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CORTICAL pH AND THE BLOOD–BRAIN BARRIER

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The stability of the cerebrospinal fluid (c.s.f.) pH in metabolic acidosis and alkalosis has led to the postulate of a barrier to HCO_3^- (Gesell & Hertzman, 1926; Davson, 1956). Much of the evidence for pH stability is controversial. Although Gesell & Hertzman (1926) demonstrated by direct recording an immediate acidic c.s.f. response to intravenous $NaHCO_3$, an acidic response was not found on the cortical surface of several mammals (Dusser de Barenne, McCulloch & Nims, 1937; Jasper & Erickson, 1941; Meyer & Gotoh, 1961), except in one case (Rapoport & Marshall, 1961). The c.s.f. has been reported to become alkaline in 15 min–1 hr after intravenous $NaHCO_3$ (De Bersaques, 1955) or, on the other hand, to become acidic (Robin, Whaley, Crump, Bickelman & Travis, 1958; Holmdahl, Nahas, Hassam & Versoky, 1961).

The pH reaction of the c.s.f. to administration of NH_4Cl is also not clear. An acidic change was reported by some authors (De Thurzo & Katzenelbogen, 1935; De Bersaques, 1955), an alkaline one by others (Winterstein & Göhkan, 1953; Robin *et al.* 1958). Alkalinization was ascribed to free diffusion of NH_3 but restricted diffusion of NH_4^+ from the blood into the c.s.f. (Winterstein & Göhkan, 1953), or to over-ventilation and blowing off of CO_2 (Robin *et al.*).

These experiments were designed to evaluate the above discrepancies and to consider the relation between the pH of blood and of brain (or c.s.f.) in terms of the concept of a blood–brain (or blood–c.s.f.) barrier.

METHODS

Cats weighing 2–3·5 kg were anaesthetized with sodium pentobarbitone (30 mg/kg intraperitoneal and 6–8 mg/kg intraperitoneal/hr), given a relaxant (Flaxedil, gallamine triethiodide; American Cyanamid, 4 mg/kg I.V. and 2 mg/kg I.V. as needed) and placed on artificial respiration. A gas mixture of 98 % O_2 and 2 % (v/v) CO_2 was administered, unless otherwise noted. The head was fixed in a holder, the skin of the skull incised, arranged to form a cup for liquid, and the bone was removed over the parietal cortex to expose the dura. A $2 \times 4\ mm^2$ area of dura was removed. The electrodes (a glass electrode juxtaposed to a pore electrode), their weight just balanced by a counterweight, were placed flatly on the exposed pia-arachnoid membranes. The skin cup then was filled with light mineral oil at 37° C. A new

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area of the cortex was studied by removing the dura over it and placing the electrodes on it.

In some experiments a filter-paper pledget (2×4 mm^2), saturated with *n*-butanol, was applied to a cortical region for 10 min. The electrodes were used on the region before and after *n*-butanol application.

After a steady recording base line had been attained for both electrodes (see below), solutions were injected rapidly (in less than 3 sec, unless otherwise noted) into a canulated femoral vein. $NaHCO_3$ solutions were freshly prepared, and not equilibrated with CO_2.

In three kittens, each less than 1 week old, injections were made into the external jugular vein, and in two cats into the internal carotid artery in a cephalad direction. In several cats saturated aqueous solutions of trypan blue were injected in order to determine, by visual observation of the cortical surface, vein-to-cortex circulation times.

The carbonic anhydrase inhibitor acetazolamide was given I.V. pre-operatively to two cats in a dose of 500 mg/kg; after preparation, 0·9 N-$NaHCO_3$ (1 ml./kg) was injected rapidly and records were taken. In a third cat records were taken before and after (up to 3 hr) an I.V. injection of acetazolamide 500 mg/kg. In three cases experiments were done with rabbits, prepared as above.

The electrode pair. The pH electrode was constructed of a lead glass capillary tube (Corning 0120, 2·3 mm outer diam.) which was sealed on one end by a thin film of pH-sensitive glass (Corning 015), after the method of MacInnes & Dole (1929). Its active surface was less than 1 mm^2. The capillary tube was filled with buffer solution (6·7 g NaH_2PO_4, 3·4 g KH_2PO_4, 8·8 g NaCl/1000 ml.) into which a Ag-AgCl wire was sealed by epoxy cement. The capillary pore reference electrode was of similar dimensions (Marshall, 1959), with a Ag-AgCl wire lead, and was filled with 0·9 % NaCl solution. When placed together flatly on the pia-arachnoid surface, the electrode pair covered an area of about 1×2 mm^2.

The DC resistance of the pH electrode ranged from 1000 to 10,000 MΩ. The temperature coefficient for the electrode pair (+0·3–0·4 mV/°C) was determined by use of a buffer with known pH values over the range 25–45° C (Bower, Paabo & Bates, 1961). The potential difference (p.d.) between the pore electrode and a saturated KCl calomel electrode, determined in NaCl solutions of varying ionic strength, μ, gave an error estimate of 0·5 mV/0·01μ for pH recording with the electrodes at the brain surface. This error was due to the changing liquid junction potential of the pore electrode.

In four animals in which cortical temperature was monitored (with a Yellow Spring Telethermometer, Model 43 Ta and a hypodermic needle probe), under the experimental conditions, no consistent changes in temperature occurred with I.V. injection of solutions. It is probable that the oil bath acted as a temperature buffer. Moreover, amounts of NaCl equivalent to those of various salts injected during the experiment gave no apparent pH change at the cortical surface. Since changes of the order of 3 mV occurred after I.V. $NaHCO_3$ (see below), it is unlikely that they could be accounted for by changes in temperature or in ionic strength.

The pH electrode was shown to record faithfully the pH of buffer-saturated filter paper under oil. The pH electrode was used if it read between −50 and −59 mV/pH unit, in the pH range 4–8. It was calibrated with solutions of known pH before and after each experiment. The reading of the electrode pair did not change more than ±1 mV when kept in a buffer solution at pH 7 for 1 hr.

The response time to an applied step function for the electrode in the circuit described below was less than 0·1 sec. On the cortex, changes of 0·01 pH units could be recorded easily over a 1 min period.

Recording apparatus. The pH electrode was led into a single-ended, unity-gain amplifier (Bak, 1958), whose input resistance exceeded 10^{12}Ω. The amplifier output was led into a DC, AEL, Model 251-A differential amplifier whose output, in turn, was fed into an Offner, Type R, pen recorder (Fig. 1). The pore electrode led directly into a second differential

amplifier, and DC and AC (capacitor-coupled) records were recorded by the pen recorder. One end of each of the two amplifiers was earthed. The input leads to a third AEL amplifier were taken from the Bak amplifier (pH electrode) and from the cortical pore electrode, and the p.d. between these electrodes was monitored.

The animal was earthed through an indifferent pore electrode on an exposed region of its nose. The high resistance of the pH electrode and the possible effects of earth loop currents required that the indifferent electrode should be the only earth contact. Complications arose if blood and cortical pHs were measured concurrently, when unpredictable shifts of 10–60 mV occurred on the cortical pH record. Wetting, which established new contact potentials, also gave base-line shifts in the presence of earth loops.

In three animals blood pH was recorded by attaching a closed external circuit between the femoral vein and femoral artery of one leg, heparinizing the animal, and circulating

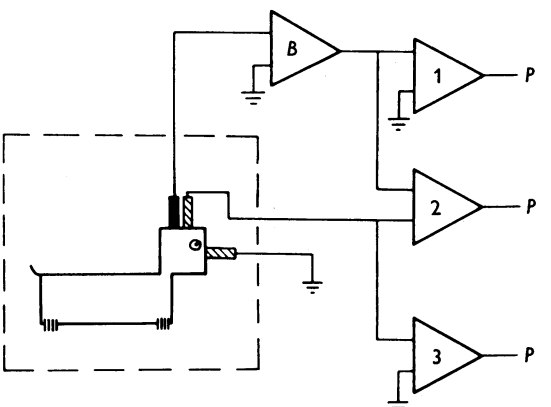

Fig. 1. pH and DC recording from the cerebral cortex. The electrode pair (the pH ▮ and the pore ▒ electrode) is on the pia-arachnoid. A grounded pore electrode is on the animal's nose. The pH electrode leads to a Bak amplifier, *B*, input resistance $> 10^{12}\Omega$. The differential amplifiers, 1, 2 and 3, lead to Offner pen recorders, *P*. They monitor the following p.d.s: (1) pH+DC p.d., (2) pH p.d. and (3) DC p.d. (approx. −55 mV/pH unit).

the blood through a glass electrode-calomel electrode pH apparatus. Because of earth-loop complications, it was convenient to measure first the pH response of the blood, then the response at the cortical surface, to identical quantities of I.V. test solution. The electrical recording system for blood pH resembled that described for recording cortical pH.

In all experiments blood pressure was recorded by a Statham transducer from a canulated femoral artery; the blood was separated from the transducer by a column of non-conducting mineral oil.

Theory of recording. The pore electrode recorded the DC p.d. (against the indifferent reference point); the pH electrode recorded the DC p.d. as well as the electrochemical p.d. due to a change of H^+ activity. For processes occurring simultaneously in the cortical area under the electrode pair, the 'true' cortical pH change, derived by subtraction of the pore electrode from the pH electrode p.d., was given by the output of the third differential amplifier.

Zero spatial and temporal gradients of the pH and DC p.d.s were demonstrated under the experimental conditions, except in some instances (reported below) when I.V. NH_4^+ salt solutions were used. Then, the third amplifier did not give the exact cortical pH change.

RESULTS

pH responses to acids, bases and CO_2

Intravenous injection (1 ml./kg) of 0·3 N solutions of lactic acid, acetic acid or HCl acidified both the blood and the cortical surface. 0·3 N Na_2CO_3, NH_4OH or NaOH produced opposite, alkaline changes. Equivalent NaCl or glucose solutions were without effect. An increase in the inspired pCO_2 acidified the cortical surface and the blood; a decrease produced alkalinization. $NaHCO_3$ (0·9 N, pH 8·3, 1 ml./kg) alkalinized the blood (to a maximum of 0·10–0·15 pH units) and transiently acidified the cortical surface

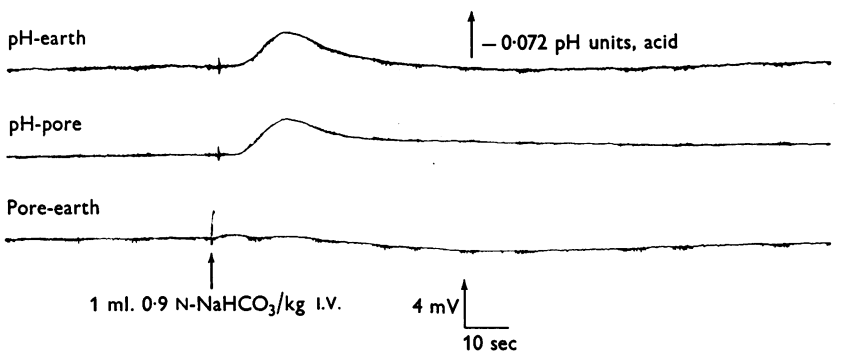

Fig. 2. The cortical pH and DC responses to I.V. $NaHCO_3$. The pH electrode-earth record gives the pH + DC p.d. The pore electrode-earth record gives the DC p.d. The pH electrode-pore electrode gives the pH p.d., −55 mV/pH unit (change in the positive direction represents an acidic response). After injection of $NaHCO_3$ (in less than 3 sec), there is an acidic response and a slower negative DC shift. e.e.g. is superimposed on all records.

(Fig. 2). In seventy-nine cats the mean maximum absolute value of the cortical acidic response to this quantity of $NaHCO_3$, for the region exposed first in the experiment, was 0·056 ± 0·020 (S.D. of an observation) pH units. The amplitude of the maximum acidic response was roughly proportional to the quantity of $NaHCO_3$, when that parameter was varied.

A cortical pH response followed the beginning of an I.V. injection of any of the above acidic or alkaline solutions by an interval of 5–7 sec. (This is the approximate femoral-vein-to-cortex circulation time as measured with trypan blue, and there was no delay after intracarotid injection.) A response reached a maximum value 5–12 sec after it began (Fig. 2). Transient B.P. changes which occurred upon injection of $NaHCO_3$ or of the other substances were not related apparently to e.e.g., DC or pH cortical changes. Higher concentrations of strong acids and bases were avoided so as to obviate significant B.P. effects.

After the peak acidic response had been reached, the pH curve returned towards the base line at a maximum rate of $2{\cdot}2 \pm 0{\cdot}2$ (S.E. of the mean) $\times 10^{-3}$ pH units/sec (140 observations). It usually levelled off 1–3 min after the peak acidic response, but often did not reach the extrapolated base line for the duration of an observation (up to 10 min). The acidic response was evoked also on the surface of the parietal cortex of the rabbit.

DC shift

After 0·9 m-mole/kg $NaHCO_3$, there was a DC shift which reached $-1{\cdot}1 \pm 0{\cdot}1$ (S.E. of mean) mV in 30–60 sec. The DC level returned to its initial value 1–3 min after this negative shift. The shift was concurrent with the return of the pH curve towards base line (Fig. 1), but its amplitude was not correlated ($P > 0{\cdot}1$) with the maximum pH change.

Negative or positive DC shifts often followed the I.V. injection of the other acidic and alkaline solutions. Electroencephalograph changes were not related to the DC shift or the pH changes, although increased e.e.g. activity has been reported in metabolic alkalosis (Dusser de Barenne *et al.* 1937). Consistent temperature changes did not occur.

Responses elsewhere

In three cats the acidic response to I.V. $NaHCO_3$ was obtained from an area of pial surface, after the arachnoid membrane had been stripped, with no obvious bleeding, under the dissecting microscope. In three kittens, each less than 1 week old, the cortical acidic response to I.V. $NaHCO_3$ did not differ qualitatively from the response in the adult cat. No clear pH response, or else a slow alkaline change, was recorded on the surface of the trapezius muscle, of a lateral thigh muscle, of the thyroid gland or of the dura.

pH responses to phosphate and ammonium salts

Table 1 summarizes results on the cortical pH response, before and after *n*-butanol treatment of the cortex, to I.V. phosphate salt solutions. It is apparent that the treatment augmented the initial pH response to a specific solution but did not change the direction of that response.

In seven cats, NH_4^+ salt solutions at different pH values were injected I.V., and the cortical responses monitored. The solutions were 0·9 M-NH_4Cl (pH 5·2), 0·9 M ammonium acetate (pH 7·1), 0·15 M diammonium citrate (pH 5·0), 0·9 M-NH_4HCO_3 (pH 7·7), 0·3 M-$NH_4H_2PO_4$ (pH 4·6) and 0·15 M-$(NH_4)_2$-HPO_4 (pH 7·9). 0·3 M and 0·9 M-NaCl were controls. These NH_4^+ solutions produced pH and DC changes that were often complex and biphasic, and not entirely reproducible. B.P. changes were complex

and longer lasting than after $NaHCO_3$ injections (in agreement with Winterstein & Göhkan, 1953).

After NH_4Cl, ammonium acetate, or $(NH_4)_2HPO_4$ injection an initial alkaline response, reaching a maximum in 10–20 sec, was followed by an acidic reversal that attained its maximum 35–60 sec after the start of injection and lasted for several minutes. Maximum changes were from 0·01 to 0·06 pH units in both directions. The pH response to NH_4HCO_3 was obviously acidic, but turned alkaline after topical application of *n*-butanol. As in the case of the acidic sodium phosphate salt solutions, *n*-butanol application to the cortex augmented the cortical acidic responses to NH_4Cl in five of six trials, and to $NH_4H_2PO_4$ in four of five trials.

TABLE 1. Effect of *n*-butanol on the cortical pH response to I.V. phosphate salt solutions

Salt	pH of solution	$\Delta\ \overline{pH}_{initial}$	$\Delta\ \overline{pH}_{treated}$	$\bar{d}$	P
NaH_2PO_4	4·2	−0·047	−0·126	−0·079	<0·01
Na_2HPO_4	9·2	0·006	0·015	0·009	<0·1
Na_3PO_4	12·2	0·073	0·195	0·123	<0·01

Eight observations on eight animals for each solution. A positive pH change is alkaline, a negative one acidic. $\Delta\ \overline{pH}_{initial}$ is the mean pH response to 1 ml./kg of a particular salt, 0·3 M, given I.V. $\Delta\ \overline{pH}_{treated}$ is the mean pH response to an identical quantity of salt after the region had been treated with *n*-butanol for 10 min. $\bar{d}$ is the mean of the paired values, $\Delta\ pH_{treated} - \Delta\ pH_{initial}$. P is the probability that $\bar{d}$ is not different from 0. The pH response to each of the salt solutions was augmented without a change of direction after treatment of the cortex with *n*-butanol.

Hypercapnia and hypoxia

CO_2 (10 % (v/v)) in O_2 was administered to three cats for periods of 30, 45 and 50 min respectively. The acidic response to a fixed test dose of I.V. $NaHCO_3$ was evoked before and during the CO_2 administration, and was not altered qualitatively. The cortical acidic response decreased qualitatively in three cats after 25 min of 20 % (v/v) CO_2 administration, and was shown to return to its initial level in two of the cats upon resumption of 98 % (v/v) O_2 administration. Thus, only the higher CO_2 concentration affected the response.

2·8 % (v/v) O_2 in N_2, administered for 10, 24, and 15 min, respectively, to three animals, extinguished the e.e.g. but did not affect the maximum acidic response, which could be evoked during the period of hypoxia. B.P. was maintained during this period.

In none of the three cats to which acetazolamide had been given, was there a qualitative alteration of the cortical acidic response to I.V. $NaHCO_3$ although the brain carbonic anhydrase probably had been almost entirely inhibited (Millichap, Woodbury & Goodman, 1955; Gray, Maren, Sisson & Smith, 1957).

DISCUSSION

The cortical acidic response to I.V. $NaHCO_3$, in 'Flaxedilized' animals under constant ventilation, is independent of respiratory mediation. Since increased respiratory excursions will occur concurrently with c.s.f. acidification in spontaneously breathing animals (Gesell & Hertzman, 1926), it is likely that the respiratory effect is a consequence of the acidification, either at a peripheral, or at a medullary level (von Euler & Söderberg, 1952). The acidic response suggests that the neuronal cells and the medullary chemosensitive cells, as well as the extracellular space of the brain, are in rapid CO_2-mediated acid-base relation with the blood. There is no reason to consider the extracellular space of the brain to be different in this respect from the c.s.f. space, as postulated by Robin *et al.* (1958).

The results do not agree with previous measurements of cortical pH, which may have been complicated by disruption of pial vasculature (Jasper & Erickson, 1941) or of cortical structure (Meyer & Gotoh, 1961). The pH electrode would have recorded from blood admixed with brain or c.s.f., and may have been affected by a demarcation potential (Marshall, 1959).

Intravenous $NaHCO_3$ will combine with blood buffers in the manner indicated by the equation

$$HCO_3^- + H^+ \rightarrow H_2CO_3 \rightarrow H_2O + \uparrow CO_2 \qquad (1)$$

Acidification of the c.s.f. will occur if the ratio of (entering HCO_3^-): (dissolved CO_2) is much less than 20:1, according to the Henderson-Hasselbalch equation. Since acidification may last for up to 1 hr in the face of excess blood HCO_3^- (Holmdahl *et al.* 1961), it is probably due to severe restriction of HCO_3^- movement from blood to brain (or to c.s.f.). It is reasonable to consider that the fluid on the arachnoid surface behaves as the subarachnoid c.s.f. with respect to CO_2, because the acidic response to $NaHCO_3$ was independent of the presence of the arachnoid membrane.

For an I.V. injection of 0·9 m-mole $NaHCO_3$/kg, the c.s.f. pH change was calculated by assuming that the injected HCO_3^- was distributed throughout the blood volume and that it reacted immediately with blood buffers (equation 1) to give CO_2 (Swanson *et al.* 1958), which in turn, determined the c.s.f. pH change. A c.s.f. pH change of $-0{\cdot}21$ to $-0{\cdot}09$ pH units was calculated for a blood pH between 7·2 and 7·6. The buffer value for c.s.f. is about that of separated plasma (Davson, 1956), i.e. $-9{\cdot}4 \times 10^{-3}$ pH units/mm Hg pCO_2 (Davenport, 1950). The mean pH change of $-0{\cdot}056$ units, smaller than the expected change, may be due to significant CO_2 loss through the lungs.

Further calculation suggested that changes in blood [H^+] do not alter c.s.f. pH under the experimental conditions, because of the small concentration gradient of H^+ between blood and c.s.f. The rate of HCO_3^- penetration into the c.s.f. or brain was estimated from values for Cl^- penetration (Woodbury, 1958), assuming a non-specific barrier. For H^+ to be as effective as HCO_3^- on c.s.f. pH, it would have to enter much faster than D_2O (Sweet, Selverstone, Soloway & Stetten, 1950), which is unlikely. A small concentration gradient is thought also to make H^+ unimportant in the regulation of muscle pH (Caldwell, 1956).

According to equation (1), blood and c.s.f. pCO_2 would decrease (pH increase) upon the addition of an alkaline solution (not containing HCO_3^-) to the blood, and pCO_2 would increase (pH decrease) upon adding an acidic solution. In the face of restricted HCO_3^- movement, all transient cortical pH responses to changes in blood pH may be mediated via pCO_2 changes alone.

Since blood pCO_2 and [HCO_3^-] changes, in the above cases, would tend to sum in their effect on c.s.f. pH, a facilitation of HCO_3^- exchange between blood and c.s.f. should augment a cortical pH response to a particular acidic or alkaline I.V. solution. This is what occurs in the experiments with acidic or alkaline phosphate salts. *n*-Butanol is thought to break down the blood–brain barrier to HCO_3^-, since it reverses the acidic response to I.V. $NaHCO_3$ (Rapoport, 1964), as it did the acidic response to NH_4HCO_4 in these experiments.

The phosphate salt experiments provide a control to the demonstration that the cortical pH changes less than does blood pH during I.V. HCl infusion (Tschirigi & Taylor, 1958), from which it was concluded that a barrier to HCO_3^- existed. Gesell & Hertzman (1926), in an analogous experiment, showed that blood pH changes more than muscle tissue pH during I.V. Na_2CO_3 infusion, or after administered CO_2.

Previous long-term c.s.f. responses to $NaHCO_3$ (Collip & Backus, 1920; De Bersaques, 1955) or to HCl (Cestan, Sendrail & Lassalle, 1925) were probably complicated by blowing off of CO_2 (Robin *et al.* 1958). Similarly, the alkaline c.s.f. change after NH_4Cl (Winterstein & Göhkan, 1953), not confirmed in these experiments, probably followed hyperpnoea.

The blood–brain barrier

A blood–brain barrier to inorganic ions has been postulated to explain the long equilibrium time between blood and brain (or c.s.f.), when compared to that between blood and other extracellular fluids (Wallace & Brodie, 1940; Greenberg, Aird, Boetler, Campbell, Cohn & Murayama, 1943; Woodbury, 1958). The barrier to HCO_3^-, as demonstrated by the cortical acidic response, may belong to this 'ionic' category. Proof of a

true 'impermeability' for HCO_3^-, in the sense used by Teorell (1949), would necessitate direct flux determinations.

There is no indication of active transport of HCO_3^- at the cortical surface, because the acidic response is independent of several metabolic inhibitors (Rapoport, unpublished) and of normal O_2 tensions, although active transport of HCO_3^- and Cl^- has been considered for glial tissue (Giacobini, 1961). The results reported here do not indicate that carbonic anhydrase is involved in the cortical acidic response.

A physical restriction to diffusion of inorganic ions would explain the above results adequately. Such a passive barrier has been proposed for restriction of organic ions (Brodie, Kurz & Schanker, 1960), and may exist also for trypan blue (Broman & Lindberg-Broman, 1945), for albumin (Lending, Slobody & Mestern, 1961), for fluorescein (Baldwin, Galindo & Farrier, 1962) and for ^{35}P phosphate (Ernster & Herlin, 1961).

The resistance of the acidic response to 10 % (v/v) CO_2 agrees with the barrier resistance to trypan blue (Clemedson, Hartelius & Holmberg, 1958), to ^{24}Na (Fishman, 1959), and to albumin (Lending *et al.* 1961). Reduction of the cortical acidic response with 20 % (v/v) CO_2 may have been due to the mass action effect of CO_2 (equation 1) or to intracerebral petechiae (Clemedson *et al.* 1958).

The absence of the acidic response to I.V. $NaHCO_3$ in other tissues (dura, thyroid, muscle) indicates a tissue specificity, as far as was studied. Though this latter work could have been complicated by cellular damage, isotope studies have shown that HCO_3^- is indeed in very rapid equilibrium with the extracellular space of muscle (Shipley, Baker, Incefy & Clark, 1959). It should be noted that Gesell & Hertzman (1926) stated that the acidic response was present also in muscle tissue, and generalized HCO_3^- 'impermeability' to muscle as well as to the cerebral 'membranes'.

The apparent blood–brain barrier to trypan blue has been ascribed, in part, to the pore area for brain capillaries (Edström, 1958). Such an explanation cannot apply to the barrier to HCO_3^-, because the acidic response does not occur in muscle, where a similar pore area obtains (Pappenheimer, 1953).

The barrier to trypan blue was found in the foetus of the rat (Grazer & Clemente, 1957) and of man (Gröntroft, 1954), and the barrier to albumin in the puppy (Lending *et al.* 1961). In the immature kitten (Barlow, Domek, Goldberg & Roth, 1961) and rat (De Robertis & Gerschenfeld, 1961), there is an expanded extracellular space, but since the acidic response occurred in the kitten, the barrier to HCO_3^- cannot be explained on the basis of a low cerebral extracellular space (Edström, 1958).

SUMMARY

1. A method was described for measuring the pH, DC and AC potentials concurrently on the cerebral cortex of the adult cat, under conditions of controlled ventilation. Kittens and rabbits were also studied.

2. The cortical pH response to I.V. $NaHCO_3$ was acidic and was accompanied by a DC negativity, while the blood became alkaline. Such an acidic response was present in the rabbit and kitten. It was not present in a number of other tissues in the cat, including the dura.

3. The cortical acidic response was not affected, qualitatively, by I.V. acetazolamide, prolonged hypoxia or 10 % (v/v) CO_2. It was reduced reversibly by 20 % (v/v) CO_2. It was not affected by removal of the arachnoid membrane.

4. The cortical pH response to an I.V. NH_4^+ salt solution was complex. NH_4Cl did not produce an alkaline response.

5. The cortical pH response to an alkaline or an acidic sodium phosphate solution was augmented but not changed in its direction following treatment of the cortex with *n*-butanol.

6. The results were interpreted in terms of a restriction of HCO_3^- by the blood–brain barrier, through which CO_2 can pass. This restriction is probably non-specific for inorganic ions, and perhaps for other substances. It does not appear to be related to a low cerebral extracellular space, and is independent of substantial oxidative metabolism.

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