Intracellular pH and intracellular buffering power of the cat brain

In 49 cats the intracellular pH (pHi) of the brain was estimated from the distribution of 5,5-dimethyloxazolidine-2,4-dione-2-C14 (DMO) between brain, plasma, and CSF, at arterial pCO2 of 14.5-83.2 mm Hg. From six cats cortical biopsies were taken; in the remainder, brain DMO distribution was determined on postmortem samples from various brain regions, both white and gray. pHi of the samples was calculated, assuming extracellular space to be equal to either 3 or 12% of brain weight, and extracellular composition to be equal to that of either arterial, cerebral venous, or mean capillary plasma, or CSF. Bicarbonate concentration of cell water and total CO2 concentration of brain were derived from the data. Results: 1) there were no significant regional differences in pHi; 2) when extracellular space was taken as 3% of brain weight, and its composition as equal to that of mean capillary plasma, the following relations were found: a) pHi = 4.94 pH arterial + 3.471, and thus pHi = 7.13 at arterial pH = 7.40 (and arterial pCO2 = 40); b) pHi = 7.83 - .42 log pCO2 tissue, and thus Δ [HCO3−]/Δ [pH] = 36.7 mEq/kg cell water; c) Δ [total CO2]/Δ pCO2 tissue = .33 ml/100 g cortical tissue in the pCO2 range of 30-50 mm Hg. The basic assumptions of the DMO method were critically examined. A general equation was developed which expresses the transmembrane steady-state distributions of the two members of the conjugate pair of a weak acid in terms of their relative permeabilities, intracellular and extracellular H+ ion concentrations, and membrane voltage. The equation was applied to the present experiments.

METHODS

Cats (2.3-3.9 kg) were anesthetized with intraperitoneal pentobarbital, 27 mg/kg. Both ureters were ligated to prevent renal excretion. The trachea was can-
nulated and total paralysis was induced with intravenous gallamine (10 mg/kg) to facilitate controlled artificial ventilation with a respiratory pump (Harvard Apparatus Co., Dover, Mass.). A catheter was placed in the femoral artery both for monitoring of blood pressure and for sampling of blood. Rectal temperature was maintained at 37 ± 0.2 °C by intermittent use of an electric heating pad. An intravenous injection of 0.1–0.25 mc of 5,5-dimethyl-2,4-oxazolidine-2,4-dione (DMO) in 10 ml saline was given just before the animals were paralyzed. The radioactive solution as obtained from the manufacturer contained 0.5 mc DMO/ml ethyl acetate and had a specific activity of 5–7.5 mc/mmole. Before the DMO was dissolved in saline, the ethyl acetate was allowed to evaporate. 

An average dose of 3.3 mEq/kg of NaHCO₃ was given intravenously, which raised arterial pH at pCO₂ = 40 mm Hg from 7.28 to about 7.40, and blood buffer base from 31–42 to 44–50 mEq/liter. Values for blood buffer base (defined as the sum of the blood buffer anion concentrations) were derived from the Singer-Hirstings nomogram (49), assuming hematocrit = 40 and plasma protein concentration = 7.2 g/liter.

The cats were ventilated with 100% oxygen at tidal volume of 40–50 ml; minute volume was determined by pump rate. Exhaled CO₂ concentration was monitored with a Liston-Becker infrared analyzer.

pH and pCO₂ of arterial blood samples were determined at 37°C with the Instrumentation Laboratory apparatus. The glass electrode was calibrated with phosphate buffer solutions of pH 6.84 and 7.384. The pCO₂ electrode was calibrated with 2, 5, and 10% CO₂; the composition of these gases had previously been determined with the Scholander apparatus.

Two groups of experiments were performed. 1) In six cats arterial pCO₂ was maintained constant at values between 27.6 and 38.5 mm Hg. After bicarbonate injection the surface of the cerebral hemispheres was exposed and covered with cotton soaked in warm Tyrode solution. Two to three hours after the DMO and bicarbonate injections the first cortical biopsies (avg wt 23 mg) were taken from midlateral, midsuprasylvian, or midectosylvian gyri. Bleeding was controlled with Oxycel. An arterial blood sample was drawn for determination of pH, pCO₂, and plasma DMO. Subsequent biopsies and blood samples were taken at 30- to 60-min intervals. Sometimes ventilation was changed between sampling by resetting the respiratory rate. Blood pressure ranged between 105 and 125 mm Hg. A final arterial blood sample was taken after arterial pCO₂ had been stabilized at 39–80 mm Hg for 1–5 hr. The animals were then killed by decapitation and samples were taken from cerebellum, medullary portion of the floor of the fourth ventricle, including the region of the obex, inferior, and sometimes superior colliculus, occipital, parietal, temporal, and frontal cerebral cortex, caudate nucleus, and cerebral white matter. Weight of all samples ranged between 13 and 102 mg (avg 42 mg). Seven to seventeen samples were taken from each brain.

2) From 43 cats only postmortem samples were taken. These animals received heparin, 400 U/kg. a) Thirty-one cats received NaHCO₃ approximately 1 hr after
DMO administration, and were sacrificed 2–5 hr (avg 3.4 hr) later. Arterial pCO₂ was maintained constant to within 4 mm Hg for an average of 3.5 hr before death, at values varying from 14.5 to 83.9 mm Hg. Arterial pH did not vary by more than .04 units. The average pH during the last 3.5 hr was used for computation of pHi.

b) Two cats were maintained at arterial pCO₂ = 40 mm Hg for 1.5–3 hr after DMO and bicarbonate injection. Ventilation was then increased; the resulting arterial pCO₂ = 14.7 and 15.1 mm Hg was maintained for 0.5 hr before sacrifice. c) Two cats were treated in a similar way, but final arterial pCO₂ was maintained at 65 and 78 mm Hg. d) In two cats ureteral ligation and DMO and bicarbonate injection were carried out on the preceding day. The animals were then allowed to recover from the anesthetic, given free access to water, and studied on the next day in the usual way at arterial pCO₂ of 39 and 40.7 mm Hg. Blood buffer base on the date of study was 47 and 48 mEq/liter, respectively. Considerably more bicarbonate was required to accomplish this level than when all procedures were carried out immediately preceding equilibration. e) Eight cats, equilibrated at various CO₂ tensions for 3–4.5 hr, did not receive NaHCO₃. Blood buffer base ranged between 44–52 mEq/liter. Blood buffer base at time of biopsies at the highest pCO₂ (75–80 mm Hg) was about 36 mEq/liter; that at time of all other procedures was 47 and 48 mEq/liter, respectively. Considerably more bicarbonate was required to accomplish this level than when all procedures were carried out immediately preceding equilibration.

### Processing of Brain Samples

The lightly blotted samples were placed in tared 12-ml Pyrex tubes which were immediately capped. The tubes were weighed to the nearest .01 mg. The samples were dried for 24 hr at 2.3 cm Hg and room temperature. The tubes were then reweighed, the dried samples ground with 2–4 ml of distilled water, and weighing was repeated. After centrifugation 0.5 ml of the clear supernatant fluid was pipetted into counting vials which contained 15 ml of a scintillation mixture composed of 5 g PPO 2.5 diphenyloxazole, 100 mg POPOP 1.4 bis 2 (phenyloxazolyl)-benzene, diluted to 2 liters with 1,170 ml toluene an 890 ml ethyl alcohol.

### Counting

The vials were counted in a liquid-scintillation counter for 30–100 min; total count was 9,000–120,000. Distilled water, 0.5 ml, in 15 ml of the scintillation mixture served for background counting; background was 2 ± 10% of total count. Counting efficiency was 95%. Results were expressed as counts per minute per gram brain water.

### Processing of Blood and CSF for DMO Determinations

Aliquots of blood, 0.5–2 ml, were centrifuged under mineral oil. The activity of 0.5–1 ml diluted aliquots of the plasma was measured as described above. CSF was directly diluted and counted. Results were expressed as counts per minute per gram plasma or CSF water. Fractional water contents of plasma and CSF were taken, respectively, as .93 and .99.

### Accuracy of Procedures

**Vacuum drying.** Seventeen vacuum-dried brain samples were heated at 83–85 °C for 16–17 hr. Heat drying re-

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**TABLE 1.** Measured parameters of arterial blood, CSF, and brain tissue, used for computation of pHi of postmortem brain samples

<table>
<thead>
<tr>
<th>pH Arterial</th>
<th>pH CSF</th>
<th>pH CSF</th>
<th>pH CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.63–7.74 (12)</td>
<td>2.18–2.9 (10)</td>
<td>7.49–7.55 (7)</td>
<td>.460–.560 (12)</td>
</tr>
<tr>
<td>7.44–7.57 (4)</td>
<td>3.9–5.5 (4)</td>
<td>7.34–7.44 (7)</td>
<td>.533–.665 (12)</td>
</tr>
<tr>
<td>7.37–7.44 (10)</td>
<td>4.9–5.5 (7)</td>
<td>7.30–7.39 (7)</td>
<td>.573–.681 (10)</td>
</tr>
<tr>
<td>7.21–7.30 (6)</td>
<td>61 (1)</td>
<td>7.28 (1)</td>
<td>.554–.706 (6)</td>
</tr>
<tr>
<td>7.17–7.23 (6)</td>
<td>7.19–7.24 (4)</td>
<td>621–.734 (6)</td>
<td></td>
</tr>
</tbody>
</table>

1 mg NaHCO₃ (avg 3.4 mEq/kg) was given to all cats. Blood buffer base 44–52 mEq/liter. Number of cats in parentheses.

* Ratio of DMO concentration in brain water (Cᵦ) over that in arterial plasma water (Cₑ). Each value of Cᵦ is the average of measurements on 7–23 brain samples.
TABLE 2. Regional intracellular pH

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Art. pH</th>
<th>Art. pCO₂</th>
<th>Art. pCO₂</th>
<th>Art. pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>7.17±0.05 (51)</td>
<td>7.15±0.004 (44)</td>
<td>7.02±0.004 (17)</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>7.17±0.009 (40)</td>
<td>7.13±0.004 (12)</td>
<td>7.02±0.007 (8)</td>
<td></td>
</tr>
<tr>
<td>Floor 4th ventricle</td>
<td>7.26±0.006 (24)</td>
<td>7.13±0.006 (19)</td>
<td>7.02±0.015 (9)</td>
<td></td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>7.24±0.007 (20)</td>
<td>7.13±0.006 (14)</td>
<td>7.02±0.008 (8)</td>
<td></td>
</tr>
<tr>
<td>Cerebral white matter</td>
<td>7.23±0.008 (22)</td>
<td>7.11±0.010 (13)</td>
<td>7.02±0.013 (8)</td>
<td></td>
</tr>
</tbody>
</table>

W/W₁ = .045, pH₁ = pH₂. All cats had received NaHCO₃. Blood buffer base 44-52 mEq/liter. Values are given ± se. Number of samples given in parentheses.

Results in an additional water loss amounting to 0-4.5% (avg 2.5%) of the loss after evacuation alone (10-10% of DMO was lost during heat drying).

DMO loss during vacuum drying. DMO loss from 22 µl aliquots of DMO-C₁⁴ in water on vacuum drying amounted to 1.7-4% (avg 2.5%).

The effects of these two errors on DMO concentration in tissue water balance, so that no correction was necessary.

Effect of CO₂ loss from blood during centrifugation on plasma DMO concentration. This can be computed from the relation between plasma pH and red cell pH (11) and from the equation relating the pH in the two phases to DMO distribution (ref. 53, see below). At hematocrit = 40 a fall in pCO₂ of 20 mm Hg from a value of 40-80 mm Hg would lead to an increase in plasma DMO of about 2%. No significant difference in plasma activity was observed when CO₂ escape during centrifugation of blood was prevented by completely filling and capping the tube.

Effect of temperature fall of blood after withdrawal on plasma DMO concentration. At pCO₂ = 40 mm Hg, plasma pH = 7.40, red cell pH = 7.19, and hematocrit = 40, it can be calculated that plasma DMO concentration rises by only 1.5% when temperature is lowered from 37 to 25 C.

Protein binding of DMO. About half of the cats received 80 mg/kg of untagged DMO, in addition to the usual radioactive material. Under comparable conditions of arterial pH and pCO₂ there were no systematic differences in pH₁ between the two groups.

Calculation of Intracellular pH (pHi)

pHi was calculated from the equation derived by Waddell and Butler (53). This equation expresses pH₁ in terms of pK of DMO (6.13), extracellular pH (pHe), ratio of DMO concentration (count/min per g) in brain water over that in extracellular water (C₁/Cₑ), and weight ratio of extracellular over intracellular water (Wₑ/W₁). Three assumptions are implicit in the equation: a) the aqueous phases of extracellular and intracellular fluid are homogeneous; b) the concentrations of the undissociated form of the weak acid DMO are equal in extracellular water and intracellular water; and c) the dissociation exponent, pK₁, of DMO is equal in the two aqueous phases.

Two different assumed values for Wₑ/W₁, namely, .045 and .29, were substituted, and four separate calculations were carried out at each value of Wₑ/W₁, taking for extracellular DMO concentration and for extracellular pH, respectively, DMO concentration and pH in arterial plasma, CSF, cerebral venous plasma, and mean cerebral capillary plasma. Since cerebral venous blood was not sampled, venous pH was derived from arterial pCO₂ and pH and from CSF pCO₂, assuming the latter to be equal to venous pCO₂ (3, 28). The following relation was used (47): Δ log pCO₂/Δ pH = 1.4, where Δ log pCO₂ = difference between the logarithm of arterial and venous (CSF) pCO₂, and Δ pH = difference between arterial and venous pH. In order to account for venous oxygen desaturation, 0.01 pH unit was added to the pH thus derived. Mean capillary pCO₂ was taken to lie halfway between arterial and venous pCO₂ (17). Mean capillary pH was then obtained in a way similar to venous pH. Total DMO concentrations in venous and capillary plasma water were considered to be equal to the concentration in arterial plasma water, a corollary of the assumed equilibrium state of DMO distribution between blood and tissue at the time of sampling.

The rationale of the various assumptions will be examined in the discussion.

Calculation of Intracellular Bicarbonate Concentration

Bicarbonate concentration in intracellular water was derived from pCO₂ and pH₁ by means of the Henderson-Hasselbalch equation (solubility factor of CO₂ in brain water = .03136 mmole/kg per mm Hg (44), pK' of carbonic acid in brain = 6.12 (44)). Wₑ/W₁ was taken as .045. Four sets of values for pH₁ and pCO₂ were used, based on the four possible compositions of extracellular fluid. Intracellular pCO₂ was assumed to equal extracellular pCO₂, with one exception: at extracellular fluid = mean capillary plasma, intracellular pCO₂ was taken to be 1 mm Hg higher than extracellular pCO₂ (17).

4 It must be admitted that recent work by Ames et al. (1) casts doubt on the validity of assigning one single value to the ionic composition of CSF, since the fluid apparently undergoes exchange with the ventricular walls as it moves from the choroid plexus to the cisterna.
Buffering Capacity of Intracellular Space

Two measures of intracellular buffering capacity were derived from the data. 1) The change in the logarithm of pCO2 required to change pHi by one unit (Δ log pCO2/Δ pHi), and 2) the change in bicarbonate concentration of intracellular water required to change pHi by one unit (Δ [HCO3]ew/Δ pHi).

RESULTS

Figure 1A shows the time course of the DMO concentration in arterial plasma after intravenous injection, derived from 10 cats. The concentration 4 hr after injection is taken as unity. Arterial pCO2 was maintained constant in each cat after DMO injection. It can be seen that a constant blood DMO level is attained about 3 hr after injection. Figure 1B shows the time course of the ratio of undissociated DMO in CSF over that in arterial and in cerebral venous plasma. Each point represents a different cat. The undissociated DMO concentrations were calculated from pK of DMO (6.13) and from pH and total DMO concentration of the respective fluids. After 4 hr of equilibration the CSF/arterial ratio reaches a value of .97, the CSF/venous ratio a value of .88. From then on the ratios remain nearly unchanged for 24 hr.

Figure 2 is a semilogarithmic plot of pH of the individual cortical biopsies of six cats against arterial pH. Each symbol represents a different cat. The average pH of the postmortem samples of three of the animals is shown (arrows). For comparison, the regression line based on the postmortem samples of 37 cats (see Fig. 3) is also shown in Fig. 2. It can be seen that there is reasonable agreement between the two sets of data.

Table 1 shows the measured parameters of the cats from which only postmortem brain samples were obtained.

Table 2 tabulates pH of various regions of the brain in three groups of cats at low, intermediate, and high levels of pH. Extracellular pH was taken as equal to mean cerebral capillary pH and W/0.045. Except perhaps in the cerebral white matter at low tension, no significant regional differences in pH were found.

Figures 3 and 4 show semilogarithmic plots of pH of the individual cortical biopsies of six cats against arterial pH. Each point refers to a different cat and is the average of 7-23 measurements. Sample standard error ranged between .003 and .010 pHi units. All animals had received bicarbonate. W/0.045. The values in Fig. 3 were derived on the assumption that pH equals arterial pH (dots) and cerebral venous pH (circles); pHi is plotted against arterial pCO2 and venous pCO2, respectively. The values in Fig. 4 were derived on the assumption that pH equals mean capillary pH (dots) and CSF pH (circles); pHi is plotted here against mean tissue pCO2 (taken to be 1 mm Hg higher than mean capillary pCO2) and CSF pCO2, respectively. The variations in pHi, due to the differences in assumed extracellular composition, can be seen to be greatest at low CO2 tensions: at pCO2 = 15 the values range between 7.29 (extracellular fluid = venous plasma) and 7.43 (extracellular fluid = CSF). The linear regressions of each of the four relations are also indicated in Figs. 3 and 4, as well as the regression equations and the standard deviations of the regression coefficients. It can be seen that the regression coefficients of three of the four lines are quite similar. The 95% confidence belt for individual data for three of the regression lines has a width of ± 0.6 pH units; the belt for the line based on equality of CSF and extracellular fluid has a width of ± 0.8 pH units. The belt for one of the regression lines is shown in Fig. 4.

At comparable values for arterial pCO2 and pH, the values for pHi are not affected by the length of time
which elapsed between bicarbonate injection and death (2–5 hr). The values for pH i of the two cats which were hyperventilated for only 0.5 hr, of the two cats which were hyperventilated for 0.5 hr, and of the two animals which had received DMO and bicarbonate on the preceding day do not differ significantly from those of the main group; their points have been labeled, respectively, a, b, and c, in Figs. 3 and 4.

Figure 5 illustrates the dependence of the computed pH i on \( W_i/W_t \). The upper limit of pH i, derived on the assumption of no extracellular space at all, is only .01–.02 pH units greater than the value at \( W_i/W_t = .045 \). The reduction in pH i when \( W_i/W_t \) is made to increase to .20 amounts to .10 units at \( pCO_2 = 15 \), and to .04 units at \( pCO_2 = 40 \). Thus a considerable degree of uncertainty about the exact size of the extracellular compartment will introduce only a small error in the derived pH i.

The pH i has also been calculated on the basis of \( W_i/W_t = .64 \). The average value in seven cats at mean capillary \( pCO_2 = 44.6 \) was 6.90 ± .017 (Fig. 5). The value of \( W_i/W_t = .64 \) corresponds to an extracellular volume of .30 of wet weight, which is the size of the cerebral chloride space. As will be discussed, it is improbable that this space actually corresponds to the extracellular compartment.

Figure 6 shows the data obtained from 11 cats which had received no bicarbonate. pH i of the two animals which had received DMO only 0.5 and 1 hr before death (Fig. 6, arrows) did not significantly differ from those equilibrated for several hours. For comparison the regression of pH i on log \( pCO_2 \) of the cats which had received NaHCO3 is also shown. The average difference in arterial pH of the two groups was .18 units, that between the adjusted mean pH i was .08 units. Covariance analysis shows this difference in pH i to be highly significant \( (P < .001) \).

The relation between bicarbonate concentration of intracellular water and pH i is shown in Fig. 7. \( W_i/W_t \) was taken as .045. The four lines are predicated on the four different assumed compositions of extracellular fluid. The individual values based on extracellular fluid = mean capillary plasma are plotted. The degree of scatter around the regression lines is greater than in the plots of pH i against log \( pCO_2 \) (Figs. 3 and 4), because any error in pH i will result in an error in bicarbonate concentration in the same direction. The regression equations and the standard deviations of the regression coefficients are also given in Fig. 7.

The relation between total \( CO_2 \) concentration of the cerebral cortex and \( pCO_2 \) (\( CO_2 \) dissociation curve) is shown in Fig. 8. The four curves are based on assumed equality of composition of extracellular fluid to either arterial, mean capillary, or venous blood, or CSF. The abscissa represents either arterial, tissue (1 mm Hg above mean capillary), or cerebral venous (CSF) \( pCO_2 \). The individual data based on extracellular fluid = mean capillary plasma have been plotted. When composition of extracellular fluid is taken to equal that of mean capillary plasma, the slope of the dissociation curve between \( pCO_2 = 50 \) and 50 mm Hg amounts to .33 ml \( CO_2 \)/100 g cortical tissue per mm Hg tissue \( pCO_2 \).

The total \( CO_2 \) concentration of the tissue is approximately proportional to its water content. As the average fractional water content of cerebral cortex was .75, that of white matter .64, the slope of the dissociation curve between \( pCO_2 = 50 \) and 50 mm Hg amounts to .33 ml \( CO_2 \)/100 g cortical tissue per mm Hg tissue \( pCO_2 \).


discussion of principles and methods

Equalization of Concentrations of Undissociated DMO in Extracellular and Intracellular Water

In spite of the numerous applications of the DMO method, the basic assumption, that in the steady state the undissociated form of the acid is present in equal concentrations in extracellular and intracellular water, has not been critically examined, except for one attempt (21).

Theoretical considerations. Consider the steady-state distribution of a weak monovalent acid which has been added to a system of cells and extracellular fluid in an amount small enough to be without effect on either H+ ion concentrations or membrane voltage. Transmembrane movements of both members of the conjugate pair, HA and A−, are assumed to be purely passive.

The net transmembrane flux of the undissociated form of the acid per unit membrane surface equals

\[
P_{HA} ([HA]_o - [HA]_i),
\]

where \( P_{HA} \) is permeability constant (cm sec⁻¹), assumed to be the same in both directions, and \([HA]_o \) and \([HA]_i \) = undissociated acid concentrations in the water of the exterior and interior of the cell, respectively.
The net transmembrane flux of the ion, $A^-$, per unit membrane surface under the influence of the electrochemical gradient can be expressed by employing the constant field treatment (18). This flux equals

$$P_A \frac{FV}{RT} \frac{[A]_e - [A]_i}{[H]_i} \exp - \frac{FV}{RT}$$

where $P_A$ is permeability constant ($cm sec^{-1}$), $F$ = Faraday's constant, $V$ = membrane voltage, $T$ = absolute temperature, $R$ = gas constant, and $[A]_e$ and $[A]_i$ = ionic concentrations in the water of the exterior and interior, respectively.

In the steady state the sum of the two fluxes is zero. After expressing $[A]$ into $[HA]$, $[HI]$, and $K$ (dissociation constant), the following steady-state relation is obtained:

$$P_{HA} + \frac{FV}{RT} \frac{K_P}{[H]_i}$$

$$P_{HA} - \frac{FV}{RT} \frac{KP_A}{[H]_i}$$

Either when $P_A = 0$, or when $[HI]/[HA]_i = \exp - VF/RT$, $[HA]_i - [HA]_e$. It should be noted that for the second condition, which states that the $H^+$ ions must be distributed according to the Donnan equilibrium, the magnitudes of the two permeability constants are immaterial. When $P_{HA} = 0$, $[HI]/[HA]_i = \exp VF/RT$, or $\ln ([HA]/[HA]_i) = VF/RT$; i.e., the distribution of the dissociated form is of the Donnan type. When $V$ approaches 0, $K_P$ is equal to mean capillary $pCO_2$. Upper regression line (after NaHCO$_3$) is the same as line labeled "art." in Fig. 9. Individual data from which lower regression line (without NaHCO$_3$) was derived are shown. Arrows indicate results on two cats which had received DMO only 0.5 and 1 hr, respectively, before death.

Experiment at five different values for membrane voltage. When extracellular $II^+$ ion and total DMO concentrations were taken as equal to those in mean capillary plasma water, $pHe$ was 7.40, $C_i/C_e$, 615. The 4-hr period of exposure to DMO was found to be sufficient for reaching the steady state (see below). Details of the calculations are given in the legend to Fig. 9. Both $[HA]/[HA]_i$ and $pHi$ have been plotted against $P_{HA}/PA$. Figure 9 shows that when $P_{HA}/PA$ is taken as 0.50, $[HA]/[HA]_i$ deviates by 8% or less from unity, the greatest deviation occurring at the highest membrane voltage. At this permeability ratio $pHi$ at the different voltages can be seen to vary by only 0.05 unit. If the actual permeability ratio were as low as 0.50, the assumption of $P_{HA}/PA = \infty$ (that is, $[HA]/[HA]_i = 1$) would introduce a maximal error in the derived $pHi$ of only 0.04 unit. Figure 9 also shows that at membrane voltage = 14.4 mv both $[HA]/[HA]_i$ and derived $pHi$ (- 7.16) are independent of the permeability ratio. Thus at this voltage the $H^+$ ions are distributed according to the Donnan equilibrium, as are, of course, the $A^-$ ions. Comparable conclusions can be drawn at other values for $pHe$ and $C_i/C_e$ (Table 3).

By analogy with other weak acids (and bases) (41a) the permeability of the cell membrane to ionized DMO can be expected to be very much less than that to the undisassociated acid. Additional evidence for this is provided by the fact that the renal clearance of DMO with alkaline urine is as much as 110 times that with acid urine at moderate urine flow (52). A ratio of permeabilities of 500 seems therefore not to be unreasonable.  

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**Fig. 6.** Effect of administration of 1 n NaHCO$_3$ solution (avg dose 3.5 mEq/kg) on $pHi$. $W/W_i$ was taken as 0.45, $pHe$ to be equal to arterial $pHi$. Upper regression line (after NaHCO$_3$) is the same as line labeled "art." in Fig. 9. Individual data from which lower regression line (without NaHCO$_3$) was derived are shown. Arrows indicate results on two cats which had received DMO only 0.5 and 1 hr, respectively, before death.

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6 Waddell and Butler (52) quote a maximal clearance ratio of 250. However, the two clearances were obtained at widely different urine flows, and thus are not comparable.

7 It is not possible to derive from the clearance data the exact permeability ratio of DMO for the tubular cells, nor even to set a lower limit to this ratio, as has been attempted (41). In both alka-
which regression line, based on equality of pHe and mean capillary would have to be only about 300 for this degree of accuracy of pHi measurement (see Fig. 9); this is quite acceptable. As the average cerebral membrane voltage is probably about 70 mv, the permeability ratio indicates failure of DMO equilibration between urine and blood. The transtubular voltage difference adds another complication.

The individual data refer to the cap line and acid urine, clearance increased and U/P ratio decreased with urine flow (52). Even at the highest flow measured, U/P in alkaline urine greatly exceeded the maximal theoretical steady-state ratio, namely, that which would be observed if the (distal) tubular cell were impermeable to the ionized form of DMO. This indicates failure of DMO equilibration between urine and blood. The transtubular voltage difference adds another complication.

Miller et al. (29) determined pH of incubated excised rat diaphragm simultaneously by means of the DMO method and the CO2 method. At pCO2 = 104 mm Hg in the medium, the CO2 method yielded a value for pH = 7.55, the DMO method 7.10; at pCO2 = 39 the values were, respectively, 7.03 and 6.89; at pCO2 = 105.5 the values were 6.72 with both methods. These results show a rather considerable discrepancy. It seems probable that, particularly at low pCO2, pH of the extracellular fluid of the samples was significantly lower than that of the medium, and pCO2 significantly higher, due to tissue release of CO2 and lactic acid. Such pH and p(CO2) gradients have also been found by Caldwell (8). The values for pH derived from pH of the medium would then be too high for both methods. DMO may also have failed to equilibrate between medium and extracellular fluid. This would lead to too low a value for pH derived from DMO. We must conclude that no experimental proof of concentration equalization of undissociated DMO is as yet available.

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taken as .045, pHe = pH cap, brain water = .72 of wet brain (42), and CSF = .09 of total mass (42). In Fig. 10 both the values for composite pH derived from Ponten's data, and our own, have been plotted against tissue pCO2. It can be seen that at tissue pCO2 = 40 mm Hg the two studies give identical values. At pCO2 = 80 our results are on the average .058 pH unit higher than those of Ponten, at pCO2 = 90, .07 pH unit less. These differences will be discussed below.

Uniformity of Intracellular H Ion Concentration

This assumption is almost certainly not correct. Under conditions of intracellular inhomogeneity, the methods employing weak acids (such as DMO) lead to a value of mean intracellular [OH-], rather than [H+], provided the undissociated form of the acid is evenly distributed within the cell at a concentration equal to that in extracellular fluid (7). Although the CO2 method (36) was found to yield results closely similar to our own, the dissociation constants of DMO and carbonic acid are so similar that this cannot give assurance that mean intracellular [H+] actually has been measured by either method.

It must be added that undissociated DMO might not be homogeneously distributed throughout the cells. Its concentration in capillary plasma must increase from artery to vein, and with it, presumably, its concentration in neighboring tissue water. Also, the undissociated DMO distribution across closed intracellular boundaries might deviate from unity, depending on the nature of the boundaries.

Dissociation Constant of DMO

pK of DMO is assumed to have the same value in intracellular water as in extracellular water. This has not been studied; the assumption seems reasonable in view of the fact that pK of carbonic acid, which has nearly the same value, is unaffected by the presence of brain homogenates (44).

Size of Extracellular Space

Ideally this space should be defined as the volume in which the concentrations of both undissociated and dissociated DMO are identical with those in blood (or perhaps CSF). It is possible to set an upper limit to the relative size of the "DMO space" in the following manner. At two levels of extracellular pH (pHe1 and pHe2), the extracellular (C1 or C2) and tissue (C11 and C12)

\[ \text{W}/\text{W} = \left( \frac{1}{(a - 1)/(aR_2 - R_1)} - 1 \right) \]

where a = (10pHe2 - pK + 1)/(10pHe1 - pK + 1); R1 = C11/C1; R2 = C12/C11. If pH in actually increased somewhat by the addition of base (or decreased by the addition of acid), the actual size of the extracellular space would be less than that derived from this equation. Thus the equation gives the upper limit of the extracellular DMO space. The practical difficulty of this method is that it is extremely sensitive to the measured parameters, so that the error is considerable.

We have applied the equation to two groups of experiments, rather than to a single study. One group (seven cats, mean arterial pH = 7.40) had been injected with an average of 3.3 mEq/kg of 1 n NaHCO3 solution; this amount of hypertonic solution should have a negligible effect on cell volume (48). The second group (four cats, mean arterial pH = 7.48) had received no bicarbonate. Arterial pCO2 of both groups was about 40 mm Hg. Extracellular pH was taken as equal to mean capillary pH. The values for W/Wi calculated for each of the 28 combinations ranged from .05 to .30. Though no great significance should be attached to the exact magnitude of these results, they are of interest in that the lower values of thier range are of the same order as the values based on the distribution of substances which do not penetrate the cell membrane.
The chloride concentration of myelin would have to be similar to that of the intracellular space. No data on this point are available.

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A number of workers have measured the cerebral concentrations of sulfate (2, 3), p-aminohippuric acid (10), sucrose (10, 40), iodide (40), and inulin (40) after intravenous, intraperitoneal, or intrathecal (30) injection. The size of the spaces thus determined ranged between 0.01 and 0.06 of brain weight; no significant regional differences were found (2). We therefore used a value of 0.03 to derive the ratio of extracellular over intracellular water, W_e/W_i, which was found to be 0.042 for gray matter (total water content 0.75) and 0.051 for white matter (total water content 0.64). Since these small differences have an insignificant effect on the derived pHi (Fig. 5), an average value of 0.045 for all brain regions was used for one set of calculations. The omission of a correction for blood content (1 %, ref. 40) hardly affects the derived pHi (Fig. 5).

Somewhat larger spaces (0.08–0.12) were found by Rall et al. (39) after ventricular perfusion with inulin, dextran, or sulfate. An extracellular space of 0.12 was used to derive a second ratio W_e/W_i, which amounted to 0.19 for gray matter and 0.23 for white matter. An average value of 0.20 was employed for a second set of calculations of pHi.

The assumption of a small extracellular space implies that the derived pHi represents more or less an average of the pH of neurons and neuroglia. No significant differences in pHi were found to exist between gray and white matter (Table 9). As the glial elements in gray matter occupy much more space than in white matter,
it is probable that pHi is closely similar for both neurons and neuroglia.

### DISCUSSION OF RESULTS

#### DMO Concentration in CSF

The steady-state ratio $\left[ \text{HA} \right]_{\text{CSF}} / \left[ \text{HA} \right]_{\text{gap}}$ was on the average .91 at all values for arterial $pCO_{2}$, with significant scatter (Fig. 1B). At low $pCO_{2}$ the electrical potential of CSF with respect to blood must have been slightly negative (19). As pHCSF was only a few .01 pH units lower than $pH_{\text{cap}}$, the ratio of dissociated DMO should theoretically lie within the narrow range of .93-.1.0, the exact value depending on $P_{\text{HA}}/P_{\text{A}}$. At high $pCO_{2}$, CSF potential must have been positive (19), in the two phases was nearly identical. Therefore $\left[ \text{HA} \right]_{\text{CSF}} / \left[ \text{HA} \right]_{\text{gap}}$ should have been 1 or somewhat greater (see Fig. 9). The differences between the observed and theoretical values could have been due to experimental error, but active participation of the blood-CSF barrier cannot be excluded.

#### Intracellular pH

As has been discussed, the computed value for pHi is determined by the choice both of composition and of size of the extracellular compartment. It would seem most reasonable to assume an extracellular space of the order of 3% of brain weight ($W_{e}/W_{i} = .045$), with a composition resembling that of mean capillary plasma. At arterial $pCO_{2} = 38-44$ (arterial pH = 7.39, mean capillary $pCO_{2} = 44.6$), this would lead to a value for pH of $pHi = 7.13 \pm .008$ (N = 7); at arterial $pCO_{2} = 14.5$ \textit{atp} (arterial pH = 7.68, mean capillary $pCO_{2} = 21.1$) to pH = $7.26 \pm .01$ (N = 10); at arterial $pCO_{2} = 77.9 \textit{atp}$ (arterial pH = 7.21, mean capillary $pCO_{2} = 79.5$) to pH = $7.09 \pm .007$ (N = 4), with only minor regional differences (Table 2).

Nicholls (33) determined total CO2 content of the rat brain at arterial $pCO_{2} = 36.9$ and pH = 7.47. From these data and with an extracellular space of 3% we derived a value for pHi = 7.23. Similar experiments have been performed on rat cerebral cortex by Brodie and Woodbury (4) and by Koch and Woodbury (25). The former workers sampled "heart blood" ($pCO_{2} = 39$, pH = 7.41), the latter, blood from the inferior vena cava in three groups of studies ($pCO_{2} = 34.9$, 38.5, 38.2, pH 7.46, 7.46, 7.47, respectively). pHi derived as described amounted to 7.23, 7.32, 7.31, and 7.34, respectively. These values are about .15 units higher than our results. It is possible, as Ponten and Siesjo have suggested (37), that tissue accumulation of CO2 immediately after death may be responsible for at least part of the difference.

Kibler et al. (24), using the sulfate space as a measure of extracellular volume, determined pHii in the dog brain by means of DMO at arterial $pCO_{2} = 12-34$ and 70-91 mm Hg. Their data indicate that pHii would be 6.92 at arterial $pCO_{2} = 39$. This value is significantly lower than that found by us, namely, 7.12 under similar conditions of blood acidity (blood buffer base 37 meq/liter, Fig. 6). It must be added that the data of Kibler et al. show a great deal of scatter (width of 95% confidence band ± .20 pH units).

A few data on pHii at high CO2 tensions are available. The results of Koch and Woodbury (25) (pHi = 7.17 at arterial $pCO_{2} = 68.8$ and pH = 7.44) are somewhat higher than ours, those of Kibler et al. (24) considerably lower.

As mentioned above, our pHii data differ slightly from those of Ponten (36) at the extremes of $pCO_{2}$ (Fig. 10). Siesjo (46) found an increase in CO2 content of the brain between the 1st and 3rd hr of exposure to 8% CO2. As our length of exposure was considerably longer than Ponten's, this might explain the difference in pHii at high $pCO_{2}$. An effect in the opposite direction might account for the discrepancy at low $pCO_{2}$. However, we found no difference in pHii between two cats, hyperventilated for only .5 hr before death, and the main group (c in Fig. 3). The pHii values of two cats hyperventilated for .5 hr were at the upper limit of those of the main group (a in Figs. 3 and 4). The final .5-hr period should have been sufficient for DMO equilibration at the new $pCO_{2}$, since we have demonstrated that DMO administration only .5 hr before death results in a value for pHii similar to that after 4 hr of equilibration at the same $pCO_{2}$ (Fig. 6). Therefore the differences in pHii between Ponten's and our data cannot be explained by differences in equilibration time.

Passing mention should be made of measurements on brain tissue homogenates equilibrated at various CO2 tensions. Such methods have serious drawbacks because of possible metabolic changes in the suspension, and also because the homogenate must be diluted in order to make equilibration possible. By extrapolation from the pH values obtained on suspensions diluted to various extents, Siesjo (44) obtained for the undiluted brain suspension a value of pH = 7.03 at $pCO_{2} = 40$ mm Hg. Kazemi and Mithoefer (22) attempted to derive the cere-
A. ROOS

7.20
6.90

I I I I IIlll J
30 40 50 60 70 80 90 100

FIG. 10. Relation between “composite” pH of entire skull content and tissue pCO₂. O, Data of Ponten (36); ●, our data, recalculated to yield the same information.

Thus the buffering power of brain cells is superior to that of these other tissues.

The only previous systematic work on brain buffering power is that of Ponten (36), whose data from the rat pertain to the entire supratentorial skull content including CSF. His value of 1.27 compares with ours of 1.75 computed for the same structures (Fig. 10).

A second measure of intracellular buffering power is the change in bicarbonate concentration of cell water per unit change in pH (Δ[HCO₃]extr/ΔpHi). At an assumed extracellular composition equal to that of mean capillary plasma and Wₑ/Wᵢ = 0.45, this buffer index has a value of 36.7 (Fig. 7). If bicarbonate concentration is expressed per kilogram intracellular mass, rather than per kilogram cell water, the index will, of course, be dependent on water content of the tissue. Its value for cerebral cortex then becomes 27.1, for white matter 22.4, and for the entire brain 26.0. These values compare with about 28 for true plasma, 5 for frog skeletal muscle and sciatic nerve (14), and 3.3 for frog heart (5). The values again indicate the great resistance to changes in pH of the intracellular milieu of the brain.

It should be understood that the buffering power, as expressed by either of the two ratios, includes the net effect of any movement of ions across the cell membrane in response to changes in CO₂ tension. If only CO₂ molecules entered or left the cell, the buffer ratio,
Δ [HCO₃⁻]/Δ pH, would be a quantitative index of the buffering capacity of all intracellular buffers other than bicarbonate.

Very little is known about ionic flux across brain cell membranes with changes in acidity. The rise of pH in increasing external bicarbonate concentration (Fig. 6) indicates that some bicarbonate ions are able to enter the cell. As was pointed out by Caldwell (8), even if H or OH ions could penetrate the membrane with relative ease, pH would hardly be affected by such movements within the period of observation (hours or days) because of the magnitude of intracellular buffering. Nichols (33) and Siesjo (46) observed an increase in brain CO₂ (bicarbonate) concentration during the first 1-3 hr of exposure to high pCO₂, but we failed to find significant changes in pH and intracellular bicarbonate concentration between 0.5 and 4 hr of exposure to either high or low CO₂ tensions (Figs. 3 and 4). Woodbury et al. (58) found that exposure of rats to 50 % CO₂ for 15 min hardly changed the cortical sodium content in the face of a 0.5 % rise in plasma sodium. Since they assumed an extracellular space of 25 %, based on chloride content, they interpreted their findings as a 40 % fall in intracellular sodium, from 9.6 to 5.8 mM/kg intracellular water. Such a conclusion must be seriously questioned in view of the improbability that the chloride space represents the extracellular compartment. The same workers also found a small loss in cortical potassium, whereas plasma potassium increased.

The slope of the CO₂ dissociation curve (Fig. 8) in the range of tissue pCO₂ = 30-50 mm Hg amounted to 0.33 ml CO₂/100 g cortical tissue per 1 mm Hg pCO₂. This value is somewhat greater than that of lung tissue (45, ref. 12), and considerably more than that of frog skeletal muscle and sciatic nerve (19, ref. 14) and frog heart (17, ref. 5). According to Farhi and Rahn (13) the slope of the curve of the combined tissues of the dog is 1.12, which is only one-third of that of the cat brain.

Recent evidence has been brought forth to indicate that the activity of the medullary neurones, which are concerned with the regulation of ventilation, is determined both by the pH of the blood and by that of the CSF (31, 35). It would seem probable that the activity of these structures is affected by the H⁺ ion concentration of their interior, as well as by that of the fluid surrounding them. The work reported in this paper may contribute, by its definition of the relation between extracellular and intracellular pH, pCO₂, and bicarbonate concentrations, to a better understanding of the operating conditions of these cells.

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