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CARBON DIOXIDE RETENTION: A MECHANISM OF AMMONIA TOLERANCE IN MAMMALS[1]

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Abstract. When guano bats, *Tadarida brasiliensis,* inhale ammonia in air mixtures, carbon dioxide is passively retained in sufficient amounts to neutralize alkali excess resulting from increased blood ammonia levels. There is no change in blood carbon dioxide levels in house mice, *Mus musculus.* Little brown bats, *Myotis lucifugus,* are intermediate in this respect. Passive carbon dioxide retention is clearly related to ammonia tolerance in these mammals.

INTRODUCTION

Mammals exhibit extreme diversity in tolerance, i.e. gross survival, to inhaled ammonia in air mixtures (Weedon, Hartzell, and Setterstrom 1940; Henderson and Haggard 1943; Studier, Beck, and Lindeborg 1967). The ability of bats and rabbits to withstand high levels of inhaled ammonia has prompted general studies on tolerance mechanisms in those groups (Boyd, MacLachlan, and Perry 1944; Mitchell 1964; Studier, Beck and Lindeborg 1967). Most studies concerning mechanisms of tolerance to inhaled ammonia in mammals can be divided into two general areas: (i) the interaction of ammonia and respiratory tract mucous (Studier 1966, 1969) and (ii) homeostatic mechanisms concerned with neutralization of alkali excess (Mitchell 1964; Studier 1965). Additionally, much of the work concerned with the toxic effects of hyperammonemia resulting from metabolic disorders in normal ammonium detoxification pathways also applies to the variance in tolerance to inhaled ammonia exhibited by mammals (Warren and Schenker 1964; Shorey, McCandless, and Schenker 1967; Schenker et al. 1967). Although blood pH of the guano bat does not change significantly during exposure to high levels of inhaled ammonia (Studier 1965), no evidence has been presented to indicate adjustments in homeostatic systems for maintenance of body fluid pH which could partially account for the extremely high tolerance to inhaled ammonia exhibited by this bat. The present study tests the effects of ammonia inhalation on blood carbon dioxide levels and examines changes in blood carbon dioxide concentrations as a mechanism which may partially account for some of the diversity in tolerance to inhaled ammonia.

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MATERIALS AND METHODS

Three species of mammals with widely differing tolerance to inhaled ammonia in air mixtures were tested. Guano bats, *Tadarida brasiliensis,* exhibit extremely high tolerance; house mice, *Mus musculus,* have very low tolerance; and, little brown bats, *Myotis lucifugus,* are intermediate in this respect (Studier, Beck, and Lindeborg 1967).

Adult mice of both sexes were obtained commercially. Adult male guano bats were collected in the last week of June 1967, from a cave 19 miles S of Grants, Valencia Co., N.M. Pregnant little brown bats were collected during the first week of June 1967, from Montezuma Seminary, Montezuma, San Miguel Co., N.M. Parturition in this group of little brown bats occurred in mid-July 1967. Bats were captured just after returning to their roosts at dawn and were tested before sunset on the day of capture to insure comparable nutritional and physiological states.

Preparation of gas mixtures and the gassing chambers used in this study have been described previously for determination of metabolic rate in these animals (Studier, Beck, and Lindeborg 1967). All animals were exposed for one-half hour at a given ammonia concentration and were nearly all at metabolic equilibrium (reached constant metabolic rate) by the end of the exposure period (Studier, Beck and Lindeborg 1967).

Blood was collected from the brachial artery of bats

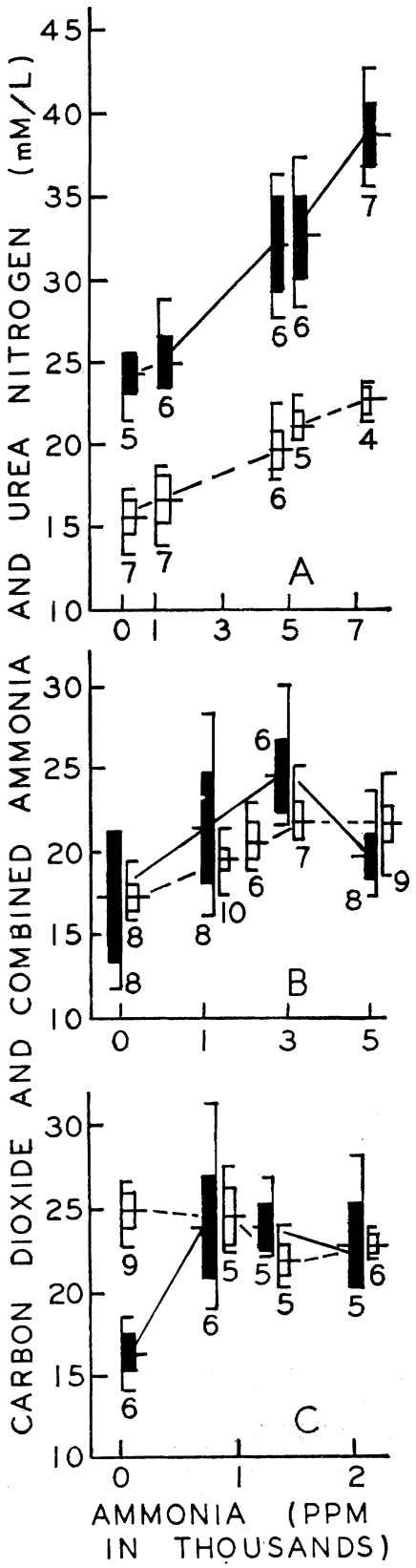

FIG. 1. Mean, range and 95% confidence interval of carbon dioxide and combined ammonia and urea nitrogen levels in the plasma of A) guano bats, B) little brown bats, and C) house mice exposed to various ammonia in air mixtures. Open symbols are carbon dioxide levels; solid symbols are combined ammonia and urea levels. Sample size is indicated beside each set of values.

and the tail of mice under oil in small heparinized capillary tubes and centrifuged. Plasma carbon dioxide determinations were made with a Natelson Microgasometer, Model #650. For determinations of combined ammonia and urea nitrogen, blood was collected into small heparinized, tapered centrifuge tubes. Ammonia and urea nitrogen content of the plasma of centrifuged samples was determined with the Natelson Microgasometer. This method is specific for ammonia and urea nitrogen. Nitrogen of uric acid is released very slowly and does not interfere with the determination. Amides undergo a Hofman rearrangement and do not interfere.

Little brown bats were tested at 0, 1,330, 1,990, 3,010 and 5,180 parts per million (ppm) ammonia. Guano bats were tested in ammonia concentrations of 0, 1,130, 4,420, 5,040 and 7,200 ppm. Ammonia concentrations used for house mice were 0, 870, 1,370 and 2,100 ppm. Ammonia concentrations were accurate to within 10 ppm; 1,000 ppm ammonia is equivalent to a partial pressure of 0.63 mm Hg at our elevation (6,500 ft). Most of these ammonia levels are well above the natural concentrations encountered by any of the species tested.

RESULTS AND DISCUSSION

Figure 1-A illustrates plasma carbon dioxide and combined ammonia and urea nitrogen levels in *Tadarida brasiliensis* after exposure to various inhaled ammonia in air mixtures. When inhaling air, guano bat plasma carbon dioxide and combined ammonia and urea nitrogen mean values are 15.5 and 24.5 mM/liter, respectively. When the level of ammonia in inhaled air is increased, plasma ammonia and urea nitrogen increases at a rate which is nearly paralleled by rises in plasma carbon dioxide, reaching a maximum at 7,200 ppm ammonia when plasma carbon dioxide and combined ammonia and urea nitrogen mean values are 22.7 and 38.1 mM/liter, respectively. Inhaled ammonia levels greater than 7,200 are lethal to guano bats within one-half hour. Metabolic rate in *T. brasiliensis* is inversely proportional to inhaled ammonia levels indicating that ammonia tolerance in guano bats involves passive mechanisms (Studier, Beck, and Lindeborg 1967). Presumably, retention of carbon dioxide both in the blood stream and in respiratory mucous could result from drastic decrease in alveolar ventilation in spite of the observed hypometabolism (Studier, Beck, and Lindeborg 1967).

The partial pressure of ammonia in alveolar air is the same as the calculated partial pressure of ammonia in arterial plasma because ammonia is equilibrated between alveolar air and the blood during its passages through pulmonary capillaries (Jacquez, Poppell, and Jeltsch 1959; Robin et al. 1959). Ammonia is exhaled by the guano bat upon its return from highly ammoniated air to normal air in amounts equivalent to the elevation in blood ammonium levels produced through inhalation of ammoniated air (Studier 1966). There is no significant rise in urinary urea and ammonium nitrogen in the California leaf-nosed bat after exposure to high inhaled levels of ammonia (Mitchell 1963). For these reasons, it appears that most of the elevation in blood urea and ammonia nitrogen found in the species tested, particularly guano bats, results wholly from increased blood ammonium levels.

The elevation of blood ammonium level of 13.6 mM/liter under the conditions described here is surprising. Although normal blood ammonium levels are about 0.06 mM/L, mice can withstand up to 5.4 mM/kg and rats can survive 7.9 mM/kg of injected ammonium acetate (Shorey, McCandless, and Schenker 1967). Since guano bats are much more tolerant to inhaled ammonia than are mice and rats, the rise in blood ammonium level reported here does not seem unreasonable.

Plasma carbon dioxide and combined ammonia and urea levels in *M. lucifugus* after exposure to various ammonia concentrations are shown in Figure 1-B. Plasma of the little brown bat contains 17.2 mM/liter carbon dioxide and 17.2 mM/liter ammonia and urea nitrogen in air and 21.8 mM/liter and 24.6 mM/liter respectively at 3,010 ppm. The mechanism of tolerance to low inhaled ammonia levels in *M. lucifugus* appears also to be passive retention of carbon dioxide in sufficient quantities to buffer rising plasma ammonia and urea levels. However, when inhaling 5,180 ppm ammonia, the plasma ammonia and urea nitrogen values drop drastically while the plasma carbon dioxide level remains constant. In low ammonia concentrations the metabolic rate of the little brown bat is inversely proportional to the ammonia concentration; however, when inhaling air containing 3,000 ppm ammonia or higher, the metabolic rate of the little brown bat is markedly higher than control values (Studier, Beck, and Lindeborg 1967). The little brown bat apparently is able to retain sufficient carbon dioxide to buffer absorbed ammonia at low inhaled concentrations but plasma carbon dioxide reaches a maximum allowable level at about 21.5 mM/liter. When inhaled concentrations of ammonia result in blood ammonium levels which rise above a level which can be buffered by retention of carbon dioxide, a secondary defense mechanism is initiated which requires high energy expenditure. This secondary defense mechanism may involve attempted removal of ammonia by renal excretion of fixed base. Ammonia concentrations of 3,000 ppm or higher are ultimately fatal to *M. lucifugus* (Studier, Beck, and Lindeborg 1967). Therefore, it appears that this secondary energy-requiring defense mechanism imposes such a large drain on energy production and reserves that it provides only a temporary mechanism of ammonia tolerance. Death may occur then from disruption of acid-base balance when metabolic rate cannot be maintained at a sufficient rate to sustain this secondary mechanism of ammonia tolerance.

Mice exhibit very poor tolerance to inhaled ammonia (Studier, Beck, and Lindeborg 1967). Figure 1-C includes the determinations of plasma carbon dioxide and combined ammonia and urea nitrogen of *Mus musculus* after exposure to varied ammonia concentrations. Mean plasma values of *M. musculus* are 24.8 mM/liter carbon dioxide in air, dropping to 22.8 mM/liter at 2,100 ppm. The metabolic rate of *M. musculus* is directly proportional to inhaled ammonia levels (Studier, Beck, and Lindeborg 1967). Mean plasma ammonia and urea nitrogen rise from 16.2 mM/liter to about 24 mM/liter where equilibrium is established. This rise of 7.8 mM/liter ammonia and urea nitrogen is well above the maximum tolerance levels of 5.4 mM/kg injected ammonium acetate found for mice (Shorey, McCandless, and Schenker 1967) suggesting that a relatively large amount of the increase found in this study is due to increased blood urea levels in the mice. It appears that mice combat the toxic effects of absorbed ammonia by an energy-requiring mechanism at all levels of inhaled ammonia because: 1) there is no apparent rise in blood carbon dioxide to compensate for the rise in plasma combined ammonia and urea nitrogen; 2) plasma ammonia and urea nitrogen reach a constant blood level; and, 3) metabolic rate is directly proportional to inhaled ammonia. Presumably, this mechanism involves renal excretion of fixed base. Toxicity of injected ammonium salts in mice appears to be dependent upon the rate of detoxification of ammonia in the brain stem (Shorey, McCandless, and Schenker 1967). Since brain ammonium levels must be at least partially dependent on blood ammonium concentration, tolerance to inhaled ammonia in house mice appears to depend upon the amount of energy necessary to maintain blood ammonium levels within a tolerable range. The metabolic response of mice and their inability to retain carbon dioxide in response to elevated levels of inhaled ammonia may explain the observations that hypercapnia and hypoxia tend to augment the toxicity of injected ammonium salts (Warren and Schenker 1960, 1962). Both of these conditions exist normally in the roosting environment of guano bats (Mitchell 1964) and apparently aid in their ability to tolerate high inhaled ammonia levels.

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