

THE ROLE OF pH GRADIENT IN THE DISTRIBUTION OF AMMONIA BETWEEN BLOOD AND CEREBRO-SPINAL FLUID, BRAIN AND MUSCLE *

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The role of ammonia toxicity in the production of the hepatic coma syndrome has not been clearly defined. No direct relationship has been demonstrated consistently between arterial or venous blood ammonia-nitrogen concentration or the arteriovenous ammonia-nitrogen difference and the state of cerebral dysfunction in hepatic disease. Recently a direct relationship between an increase in blood pH and the diffusion of ammonia from blood into tissues has been suggested (2). Other investigators have demonstrated greater toxicity of the more alkaline ammonium salts (3) and that an increase in blood pH enhanced passage of ammonia into the brain of mice (4).

The diffusion of a weak electrolyte into a cell is determined by the pKa of the weak electrolyte, the pH within and without the cell, and the relative permeability of the cell membrane to the unionized species of the electrolyte (5, 6). It is proposed that ammonia, a weak base with pKa 9.3, should be distributed between blood and cerebrospinal fluid (CSF), brain and muscle according to existing pH gradients, in a manner analogous to that demonstrated in the studies of renal excretion (7, 8), gastrointestinal secretion (9) and absorption (10) of weak acids and bases, and in the studies of distribution of weak organic acids and bases from blood to CSF (11). This study was designed to evaluate in the dog the distribution of ammonia from blood to CSF, brain and muscle under conditions of experimentally induced pH gradients between blood and CSF, brain and muscle.

* A preliminary report of these findings has been published (1).

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METHODS

The principles and methods of a study of the distribution of a drug between blood and cerebrospinal fluid and tissue have been described in detail (12). Fifty-one mongrel dogs lightly anesthetized with pentobarbital were nephrectomized bilaterally. Ammonium bicarbonate solution (0.16 N) was infused intravenously in 48 dogs; ammonium chloride (0.16 N) was infused in 3 dogs. A constant infusion of the ammonium salts was maintained and simultaneous samples of CSF, muscle and blood were taken serially in each animal experiment. Arterial blood samples were obtained through a polyethylene catheter placed in the femoral artery, while venous blood was obtained from jugular bulb and femoral vein catheters. Cerebrospinal fluid (CSF) samples were withdrawn from the cisterna magna through an indwelling percutaneously inserted polyethylene catheter according to the method of Rall, Stabenau and Zubrod (12). The ammonia content of arterial and venous blood and CSF was determined according to the method of Nathan and Rodkey (13) and is expressed as $\mu\text{g. NH}_3\text{-N}$ per ml. Muscle biopsy specimens were obtained from the quadriceps and deltoid areas. Brain biopsy samples were aspirated with a 5 mm. bore needle through a parietal craniotomy site before termination of the experiment. Both muscle and brain biopsy samples were immediately frozen in liquid nitrogen and analyzed for their ammonia content according to the method of Nathan and Warren (14). Tissue ammonia content is expressed as $\mu\text{g. NH}_3\text{-N}$ per Gm. wet tissue weight.

Glutamine concentrations of whole blood and tissue were determined according to the method of Krebs (15). Blood urea nitrogen concentrations in serum were determined according to the method of Caraway and Fanger (16).

Arterial blood and CSF pH were determined anaerobically on a Beckman model GS pH meter with a Beckman 39022 glass electrode in a 37° C. constant temperature room less than 10 minutes after withdrawal in heparinized syringes. Duplicate determinations bracketed by standards agreed within ± 0.02 pH unit.

Changes in the pH gradients between blood and CSF, during constant ammonia infusion, were induced and maintained by: intravenous infusion of 0.2 N HCl; intravenous infusion of 0.2 N NaOH; pCO₂ elevation and depression by means of controlled inhalation either of a

TABLE I
The distribution of ammonia from blood to CSF during metabolic and respiratory acidosis and alkalosis *

| Condition | NH ₄ HCO ₃ infusion† | | | | |
|-----------------------------------|--------------------------------------------|--------------|--------------|----------------------|-----------------------|
| | Control | HCl | NaOH | Respiratory acidosis | Respiratory alkalosis |
| pH blood‡ | 7.44 ± 0.008 | 7.25 ± 0.020 | 7.60 ± 0.021 | 7.10 ± 0.022 | 7.66 ± 0.028 |
| pH CSF‡ | 7.39 ± 0.012 | 7.40 ± 0.016 | 7.41 ± 0.022 | 7.00 ± 0.022 | 7.58 ± 0.048 |
| pH gradient | +0.05 | -0.15 | +0.19 | +0.10 | +0.08 |
| C _{CSF} /C _{BL} | | | | | |
| obs.§ | 0.49 | 0.38 p<0.01 | 0.83 p<0.01 | 0.45 p=0.5 | 0.52 p=0.6 |
| calc. | 1.1 | 0.7 | 1.5 | 1.2 | 1.2 |

* C_{CSF}/C_{BL} refers to steady state distribution ratio of ammonia, CSF to blood. The C_{CSF}/C_{BL} observed (obs.) is given as well as the ratio C_{CSF}/C_{BL} calculated (calc.) from observed pH of blood and CSF.

† A constant infusion of 0.16N NH₄HCO₃ was maintained during: control period; infusion of 0.2N HCl (HCl); infusion of 0.2N NaOH (NaOH); inhalation of 20 per cent CO₂ and 80 per cent O₂ mixture (respiratory acidosis); and inhalation of 100 per cent O₂ and CO₂ absorption (respiratory alkalosis). This footnote applies to Tables II, III, IV and VI.

‡ Mean value plus or minus standard error of the mean.

§ The significance of the difference between mean values when compared with the control is given as p.

|| See Discussion for formula and method of calculation.

mixture of 20 per cent CO₂ and 80 per cent O₂ or of 100 per cent O₂ with CO₂ reabsorption. A closed circuit Rand-Wolfe respirator was utilized.

In addition to separate control animals each dog served as his own control. During the first 90 minutes of infusion a steady state was readily attained in which blood, CSF and muscle ammonia concentration increased, reached a plateau, and then remained essentially the same with the passage of time and constant ammonia infusion. After this period the experimental induction of pH

change was begun and maintained for an additional 120 minutes or longer.

Ammonium ion refers to the ionized form of ammonia and unionized ammonia is designated as such, while the term ammonia refers to the total of both ammonium ion, and unionized ammonia. The term C_{CSF} refers to concentration of ammonia in CSF; C_M refers to concentration of ammonia in muscle; C_{BR} refers to concentration of ammonia in brain; and C_{BL} refers to concentration of ammonia in whole arterial blood. The ratio of C_{CSF}/C_{BL},

TABLE II
Distribution of ammonia from blood to muscle and brain during metabolic and respiratory acidosis and alkalosis *

| Condition | NH ₄ HCO ₃ infusion | | | | |
|----------------------------------|-------------------------------------------|--------------|--------------|----------------------|-----------------------|
| | Control | HCl | NaOH | Respiratory acidosis | Respiratory alkalosis |
| Muscle | | | | | |
| pH blood‡ | 7.44 ± 0.008 | 7.25 ± 0.020 | 7.60 ± 0.021 | 7.10 ± 0.022 | 7.66 ± 0.028 |
| $\frac{A-PV}{A} \times 100$ | 27.0 | 7.7 p<0.01 | 41.3 p=0.05 | 25.1 p=0.8 | 34.0 p=0.5 |
| C _M /C _{BL} | | | | | |
| obs.‡ | 0.90 | 0.73 p=0.4 | 1.8 p<0.01 | 0.73 p=0.5 | 1.5 p=0.02 |
| calc.§ | 2.7 | 1.8 | 3.9 | 1.2 | 4.4 |
| Brain | | | | | |
| $\frac{A-JV}{A} \times 100$ ‡ | 27.1 | 11.7 p=0.05 | 25.4 p=0.8 | 6.1 p<0.01 | 23.8 p=0.8 |
| C _{BR} /C _{BL} | | | | | |
| obs.‡ | 0.83 | 0.67 p=0.2 | 2.2 p<0.01 | 1.0 p=0.2 | 2.2 p<0.01 |
| calc.§ | 2.7 | 1.8 | 3.9 | 1.2 | 4.4 |

* C_M/C_{BL}, C_{BR}/C_{BL} refer to steady-state distribution ratios of ammonia between muscle and brain and blood. The observed ratios (obs.) are given as well as the ratios C_M/C_{BL} and C_{BR}/C_{BL} calculated from the observed pH of blood and the pH of muscle and brain as 7.0. $\frac{A-PV}{A} \times 100$ and $\frac{A-JV}{A} \times 100$ represent A-V ammonia concentration differences between arterial (A) and peripheral (PV) and jugular venous (JV) blood, respectively.

‡ Mean value plus or minus standard error.

§ The significance of the difference between mean values when compared with the control is given as p.

|| See Discussion for formula and method of calculation.

C_M/C_{BL} , or C_{BR}/C_{BL} refers to the distribution ratio of ammonia at steady state or near steady state.

The arterial (A) whole blood ammonia concentration minus venous (V) whole blood ammonia concentration (A-V difference) was expressed as the ratio of the difference A-V to A, times 100

$$\left(\frac{A-V}{A} \times 100\right).$$

Statistical evaluation of the data is expressed as the standard error of the mean (S.E.). The symbol \pm refers to the S.E. The "t" ratio was used to obtain the p values (17).

RESULTS

1. Ammonia distribution during acidosis and alkalosis

Steady state ratios relating concentration of ammonia in CSF (C_{CSF}/C_{BL}), muscle (C_M/C_{BL}) and brain (C_{BR}/C_{BL}) to concentration of am-

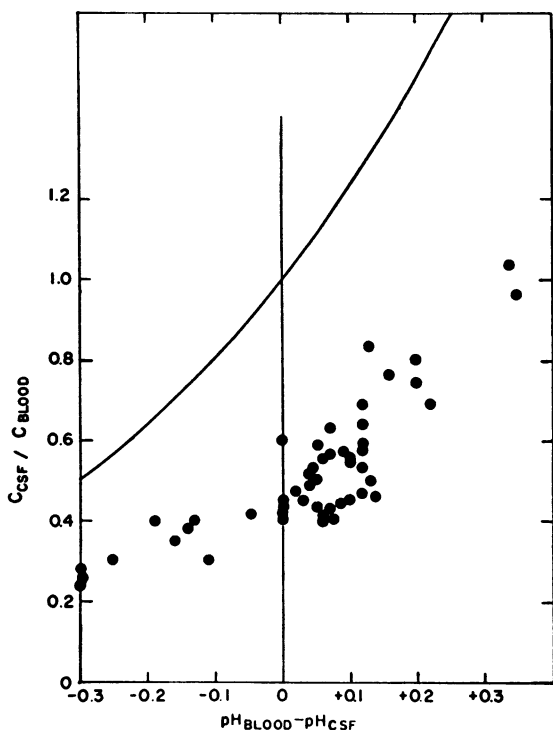


FIG. 1. THE RELATION BETWEEN pH GRADIENT AND THE OBSERVED AND CALCULATED DISTRIBUTION RATIO OF AMMONIA, CSF TO BLOOD (C_{CSF}/C_{BL})

Observed C_{CSF}/C_{BL} ratios at steady state are plotted as solid dots against the pH gradient derived from the blood and CSF pH observed during the steady state. The solid line curve represents the locus of plotted points for the predicted C_{CSF}/C_{BL} as calculated from the observed blood and CSF pH.

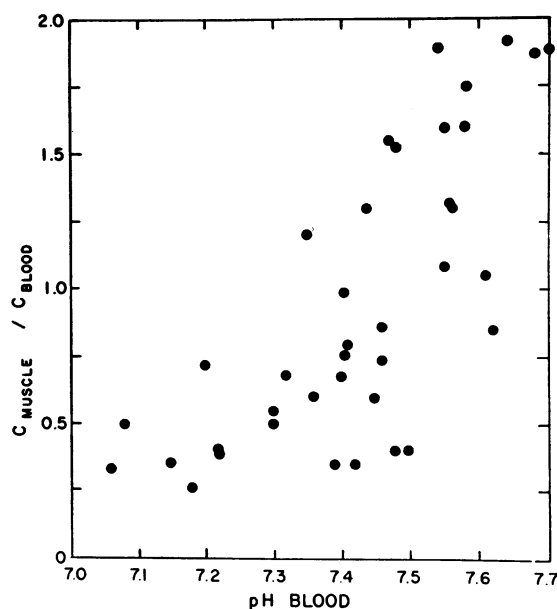


FIG. 2. THE RELATION BETWEEN BLOOD pH AND THE OBSERVED DISTRIBUTION RATIO OF AMMONIA, MUSCLE TO BLOOD (C_M/C_{BL})

Observed C_M/C_{BL} ratios at steady state are plotted as solid dots against the pH of blood observed during the steady state.

monia in whole arterial blood were determined during various phases of the study and were compared to those ratios achieved during a control infusion state (Tables I and II).

A. Cerebrospinal fluid. C_{CSF}/C_{BL} decreased significantly from the control infusion value of 0.49 to 0.38 during metabolic acidosis while during metabolic alkalosis there was a significant increase to 0.83. Respiratory acidosis and alkalosis caused no significant variation from control infusion ratio.

The mean values for blood and CSF pH are shown in Table I. Change in magnitude and or direction of pH gradient occurred only during the infusion of NaOH or HCl due to changes in blood pH without significant alteration of CSF pH. During respiratory pCO_2 changes, a pH gradient similar to that in the control state was maintained due to simultaneous changes of the blood and CSF pH. There was a direct relation between the magnitude and the direction of the pH gradient produced and the distribution of ammonia to the CSF. This is shown in Figure 1. When a large positive pH gradient (pH blood

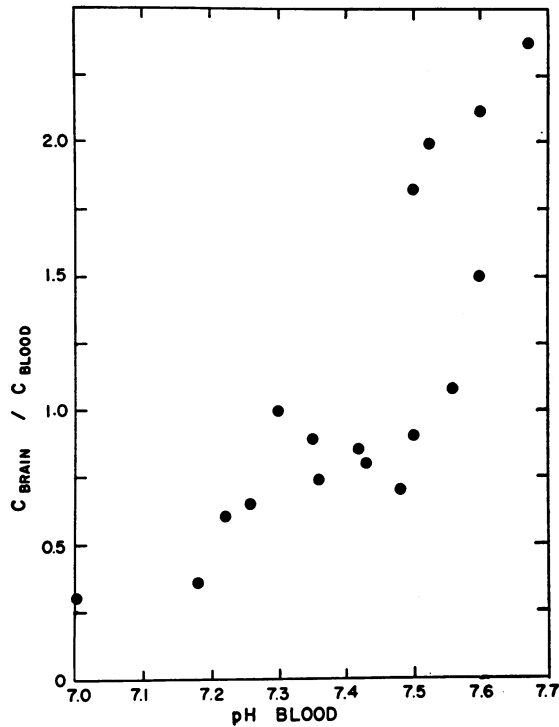


FIG. 3. THE RELATION BETWEEN BLOOD pH AND THE OBSERVED DISTRIBUTION RATIO OF AMMONIA, BRAIN TO BLOOD (C_{BR}/C_{BL})

Observed C_{BR}/C_{BL} ratios at steady state are plotted as solid dots against the pH of blood observed during the steady state.

minus pH CSF) existed an increase in C_{CSF}/C_{BL} occurred, and when a large negative gradient existed a decrease in the C_{CSF}/C_{BL} occurred.

During metabolic acidosis and alkalosis samples of aqueous humour and CSF were withdrawn simultaneously. The ratio of the concentration of ammonia in aqueous humour to the concentration of ammonia in blood in six dogs so examined was quantitatively similar to the ratio C_{CSF}/C_{BL} .

B. Tissues. The decrease of C_M/C_{BL} from control value of 0.90 during metabolic and respiratory acidosis was not significant in a comparison of group means (Table II), but was significant in a paired comparison within each experiment ($p < 0.05$). A significant increase to 1.8 during metabolic alkalosis and to 1.5 during respiratory alkalosis occurred. The direct relationship between blood pH and C_M/C_{BL} is demonstrated in Figure 2.

The data in Table II demonstrate the relation between the observed A-PV difference (arterial whole blood ammonia concentration minus peripheral venous whole blood ammonia concentration) and A-JV difference (arterial whole blood ammonia concentration minus jugular venous whole blood ammonia concentration) to the relative distribution of ammonia between blood and muscle and brain. During NaOH infusion muscle ammonia concentration increased and A-PV difference was significantly greater than during the control period. With HCl infusion the muscle ammonia concentration decreased and A-PV difference significantly decreased. Respiratory acidosis and alkalosis produced no significant variation in A-PV difference.

C_{BR}/C_{BL} increased significantly during metabolic alkalosis (2.2) and respiratory alkalosis (2.2) from the control infusion ratio of 0.83. There was an insignificant decrease in C_{BR}/C_{BL} to 0.67 during metabolic acidosis and increase to 1.0 during respiratory acidosis. Animals exposed to respiratory acidosis after periods of respiratory alkalosis had a change of C_{BR}/C_{BL} from 2.0 in alkalosis to 1.3 ($p = 0.02$) in acidosis. The direct relationship between blood pH and C_{BR}/C_{BL} is demonstrated in Figure 3.

Significant narrowing of the A-JV difference

TABLE III
Brain and muscle ammonia concentration as related to conditions of pH change as well as time and amount of ammonia infused

| Condition | NH_4HCO_3 infusion | | | | |
|---------------------------------------|----------------------|------------------|------------------|----------------------|-----------------------|
| | Control | HCl | NaOH | Respiratory acidosis | Respiratory alkalosis |
| Brain NH_3-N , $\mu g./Gm.$ * | 11.3 \pm 2.5 | 8.1 \pm 1.1 | 49.4 \pm 7.2 | 31.3 \pm 2.2 | 60.0 \pm 2.0 |
| Muscle, $\mu g. NH_3-N/Gm.$ * | 12.3 \pm 3.5 | 8.5 \pm 1.8 | 28.9 \pm 1.2 | 28.3 \pm 9.6 | 47.0 \pm 8.0 |
| NH_3-N infused, $\mu g./Kg./min.$ * | 609.8 \pm 23.4 | 570.2 \pm 68.3 | 541.4 \pm 57.1 | 585.3 \pm 66.5 | 567.5 \pm 47.4 |
| Duration NH_4HCO_3 infusion* (min.) | 313.8 \pm 20.4 | 263.2 \pm 39.9 | 262 \pm 31.6 | 365 \pm 31.8 | 300 \pm 30.0 |

* Mean value plus or minus standard error of the mean.

TABLE IV
Representative typical experiments during steady state ammonia infusion with variation of blood pH by metabolic and respiratory means

| Condition* | Time | Ammonia concentration | | | | | | pH | | Distribution ratios | | |
|----------------------------------|------|-----------------------|-------|-------|-------------|-------|-------|-------|------|------------------------------------|----------------------------------|----------------------------------|
| | | Art.† | J.V.‡ | P.V.§ | CSF | Mus. | Br.¶ | Blood | CSF | C _{CSF} /C _B L | C _M /C _B L | C _B /C _B L |
| | min. | µg./100 ml. | | | µg./100 Gm. | | | | | | | |
| Expt. 1 (10.1 Kg.)** | | | | | | | | | | | | |
| constant infusion | | | | | | | | | | | | |
| 6.5 mg. NH ₃ -N/min. | | | | | | | | | | | | |
| Control | 60 | 700 | | | 315 | 250 | | 7.50 | 7.44 | 0.45 | 0.36 | |
| | 90 | 730 | | 610 | 321 | 490 | | 7.50 | 7.45 | 0.44 | 0.67 | |
| HCl, 0.48 mEq./min. | 60 | 1,260 | 980 | 1,130 | 406 | 290 | | 7.28 | 7.48 | 0.32 | 0.23 | |
| | 120 | 1,420 | 1,070 | 880 | 436 | 150 | | 7.12 | 7.49 | 0.31 | 0.11 | |
| | 180 | 1,770 | 1,290 | 1,220 | | 600 | 820 | 7.11 | 7.46 | | 0.34 | 0.46 |
| Expt. 2 (11.4 Kg.) | | | | | | | | | | | | |
| constant infusion | | | | | | | | | | | | |
| 7.4 mg. NH ₃ -N/min. | | | | | | | | | | | | |
| Control | 60 | | | | 284 | 290 | | | | | | |
| | 120 | 753 | 428 | 452 | 297 | 730 | | 7.49 | 7.43 | 0.40 | 0.97 | |
| NaOH, 0.51 mEq./min. | 60 | 940 | | | 490 | 2,100 | | 7.75 | 7.52 | 0.52 | 2.2 | |
| | 180 | 1,740 | 1,480 | 1,370 | 1,190 | 3,260 | 3,600 | 7.70 | 7.48 | 0.69 | 1.9 | 2.1 |
| Expt. 3 (10.2 Kg.) | | | | | | | | | | | | |
| constant infusion | | | | | | | | | | | | |
| 7.6 mg. NH ₃ -N/min. | | | | | | | | | | | | |
| Control | 90 | 855 | 578 | 780 | 444 | 556 | | 7.49 | 7.42 | 0.52 | 0.65 | |
| | 120 | 1,015 | 650 | 630 | 436 | 757 | | 7.47 | 7.42 | 0.43 | 0.75 | |
| NaOH, 0.35 mEq./min. | 45 | 1,895 | 1,200 | 885 | 963 | 1,290 | | 7.58 | 7.50 | 0.51 | 0.68 | |
| | 105 | 1,510 | 1,150 | 1,180 | 1,060 | 2,710 | | 7.60 | 7.50 | 0.70 | 1.80 | |
| HCl, 0.70 mEq./min. | 45 | 2,590 | | | 919 | 2,310 | 4,000 | 7.30 | 7.29 | 0.36 | 0.93 | 1.5 |
| Expt. 4 (11.5 Kg.) | | | | | | | | | | | | |
| constant infusion | | | | | | | | | | | | |
| 3.8 mg. NH ₃ -N/min. | | | | | | | | | | | | |
| Control | 60 | 416 | 248 | 228 | 133 | 410 | | 7.43 | 7.35 | 0.32 | 0.99 | |
| | 90 | 420 | | 213 | 167 | 394 | | 7.43 | 7.36 | 0.40 | 0.94 | |
| Inhalation 100% O ₂ ; | 60 | 326 | 246 | 162 | 198 | 204 | | 7.63 | 7.49 | 0.61 | 0.63 | |
| CO ₂ absorption | 90 | 313 | 249 | 224 | 231 | 334 | | 7.63 | 7.43 | 0.74 | 1.06 | |
| Inhalation 20% CO ₂ | 60 | 485 | 460 | 368 | 234 | 207 | | 7.23 | 7.07 | 0.48 | 0.43 | |
| + 80% O ₂ | 120 | 403 | 430 | 402 | 255 | 328 | 567 | 7.09 | 6.99 | 0.63 | 0.81 | 1.4 |

* Continuous infusion of 0.16N NH₄HCO₃ was maintained during all phases of each experiment.

† Arterial blood.

‡ Jugular vein blood.

§ Peripheral vein blood.

|| Muscle.

¶ Brain.

** The weight of each dog is given after the experiment number.

during both metabolic and respiratory acidosis occurred. Metabolic or respiratory alkalosis, however, produced no significant variation from control A-JV differences though relative ammonia concentrations in brain were increased. On the whole, A-PV differences were greater than A-JV differences during the experimental phases. Three dogs infused with NaOH had negative A-JV differences during a convulsive state. The only other occurrences of negative A-V differences were during HCl infusion.

The magnitude of tissue ammonia concentration was not related to the amount and duration of ammonia infusion (Table III). A comparison

was made of mean brain and muscle concentration in several animals selected from the various categories because of similarity of weight and duration of experiment. The amounts of ammonia infused as ammonium bicarbonate in each state was similar. The average amount of NH₃-N infused was 575 ± 52.0 µg. per Kg. per minute for periods of about five hours. Though the amount of ammonia infused in each state was quite similar, brain and muscle concentrations varied markedly in the various experimental states. A high incidence of mortality occurred in dogs with a mean brain level above 40 µg. NH₃-N per Gm.

Details of the effects of pH gradient changes on

ammonia distribution in individual experiments can be obtained from Table IV. Each animal experiment is recorded in its entirety. Each dog acted as his own control. Mean ammonia-nitrogen concentrations obtained from other animals before NH_4HCO_3 infusion were: 91.1 ± 13.2 μg . per 100 ml. for blood; 35.5 ± 3.7 μg . per 100 ml. for CSF; 293.0 ± 31.2 μg . per 100 Gm. for muscle; and 353.0 ± 52.2 μg . per 100 Gm. for brain ($N = 8$). Marked increase in CSF, muscle and brain ammonia concentration occurred during NaOH infusion (Experiment 2) where large positive pH gradients were produced. In contrast there was a decrease in tissue and CSF ammonia concentration during HCl infusion (Experiment 1) where large negative pH gradients were produced. Similar but less marked changes are noted in a dog which received acid infusion after NaOH infusion (Experiment 3). Experiment 4 demonstrated changes in ammonia distribution which occurred during respiratory alkalosis followed by respiratory acidosis.

II. The pH gradient variation produced by acidosis and alkalosis

During the preinfusion state blood pH was 7.43 ± 0.011 and the CSF pH was 7.35 ± 0.012 . During the control infusion the pH of blood was 7.44 ± 0.008 , and CSF pH was 7.39 ± 0.012 . The mean pH values of blood and CSF during

the various states of pH change (Table I) reveal that during acid and alkali infusion CSF pH varied only slightly while blood pH decreased and increased. Blood pH and CSF pH decreased and increased simultaneously when acidosis and alkalosis were produced by varying pCO_2 . When the respiratory pH changes were induced during NH_4HCO_3 infusion blood pH remained alkaline to CSF pH.

Table V presents experiments in four nephrectomized dogs and one dog with intact kidneys. Two or more similar experiments were performed in each case. These experiments demonstrate the changes in CSF and blood pH during respiratory alkalosis and acidosis in an animal receiving no infusion, animals receiving a solution such as anti-pyrine which does not apparently affect pH, and in animals receiving NH_4HCO_3 infusion. It appears that pH differences between blood and CSF may vary markedly in magnitude and direction depending upon the nature of a combined metabolic and respiratory acidosis or alkalosis.

Steady states in which arterial blood concentrations remain at a constant level during acidosis or alkalosis were difficult to maintain. We have observed, during constant NH_4HCO_3 infusion in the nephrectomized dogs as well as in dogs with intact kidneys, there was an immediate large and sustained rise in arterial ammonia concentration from control levels upon administration of intravenous

TABLE V
The effect of pCO_2 changes on the pH of blood and CSF during states of mild metabolic acidosis and alkalosis

| Experiment no. | I.V. solution | pH..... | No infusion | Infusion | | | | | | | |
|----------------|---------------------------|---------|-------------|----------|------|------------------------|------|------|-----------------------|------|------|
| | | | | Control* | | Respiratory alkalosis* | | | Respiratory acidosis* | | |
| | | | | 60 | 120 | 60 | 90 | 120 | 60 | 90 | 120 |
| 5 | None† | Blood | 7.43 | 7.45 | 7.49 | 7.51 | 6.93 | 6.94 | | | |
| | | CSF | 7.35 | 7.36 | 7.49 | 7.51 | 7.03 | 7.00 | | | |
| 6 | Anti-pyrine | Blood | 7.35 | | 7.38 | | 7.02 | | 6.93 | | |
| | | CSF | 7.23 | | 7.30 | | 7.01 | | 7.04 | | |
| 7 | Anti-pyrine | Blood | 7.39 | 7.40 | 7.61 | 7.73 | 7.66 | | | | |
| | | CSF | 7.29 | 7.32 | 7.61 | 7.66 | 7.63 | | | | |
| 8 | NH_4HCO_3 | Blood | 7.34 | 7.43 | 7.44 | 7.63 | 7.63 | 7.59 | 7.23 | 7.21 | 7.09 |
| | | CSF | 7.25 | 7.25 | 7.26 | 7.49 | 7.43 | 7.46 | 7.07 | 7.06 | 6.99 |
| 9 | NH_4Cl | Blood | 7.42 | 7.30 | 7.29 | 7.60 | | 7.58 | 6.96 | | 6.93 |
| | | CSF | 7.33 | 7.33 | 7.35 | 7.70 | | 7.72 | 7.08 | | 7.09 |

* Duration of experimental period is given in minutes.

† All dogs were nephrectomized with the exception of Dog 5.

TABLE VI
Glutamine concentration in blood, muscle and brain

| Condition | No infusion* | NH ₄ HCO ₃ infusion | | | | |
|-------------------------------------|----------------|-------------------------------------------|------|-------|-----------------------|------------------------|
| | | Control† | HCl† | NaOH† | Respiratory acidosis† | Respiratory alkalosis† |
| <i>Arterial blood concentration</i> | | | | | | |
| NH ₃ -N, µg./100 ml. | 91.0 ±3.2 | 87.0 | 675 | 930 | 3,720 | 1,550 |
| Glutamine, mg./100 ml. | 11.6 ±0.5 | 7.0 | 8.7 | 6.1 | 5.4 | 1.8 |
| <i>Muscle concentration</i> | | | | | | |
| NH ₃ -N, µg./100 Gm. | 293.0 ±31.2 | 950 | 760 | 1,050 | 3,540 | 2,870 |
| Glutamine, mg./100 Gm. | 57.8 ±8.7 | 89.5 | 130 | 95.8 | 86.0 | 120.0 |
| <i>Brain concentration</i> | | | | | | |
| NH ₃ -N, µg./100 Gm. | 353 ±52.3 | 900 | 420 | 820 | 3,850 | 1,020 |
| Glutamine, mg./100 Gm. | 52 ±4.6 | 141 | 83.2 | 85.8 | 74 | 114 |

* Mean values plus or minus standard error of the mean.

† Values from each experiment.

HCl and NaOH and during inhalation of 20 per cent CO₂ and 80 per cent O₂ mixture, but not during inhalation of 100 per cent O₂ with CO₂ absorption. The rise of blood ammonia during acidosis appeared to be secondary to accumulation of ammonia due to the reduced passage into tissues. The rise following the infusion of NaOH suggested the possibility that pH change decreased enzymatic removal of ammonia either by decreasing formation of urea in the liver or of glutamine in muscle and brain. Preliminary study of blood urea nitrogen (BUN) elevation in seven nephrectomized dogs demonstrated no striking difference in the rate of BUN elevation between control infusion state and states of experimental pH change, nor was there any relation between the BUN and the blood ammonia-nitrogen concentration. A preliminary study of blood and tissue glutamine concentration involving six nephrectomized dogs has revealed blood glutamine concentrations which were all consistently lower during ammonia infusion than before infusion was begun (Table VI). While a fairly constant rise in muscle glutamine (11.3 ± 1.3 mg. per 100 Gm. per hour) occurred during control ammonia infusion, an irregular change in muscle glutamine content occurred during induced pH changes.

Although there appears to be marked glutamine production during ammonia infusion there is no direct relation between the glutamine concentration and the ammonia concentration in blood, muscle or brain.

DISCUSSION

The relation between ammonia toxicity, hepatic disease, and cerebral dysfunction has been recently reviewed by Warren (3). The index of ammonia toxicity in hepatic disease has always been in terms of blood concentration with the assumption that tissue concentration was a direct function of blood ammonia concentration (18-24).

Gyorgyi and Kleinschmidt (25) in 1926 noted that Eck fistula dogs in meat intoxication were usually alkalotic and that they improved when given hydrochloric acid *per os*. Vanamee and co-workers (26) and Robin, Whaley, Crump and Travis (27) have recently shown that the majority of patients in hepatic coma are in a respiratory alkalosis and have suggested that alkalosis might enhance ammonia toxicity. Lawrence and co-workers (2) have hypothesized an increased passage of ammonia into the tissue with an increase in blood pH, and have attempted to prove this by studying A-V differences. War-

ren and Nathan (4) demonstrated an increased rate of passage of ammonia into the brain of the mouse with a rise in blood pH.

Some clinical confirmation of the experimental findings have been published. Recent studies on blood A-V ammonia differences in patients with elevated blood ammonia concentrations appear to contradict the assumption that tissue uptake is always proportional to blood ammonia concentrations (28, 29). Occasional case reports have appeared in the literature of patients given intravenous NH_4Cl for severe alkalosis with the consequent development of symptoms of ammonia toxicity (30, 31).

The purpose of this study was to investigate the role of pH gradients between blood and CSF, brain and muscle in the distribution of ammonia from blood to the cerebrospinal fluid and those tissues. Milne, Scribner and Crawford (32) have reviewed the use of a pH gradient drug distribution hypothesis in renal tubular excretion and absorption, gastrointestinal secretion and absorption and pancreatic secretion of weak organic acids and bases. Waddell and Butler (8) demonstrated the role of pH in controlling the passage of an organic acid, phenobarbital, into the brain. Stabenau, Rall and Zubrod (11) demonstrated that certain acidic and basic compounds with pKa near the physiologic pH range had distribution ratios between plasma and CSF which are related to a normal pH gradient between blood and CSF, and that experimental changes in that gradient caused predictable changes in such distribution. In addition these authors discussed in detail the application of the pH gradient hypothesis to the transfer of drugs and metabolites into the cerebrospinal fluid.

The pH gradient drug distribution hypothesis explaining the transfer of weak electrolytes from blood to CSF, brain and other tissues is essentially that amounts of ionizable compounds existing in two fluid compartments separated by a membrane completely permeable to the unionized species of the compound and relatively impermeable to the ionized species will be distributed unequally when a pH gradient exists between the compartments. In the case of ammonia, at steady state, the greater concentration of total compound should be on the side of the membrane where the

pH is lower. The ratio of total ammonia between CSF and blood should be the inverse ratio of the per cent unionized in each phase (11, 32).

The data in Table I have demonstrated that the pH gradient between blood and CSF varies from that of the control infusion state during alkalosis and acidosis. The direct effect of the direction as well as magnitude of such gradients on the steady state distribution ratios of ammonia between blood and CSF, brain and muscle can be calculated by using an equation first utilized by Jacobs (6) and later by others (32) to equate distribution ratios of ionizable compound to the unionized state:

$$C_{\text{CSF}}/C_{\text{BL}} = \frac{1 + 10^{\text{pKa} - \text{pH}_{\text{CSF}}}}{1 + 10^{\text{pKa} - \text{pH}_{\text{BL}}}}$$

By comparing the ratio of unionized ammonia in blood to that in CSF, as calculated from their respective pH, to the observed ratio $C_{\text{CSF}}/C_{\text{BL}}$, the role of pH gradient in ammonia distribution becomes apparent. In Figure 1 it is demonstrated that the calculated $C_{\text{CSF}}/C_{\text{BL}}$ for the pH values in the experiments is about twice that of the observed ratios through the range of pH gradient -0.3 to $+0.3$ pH unit. Milne and associates (32) have considered in detail several of the reasons why observed ratios in such a study as this would necessarily be lower than predicted ratios. Some unknown degree of permeability to the ionized molecular species may exist, leading to a falsely high calculated ratio; a rate limitation of the diffusion process itself may occur; and apparent reduction of diffusion concentration ratios may occur due to reference to arterial instead of capillary plasma concentration.¹

Simultaneous blood and CSF pH determinations as presented in this communication and in other studies (11) confirm observations of Cestan, Sendrail and Lasalle (33) and Leusen (34) that during metabolic acidosis and alkalosis the blood pH decreased and increased, respectively, while CSF pH remained unchanged. During respiratory acidosis and alkalosis blood and CSF pH decreased and increased simultaneously. A demonstration of combined metabolic and respiratory acidosis and alkalosis with resultant CSF and

¹ It should be noted that due to technical difficulties arterial whole blood ammonia-nitrogen concentration was used in this study.

blood pH changes is given in Table V. The presence of the excess of bicarbonate ion in the ammonium bicarbonate infusion lead to a blood pH more alkaline than CSF pH during both respiratory acidosis and alkalosis. When NH_4HCO_3 was infused under similar conditions the blood pH became less alkaline than CSF pH during both respiratory acidosis and alkalosis. From these data one may expect to have a small but significant pH gradient between blood and CSF during induced respiratory alkalosis or acidosis only when a previous metabolic acidosis or alkalosis is present. These findings suggest that there is delayed diffusion of infused chloride or bicarbonate ion between blood and CSF but a rapid equilibration of CO_2 . This presents a situation quite similar to that described by Wallace, Hastings and Lowry (35, 36) for the muscle cell under similar experimental consideration.

Muscle intracellular pH according to Wallace and Hastings is 6.93 ± 0.12 . Infusion of HCl produced serum pH decrease while calculated muscle pH remained the same. With NaHCO_3 infusion serum pH was raised and muscle pH remained the same or decreased slightly (35). Brodie and Woodbury (37) estimated normal rat cerebral cortical intracellular pH to be 7.04 ± 0.07 while plasma pH was 7.41 ± 0.03 . When 30 per cent CO_2 was inhaled, plasma pH decreased to 6.95 ± 0.01 and cerebral pH decreased to 6.75 ± 0.06 . In Table II the calculated distribution ratios for ammonia between blood and brain are seen to correlate in extent and magnitude of variation to the observed distribution ratios when the pH of muscle and brain is assumed to be 7.0, though with pCO_2 elevation and depression tissue pH value may decrease and increase to some extent. From our data utilizing a pH gradient distribution hypothesis, the steady state distribution of ammonia from blood to brain and muscle appears to be dependent upon a pH difference between blood and the tissues. This is substantiated by the above observations concerning the role of CSF pH (which can be measured) in the distribution of ammonia to CSF. CSF pH appears to undergo little change in metabolic acidosis and alkalosis, but does change when pCO_2 of blood is experimentally varied. Muscle and brain pH changes may well be similar to those in CSF. It

has been suggested recently that CSF is not similar to extracellular fluid but has characteristics of intracellular fluid (11).

Although ammonia is metabolized to glutamine and other compounds in muscle and brain, measurements of tissue ammonia performed during a steady state situation appear to be meaningful. Tissue ammonia concentrations reflect changes in ammonia passage into cells by diffusion in a manner analogous to passage into CSF where no metabolism is thought to occur.

This study emphasizes that knowledge of arterial blood pH is necessary in order to evaluate fully the relationship between arterial ammonia concentration and ammonia toxicity in hepatic coma. Though ammonia elevation in the central nervous system and muscle would appear to explain some of the features of hepatic coma, other compounds, *e.g.*, amino acids of relative availability, due to defects in liver anabolism or detoxication, may be abnormally distributed between blood and central nervous system and muscle due to gradients in pH. Acidic compounds with certain ionization characteristics may be less able to pass into tissues during states of alkalosis. Thus if any corrective therapy utilizing metabolic and respiratory acidosis is found useful, it may be due to alteration in the transfer of such compounds to muscle and central nervous system.

SUMMARY

1. In an effort to delineate the role of pH in the distribution of ammonia between blood and various body fluids and tissues, temporary pH gradients between blood and cerebrospinal fluid, brain and muscle were experimentally induced by means of intravenous infusion of hydrochloric acid and sodium hydroxide solution or elevation and depression of the partial pressure of carbon dioxide through respiratory means. Simultaneous brain, muscle and cerebrospinal fluid ammonia concentrations were serially determined during steady state conditions and were related to arterial whole blood ammonia concentrations at corresponding times.

2. A direct relation was observed between the diffusion of ammonia into cerebrospinal fluid and the magnitude and direction of a gradient in pH between blood and cerebrospinal fluid.

3. There appeared to be a direct and predictable correlation between alteration of blood pH and the tissue ammonia concentration. During metabolic and respiratory alkalosis brain and muscle ammonia concentrations increased two- to threefold, while during metabolic and respiratory acidosis brain and muscle concentrations remained at or decreased below control concentrations.

4. Explanation for these findings may be found in the pH gradient drug distribution hypothesis.

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