changes in hydrogen ion concentra-

tion, oxygen saturation, carbon dioxide content, and serum electrolytes to the increase in blood ammonia concentration (Table 4).

All 9 patients exhibited a fall in venous blood pH following exercise. The mean pH fell from 7.40 to 7.28. This change, which is highly significant ($p < 0.001$), was greater in the cirrhotic than in the control patients.

TABLE 3. EFFECT OF ACETAZOLEAMIDE ON THE VENOUS AMMONIA CONCENTRATION AFTER 100 "POWER GRIPS" IN CIRRHOTIC PATIENTS

Cirrhotic patients	*Resting*	*After exercise*	*Difference*
	Before Diamox		
W.C.	135	258	123
C.M.	279	330	54
S.S.	197	275	78
L.S.	116	232	116
J.S.	139	207	68
D.S.	104	305	201
J.W.	130	310	180
J.K.	167	313	146
W.P.	143	291	148
Mean ± S.D.	156 ± 55	280 ± 42	124 ± 40
	After Diamox		
W.C.	145	261	116
C.M.	256	285	29
S.S.	215	225	10
L.S.	151	370	219
J.S.	161	235	74
D.S.	108	269	161
J.W.	147	314	167
J.K.	211	324	113
W.P.	138	281	143
Mean ± S.D.	170 ± 48	285 ± 59	115 ± 65

All of the patients showed a rise in venous carbon dioxide content after exercise. The mean serum CO_2 rose from 23.0 to 25.1 mEq. per L. This change is highly significant ($p < 0.001$).

The degree of oxygen saturation decreased in 6 of 8 subjects, including the 5 control patients. In one cirrhotic it was unchanged and in a second it rose slightly. The mean venous oxygen saturation fell from 66 per cent to 55 per cent. These changes are statistically significant ($p < 0.05$).

There was no significant change in the serum levels of sodium, chloride, potassium, or magnesium.

The serum SGOT concentration was measured in 3 control patients and 3 cirrhotic patients before and after fatiguing exercise (100 "power grips"). No significant changes were detected.

DISCUSSION

Cirrhotic patients are particularly apt to develop ammonia intoxication for a number of reasons. First, the functional capacity of the cirrhotic liver

TABLE 4. EFFECT OF EXERCISE ON VENOUS pH, OXYGEN SATURATION AND SERUM ELECTROLYTES

Measurement	*No. of Patients*	*Mean and standard deviation*		
		Resting	*After exercise*	*Difference*
Venous pH	9	7.40 ± 0.03	7.28 ± 0.06	-0.12 ± 0.08
Venous O_2 saturation (vol. per 100 ml)	8	66 ± 12	55 ± 9	- 11 ± 12
Venous CO_2 (mEq./L.)	9	23 ± 2.1	25.1 ± 2.7	2.1 ± 1.1
Serum sodium (mEq./L.)	9	135.8 ± 5.6	137.9 ± 5.2	2.1 ± 2.2
Serum chloride (mEq./L.)	9	100.0 ± 7.3	99.5 ± 6.7	-0.5 ± 1.4
Serum potassium (mEq./L.)	9	4.3 ± 0.55	4.4 ± 0.46	0.1 ± 0.02
Serum magnesium (mEq./L.)	7	1.7 ± 0.2	1.7 ± 0.2	0 ± 0

to remove ammonia and to synthesize urea may be diminished. Secondly, portal-systemic anastamoses permit much of the portal venous blood, which has a high ammonia content, to bypass the liver and to increase the systemic blood ammonia concentration. In addition, some studies have suggested that the removal of ammonia by the peripheral tissues is less efficient in cirrhotics than in noncirrhotic subjects.[29] This may be related to the presence of peripheral arteriovenous shunts which diminish ammonia extraction by the peripheral tissues.[14, 26]

The present investigation indicates that ammonia metabolism in cirrhotic patients may differ from normal subjects in still another manner. Although all subjects liberated ammonia from exercising muscle, cirrhotic patients appeared to release larger amounts after lesser degrees of muscle activity than did noncirrhotics. This was clearly observed with mild to moderate amounts of muscular exertion. After fatiguing exercise, however, both cirrhotic and noncirrhotic subjects exhibited an equally large and prolonged release of ammonia into the venous blood. The local nature of this libera-

tion of ammonia was established by the absence of simultaneous elevations of ammonia in either arterial blood or venous blood draining the resting limb.

Our knowledge of the role of peripheral tissues in ammonia metabolism is based on a relatively few simultaneous measurements of arterial and venous ammonia levels. It has been observed that in the resting, fasting state the peripheral arterial and venous ammonia concentrations are equal or show a small uptake by the peripheral tissues.[4, 31] When arterial ammonia levels are elevated, the arteriovenous ammonia difference increases and may constitute as much as 40 per cent of the arterial level.[3, 4, 29] It has been postulated that this removal of ammonia by the peripheral tissues, presumably muscle, prevents elevations of systemic ammonia levels which are toxic to the central nervous system.[29-31, 34]

The explanation for the greater release of ammonia after relatively mild muscular activity in cirrhotic patients is not clear, but may be indirectly related to the extraction of ammonia by the peripheral tissues. Cirrhotic patients frequently have higher blood ammonia levels than noncirrhotics, and may, by virtue of continued uptake of ammonia by the peripheral tissues, accumulate ammonia in their muscles. The "early" release of ammonia after mild exercise may thus reflect relative saturation of the muscle with ammonia, and may not necessarily involve the same biochemical reactions as the major liberation of ammonia, which follows severe exercise in both cirrhotic and noncirrhotic subjects.

Although the exact sequence of the reactions is not firmly established, adenosine triphosphate (ATP) appears to serve as the source of energy for muscular activity. In the resynthesis of ATP from ADP, adenylic acid (AMP) is formed, and is, in turn, deaminated to inosinic acid. Embden and Parnas, and their co-workers, believed that the ammonia liberated during and after exercise was derived from the deamination of adenylic acid.[18, 21] The significance of this reaction, which does not appear to be important in energy transfer, is unknown.

During muscular exercise many other local metabolic changes take place, including increased oxygen utilization, increased carbon dioxide formation and an increase in hydrogen ion concentration. Although each of these metabolic alterations has been associated with changes in the blood ammonia concentration, it is unlikely that any of these phenomena, per se, is responsible for the increase in venous ammonia levels after exercise.

The pH gradient between the intra- and extracellular fluids has recently been shown to be a major factor in the movement of ammonia across cell membranes.[5, 28] Could a change in the pH gradient account for the increase in venous ammonia values after exercise?

Normally, the pH of the intracellular fluid of muscle is lower than that of extracellular fluid.[33] In the presence of extracellular acidosis there is a decrease in this pH gradient. This favors the passage of ammonia from the intracellular to the extracellular fluid, resulting in an increase in the blood ammonia concentration.[5, 22] Conversely, extracellular alkalosis which increases the pH gradient is associated with the movement of ammonia into the cells and a decrease in the blood ammonia concentration.[5, 18] Fatiguing muscular exercise, which produces a profound local intracellular acidosis[20] also increases the pH gradient. Such a gradient would result in a decrease in blood ammonia concentration and consequently could not explain the increase in venous ammonia levels.

Several observations have suggested that blood ammonia concentrations may parallel the degree of oxygen saturation.[14, 15] Could the increased venous ammonia values after exercise be associated with variations in the venous oxygen saturation? This seems unlikely for several reasons. First, the magnitude of the increase in blood ammonia levels after exercise is much greater than that observed with changes in oxygen saturation. Secondly, the direction of change is opposite to that to be expected with changes in oxygen saturation.

There is some evidence to suggest that the blood ammonia concentration rises as the carbon dioxide tension falls *in vitro*.[10, 27] However, the simultaneous rise in both venous ammonia and carbon dioxide after exercise does not support this relationship *in vivo*.

Muscular exercise is accompanied by a local increase in blood flow which would tend to diminish, rather than enhance, an increase in the venous ammonia concentration. Similarly, the shunting of arterial blood through arteriovenous anastamoses cannot explain the rise in venous ammonia levels after exercise since the arterial ammonia levels are much lower than the venous levels.

There is no evidence to indicate that the release of ammonia during exercise is related either to the uptake of ammonia by the peripheral tissues in hyperammonemic states,[3, 4, 29] or to its discharge into the venous blood under certain circumstances.[29, 31, 34] Bessman and his co-authors postulated that in these situations ammonia was reversibly utilized in the synthesis of glutamine from α-ketoglutarate.[3, 4] Dawson and his co-workers have shown that acetazoleamide may interfere with the uptake of ammonia by the peripheral tissues.[11] This drug did not modify the ammonia rise induced by exercise in our studies.

Although the mechanism of the release of ammonia after muscular exercise is not well understood, this phenomenon emphasizes several points of

practical importance. First, the common practice of having the patient repeatedly clench his fist to facilitate the filling of peripheral veins must be assiduously avoided, since as few as 50 fist clenches may cause significant elevations of the venous ammonia concentration. Although some investigators have noted this artifact,[4, 16] it has not been universally recognized. It is of interest that unusually high venous ammonia values in newborn infants were attributed, at least in part, to the physical activity of the infants during the collection of the blood.[6]

Secondly, the importance of bed rest in the management of patients with impending hepatic coma should be further evaluated. Certainly the role of the peripheral tissues in ammonia metabolism deserves further investigation.

SUMMARY

Mild muscular exercise resulted in an increase in the venous ammonia concentration of blood draining the exercising forearm of cirrhotic patients. Identical exercise in normal subjects did not elevate the venous ammonia level.

More severe muscular exercise caused a significantly greater increase in venous ammonia values in cirrhotic patients than in noncirrhotic subjects.

Severe fatiguing exercise resulted in large elevations in the venous ammonia concentration which were approximately equal in both cirrhotic and noncirrhotic subjects. This exercise-induced rise was not modified by the use of acetazoleamide.

These phenomena are of theoretical and practical importance. Further investigations are needed to clarify the mechanism and significance of these observations.

ACKNOWLEDGMENTS

The authors are indebted to Drs. Massimo Calabresi, Nicholas Alexander, Donald Buchanan, David Seligson, and Gerald Klatskin for their help and suggestions, and to Dr. Colin White for his assistance with the statistical analyses.

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