8. This work was supported in part by funds from the National Science Foundation and from the Atomic Energy Commission [No. AT (04-3-41)]. One of us (E. H.) is indebted to the Rockefeller Foundation for a travel grant which has aided continued cooperation on this and related problems.
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Partial Pressure of Ammonia in Alveolar Air

Abstract. The partial pressure of ammonia in alveolar air was measured and found to be the same (within the limits of experimental error) as the calculated partial pressure of ammonia in arterial plasma. It is likely that ammonia is equilibrated between alveolar air and the blood during its passage through the pulmonary capillaries.

The feeding of ammonium salts to cirrhotics or to dogs and human beings with portocaval shunts induces symptoms which are similar to those of hepatic coma (*1*). Ever since the work of Hahn *et al.* (*2*), evidence has been presented at times linking elevated blood ammonia levels and hepatic coma. However, Conway's (*3*) extensive studies and his conclusion that there is no ammonia in normal blood have led to controversy over the interpretation of measurements of blood ammonia. Recently we have suggested (*4*) that it might be the free rather than the total blood ammonia which is of significance in the clinical manifestations of hepatic coma, and that therefore we might find a better correlation with the partial pressure of ammonia (P_{NH_3}). Measurement of alveolar P_{NH_3} and comparison of it with the P_{NH_3} calculated from the plasma *p*H and total ammonia would help in the resolution of this problem. Poppell (*5*) has demonstrated the presence of ammonia in the expired air of dogs with Eck fistulas and of normal dogs. This encouraged us to attempt more quantitative measurements (*6*).

Mongrel dogs with portocaval shunts were deeply anesthetized with Nembutal (*7*). Because of the possibility of ammonia formation by bacterial action in the mouth, tracheal intubation was used. A double glass cannula with inflatable balloon was inserted into the trachea to within a few inches of the carina. The dog was ventilated via one side of the cannula with H_2SO_4-washed air by means of a variable speed respirator. End-expiratory air, sampled through the other half of the cannula by means of a variable speed pump (*8*), was passed through two ammonia absorbers in series and collected into a Douglas bag. The sampling was controlled by a valve in the cannula which was synchronized with the respirator to open at the end of expiration and close before the start of inspiration. During the gas-sampling period, two samples of arterial blood were taken anaerobically into heparinized syringes. Temperature was recorded by an oesophageal telethermometer. In test runs in which a 5-gallon gas reservoir was used in place of the dog, no ammonia was ever picked up in the ammonia absorbers.

The *p*H of the blood was measured with a Cambridge model R *p*H meter with a water-jacketed glass electrode. Plasma CO_2 was determined by the Van Slyke (*9*) manometric method, and the P_{CO_2} was calculated from the factors given by Milch *et al.* (*10*). The CO_2 in the gas sample was measured by the Scholander micromethod (*11*). The total plasma ammonia was measured by a modification of the method of Seligson and Hirahara (*12*). The P_{NH_3} of plasma was calculated, using the solubility coefficients for human plasma (*13*). The ammonia in the expired air was collected in a column of glass beads moistened with .02*N* H_2SO_4, in a 250-ml cylindrical separatory funnel. The ammonia was then released with saturated K_2CO_3, diffused to a drop of acid on a glass rod in the stopper, and determined with Nessler's reagent.

The results are summarized in Table 1. The last two columns show, respectively, the measured alveolar P_{NH_3} and the arterial P_{NH_3} calculated from the plasma total ammonia, the *p*H, and the solubility coefficient of ammonia (*13*). The correspondence between the two is very good, considering the cumulative errors in the experiments and particularly considering the fact that the alveolar P_{NH_3} is an average over a fairly long collection period whereas the calculated plasma P_{NH_3}'s are for two points in the collection period.

Recently we have measured the ammonia in the alveolar air of two normal dogs and have again obtained adequate checks between the measured alveolar P_{NH_3} and the calculated arterial P_{NH_3}. Although equilibration periods of at least 1 hour were used prior to the collection periods, arterial blood *p*H's varied by ± .02 *p*H units, and occasionally by more, and the plasma total ammonia often changed somewhat. The check between the alveolar P_{NH_3} and the calculated plasma P_{NH_3} is independent corroborative evidence that the total plasma ammonia measured by our method (*12*) is of the correct order of magnitude and corresponds to the amount present in plasma in vivo. Our measurements of normal human blood ammonia have checked with those of Calkins (*14*), who found, by means of Conway's method, a mean value of 0.8 μg of NH_3 nitrogen per milliliter of normal human blood. It would appear that the statement of Conway that there is less than 0.1 μg of NH_3 nitrogen per milliliter of normal blood may be incorrect. The difference in results may be accounted for by variations in the technique of handling the blood samples, since Conway's method is sound.

Our measurements establish that (i) ammonia is present in alveolar air and (ii) the amount present is of the order

Table 1. Alveolar P_{NH_3} experiments. Each experiment occurs as a double entry corresponding to the two arterial blood samples. In each experiment there was one gas collection. The two arterial samples were taken during the gas collection period.

Date	Dog No.	Temp. of dog (°C)	Gas			Arterial			Alveolar air NH_3 (10^{-4} meq)*	Arterial plasma NH_3 (meq/lit.)	Alveolar air P_{NH_3} (10^{-4} mm-Hg)	Calcd. arterial P_{NH_3} (10^{-4} mm-Hg)
			Collection time (min)	Vol. (lit. STPD)	P_{CO_2} (mm-Hg)	*p*H	CO_2 (meq/lit.)	P_{CO_2} (mm-Hg)				
2/5	x874	37	202	101	34.8	7.41	26.7	41.3	5.4	0.178	1.0	1.0
2/5	x874	36.9	202	101	34.8	7.43	25.8	38.2	5.4	0.112	1.0	0.7
2/10	B45	37.3	135	72.9	29.4	7.41		35.9	3.9	0.26	1.0	1.5
2/10	B45	37.3	135	72.9	29.4	blood sample lost			3.9		1.0	
2/12	x874	37.0	120	68.4		7.41			4.6	0.212	1.1	1.2
2/12	x874	36.8	120	68.4		7.40			4.6	0.213	1.1	1.2
2/17	x874	37.0	149	65	30.5	7.38	18.0	29.8	3.0	0.131	0.7	0.7
2/17	x874	37.2	149	65	30.5	7.43	21.1	31.3	3.0	0.123	0.7	0.8
2/19	x1100†	39.0	80	50.7	18.5	7.54	16.6	19.3	2.0	0.097	0.6	0.9
2/19	x1100†	38.8	80	50.7	18.5	7.53	15.6	18.6	2.0	0.099	0.6	0.9
2/24	x1100	37.0	110	57.4	42.3	7.38	27.7	45.9	1.5	0.119	0.4	0.6
2/24	x1100	37.3	110	57.4	42.3	7.39	25.8	41.8	1.5	0.132	0.4	0.7
2/26	x916‡	36	110	81		6.99	28.4	107.6	2.4	0.40	0.5	0.8
2/26	x916‡	36.2	110	81		6.99	27.3	103.4	2.4	0.185	0.5	0.4
3/3	x1100‡	37.8	100	65.4		7.00	32.5	123.1	1.6	0.150	0.4	0.4
3/3	x1100‡	37.6	100	65.4		7.02	31.9	118.6	1.6	0.188	0.4	0.5

* The volume of alveolar air collected was calculated as the volume of gas collected multiplied by the ratio of the gas P_{CO_2} to the arterial P_{CO_2}. In the three experiments in which gas P_{CO_2} was not measured, the volume of gas collected was used for the alveolar air collected.
† Hyperventilated.
‡ Ventilated with a mixture of 10 percent CO_2 and 90 percent O_2.

of magnitude expected if the ammonia in the blood were to equilibrate with the alveolar air during its passage through the pulmonary capillaries. Considering the high diffusibility of NH_3, this was to be expected. The measurements of Robin *et al.* (*15*) on dogs infused with ammonium acetate lead to the same conclusion. We have recalculated the arterial P_{NH_3} for dogs 5 and 7 of their Table 2, using our values for the solubility coefficient and K_a' (*13*), and obtain 4.5×10^{-4} and 3.2×10^{-4} mm-Hg for dogs 5 and 7, respectively; this indicates that Robin *et al.* obtained a better check than was suggested by their calculations.

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6. In the course of this work we learned that E. D. Robin, D. M. Travis, T. A. Bromberg, C. E. Forkner, Jr., and J. M. Tyler had independently started similar measurements on dogs infused with ammonium acetate to elevate the blood ammonia. Their work is also reported in this issue. Our studies were supported in part by grants C-2697 and CS-9261 from the U.S. Public Health Service, and by the Andre and Bella Meyer Fund.
7. We wish to thank Dr. Walter Lawrence for allowing us to use his portocaval shunt preparations for these measurements.
8. All-glass tubing was used from the tracheal cannula to the ammonia absorbers. The tubing was warmed to prevent condensation of water before passage of the air through the ammonia absorbers.
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13. The solubility coefficients for human plasma are as follows: 0.91 and 0.89 lit. of NH_3 (STPD) per liter of plasma at a partial pressure of NH_3 of 1 mm-Hg and at temperatures of 37° and 38°C, respectively. The K_a' for ammonia in plasma was taken to be 9.5×10^{-10} at 37°C and 10.1×10^{-10} at 38°C. (J. A. Jacquez, J. W. Poppell, R. Jeltsch, *J. Appl. Physiol.*, in press.)
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Ammonia Excretion by Mammalian Lung

Abstract. The intravenous administration of ammonium acetate to dogs results in measurable levels of free ammonia in expired air. Simultaneous measurement of the physiologic dead space permits the calculation of the partial pressure of ammonia in alveolar air. This finding has implications for ammonium metabolism and transport.

Studies in fish have shown that ammonia excretion can occur by means of diffusion across the gill membranes (*1*). The excretion of ammonia by mammalian lung has not been previously investigated (*2*). The concentration of ammonium ion in normal human and dog blood is small (*3*). According to current theory, blood ammonium should be present in at least two forms: as ammonium ion (NH_4^+) and as the free gas, ammonia (NH_3). The pK of this buffer system in 0.15M saline at 38°C is 9.5; hence, at the usual pH of mammalian blood, the quantity of free ammonia (NH_3) would be small.

If the concentration of total ammonium in the blood is increased, it should be possible to elevate the concentration of free NH_3 in pulmonary capillary blood sufficiently so that it would appear in a measurable quantity in expired air, having traversed the alveolar membrane by simple diffusion. During a steady state, simultaneous measurements of the fractional concentration of NH_3 in expired air and the size of the dead space of the lung should provide a quantitative estimate of the partial pressure of NH_3 in alveolar air. This report describes experiments in which these measurements have been performed (*4*).

Seven mongrel dogs weighing approximately 15 kg each were studied. Following Nembutal anesthesia, 100 milliequivalents (meq) of $NaHCO_3$ was administered to each dog intravenously to elevate blood pH and increase the fraction of total ammonium present as NH_3. The air expired by the dog was bubbled through 10 ml of 0.1N HCl for 20 minutes to serve as a control. After the control period, 0.2M ammonium acetate was infused intravenously at a constant rate for periods of time ranging from 46 to 90 minutes. During the administration of ammonium acetate the air expired by the dog was permitted to bubble through a fresh solution of 0.1N HCl; this converted any free NH_3 in the expired air to NH_4Cl. Midway during the experimental period, measurements of arterial CO_2 tension, expired air CO_2 tension, and the volume of expired air were made by standard methods (*5*).

The concentrations of ammonium present in the control and experimental samples were determined by nesslerization. The volume of the respiratory dead space was calculated by means of the Bohr equation (*6*). On the assumption that NH_3 was distributed in the same dead space as CO_2, it was possible to calculate the partial pressure of ammonia in alveolar air, as follows:

$$\frac{\text{Mg of } NH_3 \text{ excreted}}{\text{min}} = \frac{(\text{mg of } NH_3/\text{ml} \times \text{vol of } 0.1N \text{ HCl})}{\text{time}}$$

$$F_{E(NH_3)} = \frac{\text{mg of } NH_3/\text{min}}{\text{ml of air expired/min}} \times \frac{22.1}{17}$$

$$V_A/V_E = P_{E(CO_2)}/P_{a(CO_2)}$$

$$P_{E(NH_3)} = \text{barometric pressure} \times F_{E(NH_3)}$$

$$P_{A(NH_3)} = P_{E(NH_3)}/(V_A/V_E)$$

where $F_{E(NH_3)}$ is the fractional concentration of NH_3 in expired air; V_A/V_E is the ratio of alveolar ventilation to total ventilation; $P_{E(CO_2)}$ is partial pressure of CO_2 in expired air; $P_{a(CO_2)}$ is partial pressure of CO_2 in arterial blood; $P_{E(NH_3)}$ is partial pressure of NH_3 in expired air; and $P_{A(NH_3)}$ is partial pressure of NH_3 in alveolar air.

In two dogs simultaneous measurements of arterial pH and total blood ammonium concentrations were made. By applying the Henderson-Hasselbalch equation, it was possible to *estimate* the theoretical partial pressure of ammonia in arterial blood. In four dogs the completeness of extraction of ammonia by the 0.1N HCl solution was tested by means of rebubbling expired air through a second aliquot of 0.1N HCl.

Table 1 summarizes the data obtained from the seven dogs that were studied. During control periods no measurable amount of NH_3 was found in expired air. In each dog ammonium acetate administration produced measurable quantities of NH_3 in expired air. The quantity of NH_3 in air was small, averaging 3.8×10^{-7} ml per milliliter of air. However, since the ammonia content of large volumes of air was concentrated by the technique employed, it was possible to measure $F_{E(NH_3)}$ and calculate the partial pressure of NH_3 in alveolar air. The average $P_{A(NH_3)}$ for the seven dogs was 7×10^{-4} mm-Hg. Table 2 shows the arterial levels of total ammonium in the two dogs in which it was measured and the estimated arterial NH_3 tensions [$P_{a(NH_3)}$]. The order of magnitude of the two values [$P_{a(NH_3)}$ and $P_{A(NH_3)}$] is similar.

Complete extraction of ammonia by the first aliquot of 0.1N HCl was found in the four studies in which a rebubbling technique was used.

Although the quantity of free ammonia in alveolar air (and thus, presumably, in pulmonary capillary blood) is small, its physiologic significance may be great. Jacobs (*7*) has pointed out that cells may be impermeable to a given ion but may