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.

JOHN A. JACQUEZ
Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York
J. WILLIAM POPPELL
RUDOLPH JELTSCH
Division of Experimental Surgery and Physiology and *Andre and Bella Meyer Physiology Laboratories, Sloan-Kettering Institute for Cancer Research, New York*

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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.
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19 August 1958

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 0.15*M* saline at 38°C is 9.5; hence, at the usual *p*H 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 *p*H and increase the fraction of total ammonium present as NH_3. The air expired by the dog was bubbled through 10 ml of 0.1*N* HCl for 20 minutes to serve as a control. After the control period, 0.2*M* 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 0.1*N* 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:

$$\frac{\text{Mg of NH}_3\text{ excreted}}{\text{min}} = \frac{(\text{mg of NH}_3/\text{ml} \times \text{vol of } 0.1N\text{ HCl})}{\text{time}}$$

$$F_{E(\text{NH}_3)} = \frac{\text{mg of NH}_3/\text{min}}{\text{ml of air expired/min}} \times \frac{22.1}{17}$$

$$V_A/V_E = P_{E(\text{CO}_2)}/P_{a(\text{CO}_2)}$$

$$P_{E(\text{NH}_3)} = \text{barometric pressure} \times F_{E(\text{NH}_3)}$$

$$P_{A(\text{NH}_3)} = P_{E(\text{NH}_3)}/(V_A/V_E)$$

where $F_{E(\text{NH}_3)}$ is the fractional concentration of NH_3 in expired air; V_A/V_E is the ratio of alveolar ventilation to total ventilation; $P_{E(\text{CO}_2)}$ is partial pressure of CO_2 in expired air; $P_{a(\text{CO}_2)}$ is partial pressure of CO_2 in arterial blood; $P_{E(\text{NH}_3)}$ is partial pressure of NH_3 in expired air; and $P_{A(\text{NH}_3)}$ is partial pressure of NH_3 in alveolar air.

In two dogs simultaneous measurements of arterial *p*H 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 0.1*N* HCl solution was tested by means of rebubbling expired air through a second aliquot of 0.1*N* 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 $F_{E(\text{NH}_3)}$ and calculate the partial pressure of NH_3 in alveolar air. The average $P_{A(\text{NH}_3)}$ 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 [$P_{a(\text{NH}_3)}$]. The order of magnitude of the two values [$P_{a(\text{NH}_3)}$ and $P_{A(\text{NH}_3)}$] is similar.

Complete extraction of ammonia by the first aliquot of 0.1*N* 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

Table 1. Alveolar ammonia tensions and related data in seven dogs following ammonium acetate administration.

Dog No.	Ammonium acetate administered (meq)	Time of administration (min)	Total NH_3 excretion (mg) Control	Experimental	$F_{E(NH_3)}$ (10^{-7} ml/ml)	$P_{E(NH_3)}$ (10^{-4} mm-Hg)	$P_{a(CO_2)}$ (mm-Hg)	$P_{E(CO_2)}$ (mm-Hg)	V_A/V_E	pH	$P_{A(NH_3)}$ (10^{-4} mm-Hg)
1	80	90	0	0.073	1.7	1.3	41	12	0.29	7.39	4
2	125	60	0	0.098	4.4	3.3	56	19	0.33	7.36	10
3	100	46	0	0.093	4.7	3.6	41	17	0.41	7.59	9
4	100	64	0	0.079	6.1	4.7	31	16	0.51	7.50	11
5	100	71	0	0.052	1.9	1.5	54	16	0.30	7.48	5
6	115	60	0	0.063	3.3	2.5	37	16	0.43	7.58	6
7	100	70	0	0.044	3.0	2.3	58	22	0.26	7.40	6

be permeable to the free base of this ion. Robin *et al.* (*8*) have shown that the spinal fluid compartment is permeable to CO_2, a gas, but is relatively impermeable to bicarbonate ion. Free ammonia, a gas, presumably is freely diffusible. This may be the form in which ammonium is transported across cell membranes. The diffusivity of NH_3 may be compared to the diffusivity of O_2 and CO_2 by means of Graham's and Henry's laws. Such calculations show that NH_3 is approximately 30,000 times as diffusible as O_2 and 1500 times as diffusible as CO_2. Under these circumstances it would be expected that NH_3 equilibrium would occur very rapidly indeed across the alveolar membrane. Alveolar and pulmonary capillary NH_3 tensions would thus be essentially equal.

It is important to emphasize that, at present, *accurate* calculations of arterial NH_3 tensions from experimentally determined total arterial ammonium levels and arterial pH are not possible. Such calculations would require an accurate value for the equilibrium constant, K_a, of the reaction

$$NH_4^+ \rightleftarrows NH_3 + H^+$$

in blood or plasma, which is certainly different from its value in aqueous solution. Until an acceptable plasma K_a is available, only order-of-magnitude comparisons between alveolar and arterial NH_3 tensions are warranted.

During the past 5 years major emphasis has been given to the role of ammonium metabolism in man. Significant concentrations of total ammonium have been found in the blood of patients with various types of liver disease. An increase in blood ammonium has been implicated in the pathogenesis of hepatic coma. However, correlations between blood ammonium concentrations and the degree of hepatic coma are imperfect. It is generally accepted that the measurement of blood ammonium presents great technical difficulties. Since there may be spontaneous generation of ammonium from nitrogenous substances in blood, the exact significance of blood ammonium levels, after shedding, is difficult to assess.

Table 2. Comparison of estimated arterial and experimentally determined alveolar ammonia tensions.

Dog No.	Arterial ammonium concentration (μmole/lit)	Arterial pH	Estimated arterial ammonia tension $P_{a(NH_3)}$ (10^{-4} mm-Hg)	Alveolar ammonia tension $P_{A(NH_3)}$ (10^{-4} mm-Hg)
5	610	7.48	1.7	5
7	520	7.40	1.2	6

The demonstration of free NH_3 in alveolar air may be taken to indicate that under special circumstances there are significant levels of circulating ammonium. Jacquez, Poppell, and Jeltsch have demonstrated free NH_3 in the alveolar air of dogs with Eck fistulas (*2*). Preliminary studies in some patients with hepatic coma have shown measurable levels of ammonia in expired air (*9*). By utilizing the lung of such a patient as a tonometer, it may be possible to reassess the quantitative aspects of ammonium metabolism and its relation to hepatic coma.

It is interesting to note the similarities between NH_3 and CO_2. Both are gases which are involved in metabolic processes. Under physiologic circumstances neither is present in significant quantity in inspired air. Both gases are highly diffusible. Each gas exists in equilibrium, with an ion constituting a buffer pair. As with CO_2, the study of the excretion of NH_3 by mammalian lung may prove to be a useful approach for further investigation of gas exchange.

EUGENE D. ROBIN, DAVID M. TRAVIS,
PHILIP A. BROMBERG,
CLAUDE E. FORKNER, JR.,
JOHN M. TYLER

Department of Medicine, Harvard Medical School, and Medical Clinics, Peter Bent Brigham Hospital, Boston, Massachusetts

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14 August 1958

A Case of Ovotestes in the Sea Urchin Strongylocentrotus Purpuratus

Abstract. A hermaphroditic sea urchin, *Strongylocentrotus purpuratus*, with three ovotestes and two testes is described. Neither cleaving eggs nor embryos were found *in corpore*. Fertilization *inter se* gave normal larvae. The specimen was collected from Palos Verdes, Calif., a region which has yielded an unusually large number of hermaphroditic *S. purpuratus*.

In a former report we listed known cases of hermaphroditism in echinoids, discussed the exceptional occurrence of ovotestes, and mentioned other publications in which such cases are recorded (*1*). On 19 Jan. 1958 one of us (R.A.B.) collected 30 specimens of *Strongylocentrotus purpuratus* at Palos Verdes, Los Angeles County, Calif. This is in the region which has yielded an unusually large number of hermaphroditic *S. purpuratus* (1 to 500), according to the report of Albert Tyler (*2*). One of the specimens collected at Palos Verdes, when opened, was found to contain both ovaries and testes. This specimen weighed 24.4 g and had a test diameter of 38.4 mm. The five gonads comprised two testes and three ovotestes. In the latter, the ovary occupied the dorsal half and the testes the ventral half, hence only the eggs could escape. The gonads were ripe and easily broken. No