PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **187**, 278–281 (1988)

Rat Metabolic Adaptation to Ammonia Inhalation (42664)

A. MANNINEN,* S. ANTTILA,† AND H. SAVOLAINEN‡

**Regional Institute of Occupational Health, P.O. Box 93, 70701 Kuopio, Finland; †Department of Pathology, University of Oulu, Oulu, Finland; and ‡Institute of Occupational Health, Helsinki, Finland*

Abstract. Rats exposed to 25 or 300 ppm NH_3 vapor for 5–15 days 6 hr daily showed dose-dependent blood ammonia after 5 days. Brain and blood glutamine were also increased at the same time in rats exposed to 300 ppm. The blood ammonia concentration of the exposed rats resumed control levels at 10 and 15 days while hepatic citrulline synthesis increased above that of the controls. The animals exposed to 300 ppm NH_3 showed initially a slight acidosis. As circulating ammonia decreased, brain and blood glutamine returned to the control range. Our findings support the idea that ureagenesis is aimed mainly at the removal of NH_4^+ with only secondary and small effects on the acid–base balance. © 1988 Society for Experimental Biology and Medicine.

Chronic ammonia inhalation by domestic animals and their tenders is common in modern agriculture (1). Ammonia vapor at normal concentrations has been regarded as only a nuisance (2); however, very high concentrations cause caustic damage of the upper respiratory tract and pulmonary edema (3). Moreover, moderate doses of ammonium salts have very interesting effects on cultured astroglial cells (4), on macrophage functions (5), and on ureagenesis in metabolic acidosis (6).

Apart from the assertation that the urea cycle in liver may be crucial in acid–base balance (7), it is clear that ureagenesis is aimed primarily at removal of the ammonium ion (8). Acute administration of ammonium chloride causes metabolic acidosis (8). Eventually this situation would probably prove to be intolerable, so it is reasonable to expect metabolic adaptation by the exposed animals toward sustained absorption of ammonia by inhalation. Notably, increased ureagenesis could be a logical reaction. Although costly in chemical energy, 4 ATP moles being consumed for every mole of urea produced, the product is electrically neutral, highly soluble in water, and virtually nontoxic.

This paper examines the effects of subacute inhalation of ammonia on its own inactivation by the urea cycle at the two concentration levels relevant to facilities for domestic animals.

Materials and Methods. *Animals.* Female 14-week Wistar rats were housed in 1-m^3 cubicles in metal cages with bottom grates on a 12-hr light/12-hr dark cycle. The cubicle air exchange was 10 to 15 times per hour. All animals received animal chow (Ewos, Finn Ewos) and tap water *ad libitum.*

Exposures. Rats were divided into nine groups by a randomization procedure: each group included five animals. The animals were exposed to gaseous ammonia in the exposure chambers. The gas was led through an adjustable flowmeter to maintain two different concentrations, 25 ppm in one chamber and 300 ppm in the other chamber. The pure gas (AGA, 99.95% NH_3, Helsinki, Finland) was diluted to desired concentrations by mixture with air through a hole in the bottom of the chamber. The vapor was expelled through a hole in the chamber roof. There were three cages in one chamber at the beginning of the test. The exposures lasted for 5, 10, or 15 days. The animals were exposed 6 hr daily. The ammonia concentrations were controlled by an IR Miran 1B spectrometer and Drager diffusion tubes (Drager Ammoniak 10/a-D, Dragerwerk. Ag. Lubeck). Control rats were sham exposed to the air flow simultaneously.

Analyses. All animals were weighed initially and before being sacrificed. Animals were anesthetized with pentobarbital (60 mg/ml, 60 mg/kg ip) and blood samples were taken for NH_4^+, urea, and glutamine analyses

0037-9727/88 $1.50

Copyright © 1988 by the Society for Experimental Biology and Medicine.
All rights reserved.

and for pH measurements. Brain, liver, and kidney were removed at the autopsies and frozen at −20°C. Blood ammonia concentrations were analyzed by Blood Ammonia Checker (9). Blood pH measurements were done by electrode (Radiometer, Copenhagen). Glutamine was analyzed by LKB Biochrom 4151 Alpha Plus amino acid analyzer. For brain NH_4^+ and glutamine, cerebral hemispheres were homogenized in 7 vol of water. The combined activity of the liver carbamoyl phosphate synthetase and ornithine transcarbamylase was measured according to Welbourne and Joshi (6) and Lusty (10). Protein measurements were made according to Lowry *et al.* (11).

Histopathology. The animals were autopsied immediately after killing. The thoracic cage was opened, the trachea was canulated, and the aorta was cut above the diaphragm. The lungs were inflated with air and perfused with Karnovsky's fixative (19) via the pulmonary artery. The block containing the lungs and heart was then put into the same fixative for overnight. Part of the liver and one kidney were fixed in 4% formalin for light microscopy, and small pieces of liver were fixed in 4% formaldehyde–1% glutaraldehyde solution for electron microscopy. For light microscopy, the tissue samples were conventionally embedded in paraffin, and the sections were stained with hematoxylin and eosin. For electron microscopy, the tissue was postfixed in osmium tetroxide, embedded in an epoxy medium, cut to 60-nm sections, and stained with uranyl acetate and lead citrate.

Statistical analyses were made by two-way analysis of variance and with Student's t test (12).

Results. Body weights of the exposed rats did not differ from those of controls (data not shown). Blood NH_4^+ (Y, nmol/g) increased linearly according to the NH_3 dose (X, ppm) at 5 days of exposure ($Y = 0.23X + 37.50$, $r = 0.89$, $N = 15$). The blood NH_4^+ decreased significantly at later time points in the exposed rats (Table I) so that the apparent dose response was lost. The blood glutamine concentration in rats exposed to 300 ppm was slightly above the control levels at 5 days (Table I). This difference leveled off later on; at no time did the blood urea values of the exposed rats differ from those of the controls (data not shown).

The blood pH was 7.43 ± 0.06 (±SD, $N = 5$), after 5 days for the control rats, 7.34 ± 0.04 (±SD, $N = 5$, $P < 0.05$) in rats exposed to 25 ppm NH_3, and 7.36 ± 0.03 (±SD, $N = 5$, $P < 0.05$) in those exposed to 300 ppm NH_3. These differences leveled off at later time points (data not shown). At the end of the experiment the plasma bicarbonate values had not differed from those of the controls: rats exposed to 25 ppm, 24 ± 0.9 mM ($N = 5$); to 300 ppm, 23 ± 2.2 mM ($N = 5$); and controls, 24 ± 0.9 mM, $N = 5$).

Brain ammonia values in the exposed rats did not differ from those of the controls at any time point, while brain glutamine concentrations in the former increased after 5 days (Table II) and then decreased later.

The hepatic citrulline synthesis by the exposed rats did not differ from that of the

TABLE I. BLOOD AMMONIA AND GLUTAMINE IN NH_3 VAPOR EXPOSURE

Time (days):	5		10		15	
NH_3 vapor (ppm)	Ammonia (nmol/ml)	Glutamine (nmol/ml)	Ammonia (nmol/ml)	Glutamine (nmol/ml)	Ammonia (nmol/ml)	Glutamine (nmol/ml)
0	35 ± 18	410 ± 50	43 ± 12	520 ± 60	32 ± 7	500 ± 50
25	44 ± 18	470 ± 40	57 ± 18	540 ± 40	21 ± 4	500 ± 90
300	105 ± 14***	590 ± 70*	51 ± 30	590 ± 100	35 ± 19	570 ± 50

Note. Each figure is the mean of five determinations ± SD.
* Differs from control at $P < 0.05$.
*** Differs from control at $P < 0.001$.

TABLE II. BRAIN AMMONIA AND GLUTAMINE IN NH_3 VAPOR EXPOSURE

Time (days):	5		10		15	
NH_3 vapor (ppm)	Ammonia (μmol/g)	Glutamine (μmol/g)	Ammonia (μmol/g)	Glutamine (μmol/g)	Ammonia (μmol/g)	Glutamine (μmol/g)
0	0.272 ± 0.042	10.9 ± 2.6	0.260 ± 0.039	9.3 ± 0.7	0.298 ± 0.070	9.8 ± 0.9
25	0.225 ± 0.064	15.5 ± 5.2*	0.162 ± 0.038	11.8 ± 1.8	0.268 ± 0.036	9.8 ± 0.7
300	0.212 ± 0.068	15.3 ± 1.1**	0.209 ± 0.033	9.7 ± 0.9	0.215 ± 0.059	10.0 ± 0.6

Note. Each figure is the mean of five determinations ± SD.
* Differs from control at $P < 0.05$.
** Differs from control at $P < 0.01$.

controls after 5 days of exposure (Fig. 1), while the capacity increased gradually after 10 and 15 days so that a significantly enhanced synthesis was found at the end of the experiment (Fig. 1).

Macroscopically visible large hemorrhages were noticed on the surfaces of the lungs in several of the exposed rats and in a few of the control animals. Light microscopy also showed intraalveolar fresh blood, although the difference in blood amounts in the alveoli between the groups was not statistically significant. There were no signs of alveolar damage; the tracheobronchial tree was also normal by photomicroscopy.

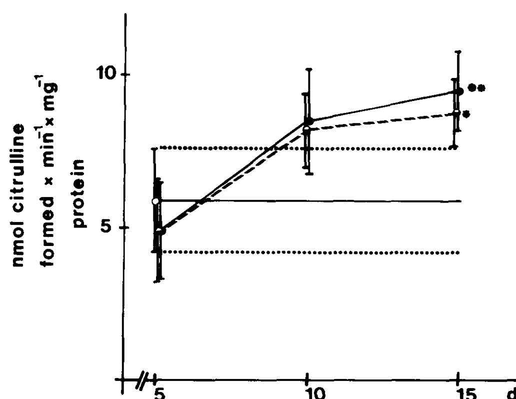

FIG. 1. Effect of ammonia inhalation on the combined activity of carbamoyl phosphate synthetase and ornithine carbamoyl transferase in liver given as formed citrulline. Open circle stands for control with bars indicating 1 SD ($N = 10$). Filled circles with bars (±SD, $N = 5$) represent data from rats exposed to 300 ppm for 6 hr daily, and semifilled circles (±SD, $N = 5$) are for rats exposed to 25 ppm ammonia vapor for 6 hr daily, respectively. Differs from control at $*P < 0.05$ and $**P < 0.01$ (two-way analysis of variance).

The histological architecture of the liver was fully preserved. No ultrastructural alterations, not even swelling of the smooth endoplasmic reticulum, could be seen. The volume fraction of the mitochondria of the cell cytoplasm, as measured by morphometric method, was similar in all groups at all times.

The histological structure of the kidney was mainly normal. However, traces of calcium casts were found in the distal tubules in all groups, but without statistical significance.

Discussion. The stable level of brain ammonia was probably due to its inactivation by the astroglial glutamate–glutamine cycle, which caused the expansion of the brain glutamine pool (13). (This pathway seems to maintain the brain ammonia within narrow limits at the expense of the expansion of the brain glutamine pool.) Brain glutamine is primarily removed by efflux, which may be partially impeded by the simultaneously elevated blood glutamine. Increased brain glutamine has been detected in dogs infused with ammonium salts (14) or in hyperammonemic rats (15, 16).

It is very interesting to note that the activity of the citrulline formation in liver increases by continued exposure to ammonia vapor. The simultaneously decreasing blood ammonia concentrations coincide with the increased ureagenesis. However, blood urea did not increase, since its excretion by the kidneys was not hindered. The blood pH, only slightly acid at 5 days, and blood glutamine resumed control ranges at the same

time, probably indicating the role of increased NH_4^+.

It seems that the substrate for carbamoyl phosphate synthetase is NH_3 rather than NH_4^+ (17). The K_m for NH_3 is 10–14 μM, which is rather high in view of the circulating NH_4^+ and which would cause 1–2.5 μM free NH_3 concentrations at the appropriate pH. The high K_m of this rate-limiting enzyme may partially explain the elevated blood ammonia concentrations from ammonia inhalation introduced in the pulmonary vein and arterial circulation before its extraction from the blood stream by liver. It is also intriguing to note that the enhanced citrulline synthesis had no effect on the circulatory bicarbonate despite the fact that it is also the cosubstrate for the carbamoyl phosphate synthetase.

The ammonia vapor concentrations used in this study were not particularly high. Sensory irritation should have been small (18), although pulmonary hemorrhages might have resulted from the exposure (20). Nevertheless, the concentrations were too low to cause histological alterations in the lung, liver, or kidney, while still causing metabolic adaptation. This implies that similar adaptive reactions can occur in domestic animals maintained in poorly ventilated sheds.

This study was supported by the National Board of Labour Protection and by the Farmers' Social Insurance Organization. We thank Mr. Heikki Pekonen and Ms. Terttu Viitanen for their technical assistance.

1. Donham KJ, Popendorf WJ. Ambient levels of selected gases inside swine confinement buildings. Amer Ind Hyg Assoc J **46**:658–661, 1985.
2. Kangas J, Nevalainen A, Manninen A, Savolainen H. Ammonia, hydrogen sulfide and methyl mercaptides in Finnish municipal sewage plants and pumping stations. Sci Total Environ **57**:49–55, 1986.
3. Walton M. Industrial ammonia gassing. Brit J Ind Med **30**:78–86, 1973.
4. Gregorius JB, Mozes LW, Norenberg LOB, Norenberg MD. Effect of cyclic AMP on ammonia-induced alterations in primary astrocyte cultures. J Neuropathol Exp Neurol **45**:396–407, 1986.
5. Brown JA, Novak EK, Swank RT. Effects of ammonia on processing and secretion of precursors and mature lysosomal enzyme from macrophages of normal and pale ear mice: Evidence for two distinct pathways. J Cell Biol **100**:1894–1904, 1985.
6. Welbourne TC, Joshi S. Hepatic enzymes of glutamine and ureagenesis in metabolic acidosis. Proc Soc Exp Biol Med **182**:399–403, 1986.
7. Walser M. Roles of urea production, ammonium excretion and amino acid oxidation in acid-base balance. Amer J Physiol **250**:F181–F188, 1986.
8. Halperin ML, Chen CB, Cheema-Dhadli S, West ML, Jungas RL. Is urea formation regulated primarily by acid-base balance in vivo? Amer J Physiol **250**:F605–F612, 1986.
9. Ratnaike RN, Buttery JE, Hoffmann S. Blood ammonia measurement using a simple reflectometer. J Clin Chem Clin Biochem **22**:105–108, 1984.
10. Lusty CJ. Carbamoylphosphate synthetase I of rat-liver mitocondria. Eur J Biochem **85**:373–383, 1978.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
12. Armitage P. Statistical Methods in Medical Research. Worcester, Blackwell, p504, 1983.
13. Phelps ME, Huang SC, Hoffman EJ, Selin C, Kuhl DE. Cerebral extraction of N-13 ammonia: Its dependence on cerebral blood flow and capillary permeability—Surface area product. Stroke **12**:607–619, 1981.
14. Hills AG, Rewid EI, Kerr WD. Circulatory transport of L-glutamine in fasted mammals: Cellular sources of urine ammonia. Amer J Physiol **223**:1470–1476, 1967.
15. Williams AH, Kyu MH, Fenton JCP, Cavanagh JB. The glutamate and glutamine content of rat brain after portocaval anastomosis. J Neurochem **19**:1073–1077, 1972.
16. Hawkinds RA, Miller AL, Nielsen RC, Veech RL. The acute action of ammonia on rat brain metabolism *in vivo*. Biochem J **134**:1001–1008, 1973.
17. Cohen NS, Kyuan FS, Cheung CW, Raijman L. The apparent K_m of ammonia for carbamoyl phosphate synthetase (ammonia) *in situ*. Biochem J **229**:205–211, 1985.
18. Wood RW. Behavioral evaluation of sensory irritation evoked by ammonia. Toxicol Appl Pharmacol **50**:157–162, 1979.
19. Karnovsky MJ. A formaldehyde-glutaraldehyde mixture of high osmolality for use in electron microscopy. J Cell Biol **27**:137–138, 1965.
20. Charles DR, Payne CG. The influence of graded levels of atmospheric ammonia on chickens. 1. Effects on respiration and on the performance of broilers and replacement growing stock. Brit Poult Sci **7**:177–187, 1966.

Received March 2, 1987. P.S.E.B.M. 1988, Vol. 187.
Accepted November 2, 1987.