Studies on the respiratory response to disturbances of acid-base balance, with deductions concerning the ionic composition of cerebral interstitial fluid

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FENCL, V., T. B. MILLER, AND J. R. PAPPENHEIMER. Studies on the respiratory response to disturbances of acid-base balance, with deductions concerning the ionic composition of cerebral interstitial fluid. Am. J. Physiol. 210(3): 459-472, 1966.-Respiratory responses to inhaled CO2 were studied on five unanesthetized goats during chronic metabolic acidosis or alkalosis. Measurements were made of alveolar ventilation, pH, [HCO3-], and [Cl-] of arterial plasma and CSF during each steady-state period of CO2 inhalation at each level of acidosis or alkalosis. Net transependymal fluxes of HCO3- and Cl- were determined. Results lead to the following principal conclusions: 1) Resting ventilation is a single function of [H+] in cerebral interstitial fluid during inhalation of CO2, perfusion of the ventriculocisternal system with varying [HCO3-], and during all degrees of chronic metabolic acidosis or alkalosis consistent with life at normal Pao2. 2) Respiratory adaptations to chronic acidosis or alkalosis are accounted for quantitatively by observed changes in ion transport between blood and CSF. 3) Concentrations of H+, HCO3-, and Cl- in cerebral interstitial fluid are equal to those in CSF, even when large differences in concentration between plasma and CSF are maintained by the blood-brain barrier.

Mechanisms underlying control of breathing during disturbances of acid-base balance have been a subject of controversy ever since the classical studies of Haldane and Priestley (11). The central points at issue concern the uniqueness of molecular CO2 or of [H+] as stimulating agents, the locus of stimulation in the CNS, and the relative role played by peripheral chemoreceptors in the over-all response. The unified theory of Winterstein (35, 36), ascribing all changes of ventilation to changes in [H+] within the central nervous system, gave way to the multiple factor theory advanced by Gray (10) following Nielson's (22) demonstration that arterial CO2 and [H+] apparently exerted independent actions on respiration during chronic metabolic acidosis. In recent years the interpretation of respiratory responses in terms of the composition of arterial blood has been questioned, owing largely to the demonstration by Leusen (17) that changes in the composition of fluid perfusing the cerebral ventricles could alter ventilation independently of the composition of arterial blood. Extensions of Leusen's experiments by Loeschcke and others (18, 20) have led to the hypothesis that ventilation may be controlled by [H+] in CSF bathing a chemoreceptive area at the surface of the medulla. This hypothesis has recently been rendered untenable, at least in its simplest form, by our own observations on unanesthetized goats (24) showing that no one of the three related variables in CSF perfusate, Pco2, [HCO3-], or [H+] was uniquely responsible for regulation of breathing. Large changes of ventilation could be produced by varying Pco2 and [HCO3-] at constant pH or alternatively by varying Pco2 and pH at constant [HCO3-]. Pulmonary ventilation became a single function of pH only when the pH was referred to a locus part way along the concentration gradient of HCO3- between CSF-perfusate and interstitial fluid close to cerebral capillaries taking part in the exchange of HCO3-. The absolute values of pH or [HCO3-] in interstitial fluid could not be determined from our data, which were consistent with several possible concentration profiles between CSF and cerebral capillaries. Two hypothetical profiles were discussed which led to unique relations between ventilation and pH; in hypothesis A, the normal concentration difference of HCO3- between blood and CSF was assumed to be maintained by an ion pump located at the blood-brain barrier and in this case the ventilation was a single function of interstitial fluid pH in the range 7.32-7.24 at all values of [HCO3-] and Pco2 in CSF perfusate; in hypothesis B, the ion pump was assumed to be located at the ependymal...
walls or pial surfaces of the brain and in this case ventilation became a single function of interstitial fluid pH in the range 7.42–7.34 (Fig. 13 of ref. 24).

We have now obtained information distinguishing between these two hypotheses. The information is derived from measurements of \( \text{HCO}_3^- \) transport and control of respiration during chronic metabolic acidosis and alkalosis, under conditions where various differences in \( [\text{HCO}_3^-] \) are naturally created and maintained between CSF and blood. The results show that alveolar ventilation is a single function of pH in large cavity fluid during all degrees of chronic acidosis and alkalosis and over a wide range of inspired CO\(_2\) concentrations. Thus the ventilation breathing \( \text{CO}_2 \) during severe chronic metabolic alkalosis is not significantly different from that breathing air during severe acidosis, provided that the pH in cisternal fluid is the same in both instances. Moreover, the relationship between ventilation and pH of cisternal fluid during acidosis or alkalosis is not significantly different from the relationship between ventilation and interstitial fluid pH as determined previously from perfusion experiments, the interstitial pH being calculated on the assumption of an ion pump at the blood-brain barrier (hypothesis A of ref. 24). Combination of these results with those obtained previously leads to the three principal conclusions of the present paper, 1) that the effects on respiration of disturbances in acid-base balance are mediated by changes in \([\text{H}^+]\) of cerebral interstitial fluid, whether these changes be caused by inhaled \( \text{CO}_2 \), perfusion of the ventricles with varying [\( \text{HCO}_3^- \)], or by chronic acidosis or alkalosis; 2) that equilibrium concentrations of \( \text{H}^+, \text{HCO}_3^- \), and \( \text{Cl}^- \) in cerebral interstitial fluid are approximately the same as those in large cavity fluid, even when large differences are created between blood and CSF during chronic disturbances of acid-base balance; 3) that the respiratory response to chronic metabolic acidosis or alkalosis can be explained quantitatively by observed changes in intracerebral ion transport.

![Figure 1](image1.png)

**FIG. 1.** Typical dose schedule for maintenance of acidosis or alkalosis in the goat. \( \text{NH}_4\text{Cl} \) or \( \text{NaHCO}_3 \) given by rumen tube.

![Figure 2](image2.png)

**FIG. 2.** \([\text{HCO}_3^-]\), pH, and \( \text{P}_{\text{CO}_2} \) in CSF and blood during chronic variations of blood \([\text{HCO}_3^-] \) induced by administration of \( \text{NH}_4\text{Cl} \) or \( \text{NaHCO}_3 \). Data obtained by simultaneous anaerobic sampling of arterial blood and cisternal or ventricular CSF in 5 unanesthetized goats at rest, breathing air. The least-squares line in the bottom panel is: \([\text{HCO}_3^-]_{\text{CSF}} = 11.3 + 0.352 [\text{HCO}_3^-]_{\text{ART}} \). In the middle panel the line for arterial \( \text{P}_{\text{CO}_2} \) was fitted to the points by eye. The broken line labeled CSF was obtained from average \( \text{P}_{\text{CO}_2} \) differences between arterial blood and cisternal CSF listed in Table 1. To obtain the lines drawn through the points in the upper panel the data were grouped according to acid-base condition and the average CSF pH in each condition was plotted against the corresponding average blood \([\text{HCO}_3^-] \), a smooth line was drawn through these points, and deviations of experimental points from the line were treated statistically. The cross-hatched area includes \( \pm 1 \text{ S.E.} \), the area between the broken lines \( \pm 1 \text{ S.D.} \).

**METHODS**

1) **General.** Repeated studies of the respiratory response to inhaled \( \text{CO}_2 \) were made on each of five unanesthetized goats which were made chronically acidic or alkalotic by intraruminal administration of \( \text{NH}_4\text{Cl} \) or \( \text{NaHCO}_3 \). Each animal was provided with carotid loops and with permanently implanted guide tubes over the lateral ventricles and cisterna magna. Measurements were made of alveolar ventilation, gas exchange, pH, \([\text{HCO}_3^-]\), and \([\text{Cl}^-]\) of arterial plasma and cisternal CSF.
during each steady-state period of CO₂ inhalation at each (chronic) level of metabolic acidosis or alkalosis. Usually four to six periods of CO₂ inhalation were studied on each experimental day, the inspired CO₂ ranging from 0 to 10% for normal and alkalotic animals and 0 to 5% in metabolic acidosis. Net transependymal fluxes of HCOS⁻ and Cl⁻ were measured in two goats during ventriculocisternal perfusion with various concentrations of HCOS⁻ while the animals were in chronic metabolic acidosis or alkalosis.

Operative procedures and techniques for perfusion of the ventriculocisternal system have been described in ref. 25; respiratory measurements and analytical techniques in ref. 24, theory and procedures for measurement of CSF formation and transependymal flux rates in refs. 19 and 24. New procedures and modifications of previous techniques are described in detail below.

2) Production and maintenance of metabolic acidosis or alkalosis. Priming doses of NH₄Cl (0.5-0.7 g/kg) or NaHCO₃ (1-2 g/kg) were dissolved in 1-2 liters of warm water, mixed with 100 g of baby cereal, and introduced into the rumen through a stomach tube. The cereal was effective in preventing diarrhea. [HCOS⁻] in arterial plasma was usually determined twice or more each day and supplementary doses of NH₄Cl or NaHCO₃ were given as needed to maintain plasma [HCOS⁻] in the range 8-15 mEq/liter (acidosis) or 35-45 mEq/liter (alkalosis). Ruminants can excrete large amounts of alkali but are poorly adapted for excretion of acid. Usually 0.2 g/kg NH₄Cl, given in a single daily dose, was sufficient to maintain plasma [HCOS⁻] in the range 10 ± 2 mEq/liter, whereas daily administration of 2-4 g/kg NaHCO₃, given in multiple doses, was necessary to maintain plasma [HCOS⁻] in the range 40 ± 5 mEq/liter. Treatment was continued on each animal for 4-5 days, the experimental measurements being made on the last 2 days of each period of treatment. Typical dose schedules and variation of plasma [HCOS⁻] are shown in Fig. 1.

3) Respiratory measurements. In our previous studies (24) the respiratory inflow was supplied from an aviation demand valve which created undesirably large inspiratory pressures when respiratory minute volume exceeded 25 liters/min. For the present work the range of respiratory responses was extended to 50 liters/min and respiratory inflow was supplied from a 100-liter balanced spirometer, refilled either continuously or intermittently from a high-pressure cylinder. Maximum inspiratory and expiratory pressures were less than ±5 cm H₂O and inspiratory volumes measured from the spirometer usually agreed with the continuously recorded expiratory minute volumes within 2%.

4) Sampling procedures, measurement of pH. CSF is poorly buffered, and a change of only 0.01 pH units is associated with a 20% change in alveolar ventilation (Figs. 9, 10). Traces of acid or alkali in the sampling system or loss of CO₂ into minute air bubbles adhering to the barrel of the sampling syringe may cause spurious shifts of pH which are large compared to physiologically significant variations. The relatively large variations of pH in samples of CSF drawn from the same animal under the same physiological conditions have not been emphasized by previous investigators. Many published values for CSF pH are improbably high, leading to values of Pco₂ corresponding to improbably small or even negative cisternal arterial differences in Pco₂, particularly when buffering power of CSF is reduced in acidosis. We used the following procedure for sampling and analyzing CSF anerobically. The cistern (or lateral ventricle) was punctured through the guide tube with a sharp probe which was left in place throughout the day of experiment; the animals showed no sign of discomfort. The probe was connected to a three-way stopcock via a 4-cm length of flexible polyvinyl tubing. A 2-ml glass, capillary-tipped syringe was rinsed thoroughly with boiled saline and small air bubbles adhering to the sides were removed by tapping. The syringe was placed in the three-way stopcock with its tip pointed upward and the barrel at the level of the auditory meatus. One milliliter of CSF was allowed to enter the syringe under

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**TABLE 1. Cisternal-arterial Pco₂ differences during inhalation of various CO₂ mixtures**

<table>
<thead>
<tr>
<th>Pco₂, mm Hg</th>
<th>Cisternal-Arterial Pco₂ Difference, mm Hg (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-30</td>
<td>11.1±2.4 (117 samples)</td>
</tr>
<tr>
<td>30-35</td>
<td>10.3±0.6 (17 samples)</td>
</tr>
<tr>
<td>35-40</td>
<td>8.9±0.5 (33 samples)</td>
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<tr>
<td>40-45</td>
<td>8.9±0.6 (31 samples)</td>
</tr>
<tr>
<td>45-50</td>
<td>7.7±0.8 (26 samples)</td>
</tr>
<tr>
<td>50-55</td>
<td>7.5±0.6 (27 samples)</td>
</tr>
</tbody>
</table>

* Includes 87 measurements obtained during perfusion of the ventricular systems of 6 goats, published previously as Fig. 5 of ref. 24.
the natural pressure of CSF and immediately discarded through the side arm; 1.5 ml of CSF was then obtained for analysis. A Radiometer capillary glass electrode was fitted with an entrance tube which could be inserted to the center of the sample syringe and which also made an airtight seal over the nozzle of the syringe. The sample was discharged into the pH capillary by gentle positive pressure. Usually less than 15 sec elapsed between withdrawal of the sample and final measurement of its anaerobic pH. Successive pH readings on samples taken from the same syringe often increased by .01-.02 units in the course of a minute or two. Sampling of both CSF and arterial blood (the latter from an indwelling needle in a carotid loop) was accomplished without disturbing the animal. The total volume of CSF in a goat is 20-25 ml (25) and the rate of production of CSF is about 0.15 ml/min (12). Consequently, 3 ml samples could be withdrawn every hour without fear of causing significant alterations in the volume or composition of CSF.

Even with these precautions, the standard deviation of multiple measurements of CSF pH, made under the same conditions, was ±0.017 pH units as shown in Fig. 2. On the basis of experience with more than 100 samples of CSF, collected under the most favorable conditions, we conclude that direct estimates of CSF pH may be less accurate than indirect estimates based on the measured PCO₂ of arterial plasma, the gasometric analysis of CSF [HCO₃⁻], and average values for cisternal-arterial CO₂ pressure difference. Table 1 shows mean values for

<table>
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<tr>
<th>DATE</th>
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<th>V̇CO₂ m/min</th>
<th>STPD</th>
<th>V̇O₂ m/min</th>
<th>STPD</th>
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</tr>
</tbody>
</table>

*Calculated as in ref. 24 from total effective dead space measured on each day of experiment. V̇O₂ = 0.06 ± 0.01 (mL).
cisternal-arterial Pco₂ differences based on analysis of 160 pairs of simultaneously drawn samples of cisternal fluid and arterial blood. Comparison of column 1 with columns 2 and 3 shows that cisternal-arterial Pco₂ differences during acidosis are not significantly different from normal at any given Pco₂.

**RESULTS**

1. Distribution of ions between plasma and CSF during chronic metabolic acidosis and alkalosis. The steady-state distribution of HCO₃⁻, H⁺, and Cl⁻ between plasma and CSF during various degrees of metabolic acidosis or alkalosis are summarized in Figs. 2, 3, and 4, respectively. In Figs. 2 and 3 the degree of metabolic acidosis is indicated on the abscissa by the arterial [HCO₃⁻] which, in contrast to the acidity, is affected only secondarily by respiratory compensation. The relation between arterial pH and [HCO₃⁻] is shown in Fig. 3. Detailed values for relevant variables are given for one goat in Table 2, which contains control data and data from repeated periods of acidosis and alkalosis. Similarly detailed data for each of four additional goats are available from a table deposited with the American Documentation Institute.5

Reference to the bottom panel of Fig. 2 shows that CSF [HCO₃⁻] varies linearly with arterial [HCO₃⁻]. There is, however, a considerable degree of regulation of [HCO₃⁻] in CSF so that each 3.5 mmolal change in arterial plasma is associated with only a 1 mmolal change in CSF. The changes in [Cl⁻] are equal and opposite to those of [HCO₃⁻] as shown in Fig. 4. The CSF [HCO₃⁻] is about 5 mmolal greater than plasma [HCO₃⁻] during severe acidosis and is 16 mmolal less than that of plasma during severe alkalosis. Most of the data summarized in

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**FIG. 4.** Variation of CSF [Cl⁻] associated with variation in arterial [Cl⁻] in chronic metabolic acidosis and alkalosis. Data from 3 goats. Least-squares line is: [Cl⁻]CSF = 83.9 + 0.396 [Cl⁻]art. Concentrations were corrected to a standard 300 milliosmos/kg H₂O to allow for effects of dehydration during administration of salts. The corrections ranged from 0 to 7%.

**Table 3. Comparison between measured and calculated CSF pH values at various arterial [HCO₃⁻] in chronic metabolic acidosis and alkalosis**

<table>
<thead>
<tr>
<th>Arterial [HCO₃⁻], mmol/kg H₂O</th>
<th>Avg measured</th>
<th>Calculated†</th>
</tr>
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<tr>
<td>10</td>
<td>7.267</td>
<td>7.272</td>
</tr>
<tr>
<td>20</td>
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<td>7.288</td>
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<tr>
<td>30</td>
<td>7.305</td>
<td>7.300</td>
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<tr>
<td>40</td>
<td>7.325</td>
<td>7.320</td>
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</table>

* Taken from Fig. 2. †pHCSF = 6.122 + log \( \frac{[HCO₃⁻]_{CSF}}{0.0366 P_{co₂}} \) where [HCO₃⁻]CSF is defined by bottom panel of Fig. 2 and Pco₂ is defined by Table 1 and middle panel of Fig. 2.

Fig. 2 refer to cisternal CSF. However, it of the points refer to ventricular samples taken under normal, acidotic, or alkalotic conditions. The [HCO₃⁻] in ventricular fluid was not significantly different from that in cisternal fluid.

Changes in CSF pH are shown in the upper panel of Fig. 2. The scatter of individual points about the mean is large compared to the total change in pH but is small in terms of absolute value. Means and SE of CSF pH at average arterial bicarbonates of 13, 28, and 40 mmolal were 7.279 ± 0.003, 7.303 ± 0.005, and 7.325 ± 0.003, respectively. The differences between these means are highly significant (P < 0.005) and the continuous change of mean CSF pH indicated by the solid central line is exactly sufficient to explain the observed changes of respiration as will be shown below. An independent check of the mean values for pH shown in Fig. 2 may be obtained indirectly from the mean [HCO₃⁻] in ventricular fluid. However, they were not concerned with the significance of small changes such as those shown in Fig. 2 and it is possible that their results, based on only three samples in alkalosis and five in acidosis, are not statistically different from our own; a more detailed comparison of our results with those of previous investigators will be considered in the DISCUSSION. The conclusion, based on Fig. 2 and Table 3, that CSF pH varies slightly but continuously with plasma [HCO₃⁻], is crucial for the subsequent development of the present paper.

The arterial Pco₂ shown in the middle panel of Fig. 2 is a reciprocal measure of the steady-state alveolar ventilation, which is increased twofold as arterial [HCO₃⁻] changes from 40 mmolal in alkalosis to 10 mmolal in acidosis. The significance of this change in relation to the small change in pH of CSF will become apparent below.
2) Net flux of $[\text{HCO}_3^-]$ between CSF and blood. In normal goats the average $[\text{HCO}_3^-]$ is 26 mmol/L in arterial plasma, 28 mmol/L in cerebral capillary plasma, and only 22 mmol/L in cisternal fluid. In our previous paper (24) we showed that the concentration difference of 6 mmol/L between capillary blood and CSF is maintained without net flux. Net flux of $\text{HCO}_3^-$ occurred only when $[\text{HCO}_3^-]$ of CSF was changed experimentally by perfusion of the ventriculocisternal system with abnormal concentrations of $\text{HCO}_3^-$. In the present investigation the $[\text{HCO}_3^-]$ in plasma has been varied over a wide range with associated chronic concentration differences between CSF and plasma ranging from 5 mmol/L in acidosis to -18 mmol/L in alkalosis (Fig. 2). In order to establish the relation between $[\text{HCO}_3^-]$ in CSF and that in interstitial fluid, it is essential to determine whether or not net flux of $\text{HCO}_3^-$ occurs under these altered conditions. If net flux occurs, there will be a concentration gradient in interstitial fluid; if net flux is zero, there will be no concentration gradient in interstitial fluid, as discussed previously (24). Net fluxes of $\text{HCO}_3^-$ and Cl$^-$ were therefore measured by the technique of ventriculocisternal perfusion in each of two goats which were subjected to chronic acidosis and alkalosis. Net fluxes were calculated as described previously (24)

$$\Delta V = \Delta V_1 (c_i - c_o) - \Delta V_2 (c_i + c_o)$$

where $\Delta V = \text{net flux (µM/min)}$, $\Delta V_1 = \text{rate of perfusion (ml/min)}$, $c = \text{concentration (mm/L)}$, $C_{\text{in}} = \text{clearance of inulin (ml/min)}$, and subscripts i, o, and l refer, respectively, to ventricular inflow, cisternal outflow, and freshly formed CSF. Concentrations of $\text{HCO}_3^-$ and Cl$^-$ in freshly formed CSF ($c_l$) were assumed to be equal to the values found in CSF ($c_{\text{CSF}}$) under the given conditions of acid-base balance. The above equation excludes net addition of ions to ventricular fluid from choroid plexus secretion and net loss of ions from the system via absorption in bulk through the arachnoid villi; it refers specifically to transependymal flux through interstitial fluid containing neural elements. Results are summarized in Fig. 5, A and B, and detailed data from one animal are given in Table 4.

From Fig. 5A it is seen that zero net flux occurred when CSF $[\text{HCO}_3^-]$ was 6 mmol/L greater than in plasma during acidosis and 18 mmol/L less than in plasma during alkalosis. Similarly, zero net flux of Cl$^-$ occurred when $[\text{Cl}^-]$ was 3 mmol/L greater than in plasma in acidosis and 25 mmol/L greater than in plasma in alkalosis. Fluxes in normal animals are shown as intermediate lines, drawn without experimental points, but adapted from Fig. 10 of our previous publication (24). Reference to Figs. 2 and 5 of the present work shows that the point of zero net flux corresponds in each case to the resting, steady-state concentration difference of $\text{HCO}_3^-$ or Cl$^-$ between CSF and plasma for each acid-base condition.
### Table 4. Net flux of $\text{HCO}_3^-$ and $\text{Cl}^-$ in CSF system during chronic metabolic acidosis and alkalosis: Goat N5

#### A. MEASURED QUANTITIES
(concentrations determined during last 15 min. of indicated period)

| Condition | Time, min | Flow Rate ml/min | $[\text{HCO}_3^-]$ mM/kg H$_2$O | $[\text{Cl}^-]$ mM/kg H$_2$O | Inulin
|-----------|-----------|------------------|-------------------------------|----------------------------|-----|
|           |           | Vent. inflow $V_i$ | Vent. outflow $V_o$ | Cist. inflow $c_i$ | Cist. outflow $c_o$ | Art. plasma $c_a$ | Cist. inflow $V_i$ | Cist. outflow $V_o$ | Art. plasma $c_a$ | Cist. outflow (% of inflow)
| Acidity   | 0-62      | 1.308            | 1.441                        | 25.5                       | 23.5                       | 14.4                       | 149.0                       | 144.7                       | 131.0                       | 85.2                       |
|           | 69-160    | 1.307            | 1.428                        | 41.0                       | 36.5                       | 16.8                       | 135.0                       | 134                           | 129.0                       | 88.9                       |
|           | 165-208   | 1.304            | 1.376                        | 7.4                        | 10.0                       | 11.9                       | 163.0                       | 154                             | 125.0                       | 93.4                       |
| Alkalosis | 0-55      | 1.722            | 1.700                        | 15.5                       | 17.8                       | 45.5                       | 153.5                       | 148.0                       | 93.3                       |
|           | 61-109    | 1.823            | 1.783                        | 34.8                       | 33.6                       | 43.7                       | 136.5                       | 134.0                       | 93.4                       |
|           | 122-180   | 1.679            | 1.679                        | 50.6                       | 46.6                       | 44.7                       | 121.0                       | 120.5                       | 93.3                       |

#### B. CALCULATED QUANTITIES

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time, min</th>
<th>$[\text{HCO}_3^-]$ mM/kg H$_2$O</th>
<th>$[\text{Cl}^-]$ mM/kg H$_2$O</th>
<th>Inulin clearance C$_{IN}$ ml/min</th>
<th>Net flux $\mu$M/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\varepsilon$</td>
<td>$c_p$</td>
<td>Steady-state conc. in CSF $c_{CSF}$</td>
<td>difference $\varepsilon$ - $c_{CSF}$</td>
</tr>
<tr>
<td>Acidity</td>
<td>0-62</td>
<td>24.3</td>
<td>16.6</td>
<td>16.8</td>
<td>+7.9</td>
</tr>
<tr>
<td></td>
<td>69-160</td>
<td>38.2</td>
<td>18.9</td>
<td>17.6</td>
<td>+19.3</td>
</tr>
<tr>
<td></td>
<td>165-208</td>
<td>9.0</td>
<td>14.0</td>
<td>15.6</td>
<td>-5.0</td>
</tr>
<tr>
<td>Alkalosis</td>
<td>0-55</td>
<td>17.0</td>
<td>47.7</td>
<td>29.1</td>
<td>-30.8</td>
</tr>
<tr>
<td></td>
<td>61-109</td>
<td>34.0</td>
<td>45.9</td>
<td>28.2</td>
<td>-11.9</td>
</tr>
<tr>
<td></td>
<td>122-180</td>
<td>48.1</td>
<td>46.9</td>
<td>28.8</td>
<td>+1.2</td>
</tr>
</tbody>
</table>
fluids brought about by chronic metabolic acidosis and alkalosis involve redistribution of ions to new equilibrium concentrations. A change in acidity of blood causes a change in active ion transport between blood and CSF; net flux due to this altered rate of active transport continues until the entire extracellular compartment (interstitial fluid plus CSF) attains a concentration such that the electrochemical gradient causing passive flux exactly balances flux occurring by active transport in the opposite direction. The ion pump could be located at the blood-brain barrier or at the ependymal linings of the ventricular system. The respiratory changes considered in sections 3 and 4 below suggest that the location of this pump is at the blood-brain barrier.

3) Respiratory changes. The experiments of Nielsen (22) showed that during metabolic acidosis in man the CO$_2$ response curve is shifted to the left of normal, and the experiments of Alexander et al. (3) showed that during metabolic alkalosis the CO$_2$ response curve is shifted to the right. Figure 7 shows a repetition of these classical experiments on an unanesthetized goat. The data were obtained over a period of 3 months during which the animal was twice made acidic and alkalotic. Detailed data are given in Table 2; similar data, obtained from four other goats, are included in the table deposited with ADI mentioned previously. As shown in Fig. 7 the CO$_2$ response curve during acidosis was shifted 30 mm Hg to the left of that obtained during alkalosis, a shift which is considerably greater than reported by previous investigators who employed milder degrees of acidosis or alkalosis in man.

The same alveolar ventilations shown in Fig. 7 are plotted in Fig. 8 as a function of [H$^+$] in cisternal CSF, and it is clear that the CO$_2$ response curves were a single function of [H$^+$] in large cavity fluid whether the animal was acidic or alkalotic. The relationship between ventilation and CSF pH is nonlinear, especially at low ventilations; semilogarithmic coordinates provide a linear description of the results as shown in Figs. 9 and 10.

Figure 10 summarizes respiratory data from the five goats used in the present experiments. Since the animals varied in weight from 32.5 to 70 kg, all ventilations were corrected to a standard resting, steady-state CO$_2$ production of 300 ml/min STPD, which is the average metabolic CO$_2$ production of animals weighing 35-40 kg (24). For example, if the resting CO$_2$ production of an animal was 300 ml/min STPD, then all ventilations of this animal were multiplied by $\frac{300}{30}$ for inclusion in Fig. 9.

The data comprise 81 measurements of ventilation with...
FIG. 8. Ventilatory response to CO₂ inhalation in goat H5 in normal condition and during chronic metabolic acidosis and alkalosis. The range of corrected alveolar ventilations extends from 2.5 liters/min while breathing air in severe alkalosis to 40±10 liters/min while breathing CO₂ mixtures in all states of alkalosis or acidosis. At the lowest ventilations, obtaining during severe alkalosis, it was necessary to add oxygen to inspired gas in order to maintain normal oxygen saturations. The entire 20-fold range of alveolar ventilation shown in Fig. 10 was associated with a pH range of only 0.2 units. Two animals died from hypoventilation during chronic alkalosis; in goats, the maximum chronic CSF pH compatible with life appears to be less than 7.40.

A statistical analysis of results summarized in Fig. 10 is shown in Table 5A which gives least-square equations and correlation coefficients for the best straight lines relating log Vₐ to CSF [H⁺] for each condition separately. Table 5B summarizes an analysis of variance and shows that the regression coefficients a and b of Table 5A are not significantly different. We therefore conclude that alveolar ventilation of resting goats is a single function of CSF [H⁺] under all conditions of our experiments, namely, at all degrees of chronic acidosis or alkalosis consistent with life and while breathing CO₂ mixtures ranging from 0 to 10% at normal arterial oxygen pressures.

Respiratory data for air-breathing periods (13 periods of control, 13 during acidosis, and 10 during alkalosis) are shown separately in Fig. 11 against a background of the over-all response in order to emphasize that the slight changes of CSF pH during chronic acidosis or alkalosis shown in Fig. 2 are quantitatively sufficient to account for the observed respiratory adaptations.

Since there is no net flux of HCO₃⁻ between large cavity fluid and blood, we may assume that the composition of CSF is identical with that of interstitial fluid with respect to [H⁺] or [HCO₃⁻] if the ion pump is located at the blood-brain barrier (hypothesis A, ref. 24). In contrast, if the ion pump were located at the ventricular ependyma (hypothesis B of ref. 24) then interstitial [H⁺] would be close to that of arterial plasma and ventilation would be a separate function of CSF pH at each degree of metabolic acidosis or alkalosis. The single relationship shown in Fig. 10 suggests that the ion pump is located at the blood-brain barrier and that the concentrations of HCO₃⁻ and H⁺ in CSF are identical with those in interstitial fluid under chronic conditions.

Comparison of respiratory response to changes in blood [HCO₃⁻] with responses to alterations in [HCO₃⁻] of ventriculocisternal perfusion fluid. The relationship between alveolar ventilation and CSF pH under conditions of zero net flux for HCO₃⁻ (Figs. 8-11) implies that the ionic composition of interstitial fluid is similar to that of CSF, the concentration gradient to blood being maintained by an ion pump at the blood-brain barrier. This conclusion is consistent with hypothesis A of our previous paper (24). A direct comparison of this hypothesis with our present
ALVEOLAR VENTILATION (per 200 ml/min CO₂ production)

**TABLE 5**

<table>
<thead>
<tr>
<th></th>
<th>Intercept</th>
<th>Slope</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-2.14</td>
<td>0.0371</td>
<td>0.806</td>
</tr>
<tr>
<td>Acidosis</td>
<td>-1.88</td>
<td>0.0509</td>
<td>0.802</td>
</tr>
<tr>
<td>Alkalosis</td>
<td>-2.93</td>
<td>0.0612</td>
<td>0.810</td>
</tr>
<tr>
<td>Pooled data</td>
<td>-1.89</td>
<td>0.0351</td>
<td>0.990</td>
</tr>
</tbody>
</table>

**B: analysis of variance for testing equality of regression coefficients (30)**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deviation from null hypothesis</td>
<td>4</td>
<td>0.1712</td>
<td>0.0428</td>
</tr>
<tr>
<td>Separate regressions</td>
<td>75</td>
<td>1.7528</td>
<td>0.0239</td>
</tr>
<tr>
<td>Common regression</td>
<td>79</td>
<td>1.9949</td>
<td></td>
</tr>
</tbody>
</table>

\[ F = 0.0428/0.0239 = 1.791 \]

result is shown in Fig. 12. It is clear from this comparison that the relationship between ventilation and CSF pH during chronic acidosis and alkalosis is not significantly different from the relationship between pulmonary ventilation and the pH of interstitial fluid during perfusions of the ventriculocisternal system with varying [HCO₃⁻], it being assumed that the ion pump is located at the blood-brain barrier and that neuronal elements responsive to pH are located three-fourths of the distance along the functional concentration gradient for HCO₃⁻ between CSF perfusate and interstitial fluid at the site of ion exchange with cerebral blood. A further quantitative test of this hypothesis was obtained by perfusion of the ventricular system with low [HCO₃⁻] during chronic metabolic alkalosis and by perfusion with high [HCO₃⁻] during acidosis (Table 6). Under these conditions the pH of the perfusate varied from 7.13 to 7.53, extremes which would be incompatible with life of the animal if they referred to fluid surrounding respiratory neurons. In contrast, the pH of interstitial fluid, calculated from hypothesis A, remained within the range 7.28–7.36 and the ventilation in each case corresponded to that predicted from Fig. 12. These results emphasize the conclusion that neural elements responsive to [H⁺] are located at some distance from the large cavities.

**DISCUSSION**

1) **Composition of CSF during chronic acidosis and alkalosis.** There is now a large literature reviewed by Mitchell et al. (19) describing the regulation of [HCO₃⁻] in CSF during disturbances of acid-base balance in humans. A summary of the human data is shown in Fig. 13 for comparison with the present data on goats shown in Fig. 2. CSF [HCO₃⁻] in goats is 3–5 mmolal less than in humans at any given plasma [HCO₃⁻], and regulation of CSF [HCO₃⁻] is slightly more efficient in the goat, as evidenced by the smaller slope. The changes of PCO₂ are also about the same in man and goat, thus indicating that respiratory adaptations to chronic acidosis or alkalosis are similar. The important difference between our conclusions and those of previous investigators concerns the regulation of CSF pH. Our results show that CSF pH changes continuously as a function of plasma [HCO₃⁻]; the change is very small in absolute terms but it is statistically significant and of precisely the right magnitude to explain the observed respiratory adaptations. In contrast, previous investigators have reported that CSF pH remains unchanged during chronic metabolic disturbances of acid-base balance (cf. Mitchell et al. (19) for review). We believe that this discrepancy is only an apparent one which derives from the technical difficulty of obtaining meaningful direct pH values in samples of CSF, as discussed above in the METHODS section. Direct measurements of CSF pH were reported by each of the nine groups of investigators listed on Fig. 13, and it is true that a mass plot of their data (not shown in Fig. 13) reveals no statistically significant alteration of CSF pH as plasma [HCO₃⁻] varies from 10 to 40 mmolal. During acidosis (plasma [HCO₃⁻] = 5–13 mmolal) the mean CSF pH was 7.326, SD ± 0.065; during alkalosis (plasma [HCO₃⁻] = 29–40 mmolal) the mean CSF pH was 7.311, SD ± 0.015. The standard deviation of 0.065 pH units during metabolic acidosis is larger than the entire change of mean pH found in our experiments on goats (Fig. 2) and is perhaps an expression of the difficulty of obtaining reproducible pH measurements in the
CEREBRAL FLUIDS AND RESPIRATION DURING CHRONIC ACID-BASE DISTURBANCES

FIG. 11. Resting steady-state alveolar ventilation (corrected to standard CO₂ production of 200 ml/min, STPD) in relation to CSF (H⁺) in alkalosis, acidosis, and normal conditions and compared with the over-all respiratory response. The air-breathing periods are presented as the mean values ± SEM of VA and CSF [H⁺] for each acid-base condition. Mean values are significantly different from each other and the slope of the plot of VA vs. CSF [H⁺] breathing air is not different from that of the over-all response to inhaled CO₂. The over-all respiratory response is represented statistically as the best fit line for the pooled data shown in Fig. 10. The 95% confidence interval (C.I.) indicates that there is but 1 chance in 20 that the true relation between log VA and CSF [H⁺] lies outside of this interval.

A further complication in experiments on humans involves the fact that CSF samples are drawn by lumbar puncture. The Pco₂ in these samples will be set by the ratio of metabolism to blood flow in the spinal cord and this may not be the same as in ventricular or cisternal fluid, especially during changes of cerebral blood flow induced by the altered Pco₂ of acidosis or alkalosis. A discrepancy of only 2–3 mm Hg in CO₂ pressure between lumbar and ventricular fluid would cause a relatively large discrepancy in the pH.

An alternative estimate of CSF pH in humans is shown in the top panel of Fig. 13 which depicts the mean CSF pH calculated indirectly from the measured CSF [HCO₃⁻] of the middle panel and the mean jugular-bulb blood Pco₂ of the middle panel. The data for jugular CO₂ were obtained from references (8, 15, 16, 23, 26, 28, and 34) and it is assumed that the jugular Pco₂ is equal to that in cisternal fluid as shown by Bradley and Semple (4). The errors in determination of CSF [HCO₃⁻]

...[HCO₃⁻]interstitial = ½[HCO₃⁻]plasma + ½[HCO₃⁻]per fusate

as required by hypothesis A of our previous paper (24). The data include the 7 measurements on 2 goats in acidosis and alkalosis shown in Table 6 as well as values derived from 6 normal goats and published previously as Table 4 of ref. 24.

and jugular Pco₂ are small and the mean values so obtained should provide a relatively reliable estimate of mean CSF pH. It is clear from Fig. 13 that CSF pH, calculated indirectly, varies as a continuous function of plasma [HCO₃⁻] in humans as it does in goats.

The chronic equilibrium conditions described above are not comparable with the effects of acute administration of HCl or NaHCO₃. The small, transient net flux of HCO₃⁻ through the blood-brain barrier which follows a change in blood [HCO₃⁻] produces a rapid change in the composition of fluid surrounding neurons in the vicinity of capillaries, owing to the small volume of interstitial fluid relative to the flux. In contrast, the same
potential, first demonstrated in acute experiments (33),
have recently been shown to persist in chronic acidosis
or alkalosis (9). The potential changes are sensitive to
pH of blood but not to changes in pHi of fluid perfcuing
the ventricular system (13); it seems reasonable to relate
them to the altered distribution of ions across the blood-
brain barrier described in the present paper, but their
precise significance in terms of specific ion transport re-
 mains to be determined.

2) Dependence of ventilation on interstitial fluid pH. The
data summarized in Fig. 12 indicate that the relationship
between ventilation and [H+] in interstitial fluid during
chronic acidosis and alkalosis is identical with that
which obtains in perfusion experiments when the [H+] is
referred to a point three-fourths of the distance along the
functional concentration gradient for HCO3- from the
pericocular to the site of ion exchange with blood. This
unique relation between ventilation and interstitial fluid
pH appears to be valid over a 20-fold variation of alveolar
ventilation induced by inhalation of 0-10% CO2 per-
fusion of the ventriculocisternal system with [HCO3-]
varying from 5 to 45 mmolal, and during all degrees of
chronic acidosis or alkalosis compatible with continued
life of the animal. The results provide quantitative sup-
port for Winterstein’s “reaction theory” (36). The mul-
tiple-factor theory proposed by Gray (10) appears to be
unnecessary, at least with regard to disturbances of
acid-base balance. It follows, also, that the peripheral
chemoreceptors play no significant role in the respiratory
response to acid-base disturbances, a conclusion which
is in accord with recent studies by Katsaros (14) on
respiratory response to acid-base disturbances following
denervation of the carotid and aortic bodies. Mitchell
et al. (19) were forced to the conclusion that peripheral
chemoreceptors contribute to the respiratory response
because they could detect no significant change in CSF
pH during chronic metabolic acidosis or alkalosis. In
the interpretation of previous work it is also important
to emphasize that the small but significant changes in
CSF pH reported in the present paper would be incon-
sequential with respect to the respiratory responses of
anesthetized animals but are quantitatively sufficient to
account for the observed responses in unanesthetized
animals.

3) Ionic composition of cerebral interstitial fluid. The func-
tional characteristics of excitable tissues depend, in part,
on the ionic composition of extracellular fluid. It is
therefore of great interest to determine whether cells of
the central nervous system are exposed to a simple ultra-
filtrate of plasma, as in tissues generally, or whether
their normal fluid environment is similar to the special
composition of fluid found in the ventricles and sub-
arachnoid spaces. The brain parenchyma has no lym-
phatic drainage and it is impossible to collect tissue
exudates or capillary ultrafiltrates for direct analysis.
Indirect methods, commonly employed for analysis of
volume and composition of peripheral interstitial fluids,
are generally inapplicable to brain because of the special
permeability characteristics of the blood-brain barrier.

\[ \Delta \text{Pco}_2 (\text{jugular-arterial}) = 18.0 \cdot 0.22 \, \text{P}_{\text{Aco}_2} \]

This equation is the best straight-line fit to data published in refs.
8, 15, 16, 23, 26, 28, and 34. CSF pH in the top panel is de-

defined by

\[ \text{CSF pH} = 6.126 + \log \frac{[\text{HCO}_3^-]_{\text{CSF}}}{0.0314 \, \text{Pco}_2 (\text{jugular})} \]

The identity of jugular and cisternal Pco2 in humans has been
established by Bradley and Semple (4).
Nicholls and Kuffler (21) have devised an elegant method for analyzing the ionic composition of interstitial fluid in the avascular central nervous system of the leech. In essence, they used the resting membrane potential and the overshoot of the action potential for the fluid in the avascular central nervous system of the leech. From Table I, it may be inferred that the interstitial fluid normally has the same ionic composition as the surrounding hemolymph.

Results described in the present paper suggest that the respiratory response of the whole animal may be used as a sensitive biological assay for $[H^+]$ in interstitial fluid. The $[H^+]$ in turn defines the $[HC03^-]$, since $PCO_2$ in interstitial fluid is identical with that which can be measured in cisternal CSF (4, 24). Finally, the $[HC03^-]$ defines the $[Cl^-]$ since the latter is the only significant anion other than $HC03^-$. If this reasoning is correct, we can conclude from our results that the concentrations of $H^+$, $HC03^-$, and $Cl^-$ in interstitial fluid surrounding respiratory neurons are the same as those in CSF, even when large concentration differences between plasma and CSF are created by alkalosis or acidosis.

Similarly large concentration differences or ratios can be induced chronically in the case of $K^+$ (3). Recent studies by Csern (6) indicate that the permeability of ependymal walls to $K^+$ is very large compared to the permeability of the blood-brain barrier, thus implying that the concentration of $K^+$ in cerebral interstitial fluid is close to that in CSF, even when large concentration differences are created between plasma and CSF.

These considerations lead us to the view that the steady-state ionic composition of fluid bathing neurons in the central nervous system is close to that in the large cavities and subarachnoid spaces.

We thank Dr. Jane Worcester of the Harvard School of Public Health for help with the statistical analysis of Table 5. It is a pleasure also to acknowledge the technical assistance of James Nicholl, Jr.

REFERENCES


