The pK' of Carbonic Acid in Cerebrospinal Fluid*

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The pK' of carbonic acid in cerebrospinal fluid has been derived only at one temperature, 38° (4). The value of 6.13 obtained is considerably higher than that found for blood plasma (5). Since the necessary pH measurements in that study were performed with a colorimetric technique of limited accuracy, and because the data used in determining pK' were not presented, it was felt necessary to carry out additional studies of the pK' of carbonic acid in cerebrospinal fluid. In the present investigation, attempts were made to obtain further information regarding the absolute value of pK' and the possible influences upon it of changes in pH and temperature.

EXPERIMENTAL PROCEDURE

The general procedures employed in the study included equilibration of cerebrospinal fluid or a solution of NaCl and NaHCO3 with known tensions of carbon dioxide in a tonometer, determination of pH and total carbon dioxide concentration in the fluid, and calculation of pK'.

Fluids—Cerebrospinal fluid of normal composition was obtained from 12 patients undergoing pneumoencephalography for diagnostic purposes. The fluid was promptly frozen and stored at -20° for 1 to 9 days, and thawed just before tonometry. Cerebrospinal fluid from each patient was usually used for two determinations, although in a few instances, fluid from several patients was pooled. Cerebrospinal fluid has been demonstrated to be essentially a solution of 0.025 M NaHCO3 with 0.135 M NaCl (6, 7); this 0.16 M "synthetic cerebrospinal fluid" was employed in a number of experiments for comparison with true cerebrospinal fluid. For the preparation of the 0.025 M NaHCO3-0.135 M NaCl solutions, reagent grade NaHCO3 and NaCl were used; four different solutions were prepared in order to distribute gravimetric and volumetric errors.

Tonometric Procedure—Determination of the pK' in cerebrospinal fluid was performed at 26, 34, and 39°. The synthetic fluid was studied at 26, 37, 39, and 41°. Six separate tonometric determinations were performed on each fluid at each of the temperatures at which it was equilibrated. The solution (20 ml) was placed in a 1250-ml glass tonometer; after the tonometer had been flushed with the selected mixture of CO2 in air, it was immersed in a thermostatically controlled water bath and rotated for 30 minutes with the total gas pressure in the vessel adjusted to existing barometric pressure. After equilibration with the pCO2 of the gas phase in the tonometer, the solution was withdrawn anaerobically with the tonometer remaining under the surface of the water to prevent gas exchange resulting from change in temperature during sampling procedures. A sample of the gas was then collected from the tonometer for measurement of CO2 concentration, permitting subsequent calculation of pCO2. During withdrawal of samples of solution and gas, a rubber balloon in the tonometer was opened to room air to maintain the pressure in the vessel at the atmospheric level.

Analytical Procedures—Carbon dioxide concentration in the tonometer gas was measured in duplicate with the Scholander 0.5-cc gas analyzer (8).

The pH of the fluid being studied was determined in duplicate with a McInnes-Belcher glass microelectrode (0.2-ml capacity) mounted in an electrically shielded, temperature-controlled, Lucite chamber which contained all components of the glass electrode assembly including the calomel half cell, KCl reservoir, and silver-silver chloride electrode. The electrometer employed with the glass electrode system was a pH meter from Electronics Industries, Ltd., with rated stability of 0.002 pH unit per 12 hours, resolution of 0.001 pH unit, and accuracy of 0.002 pH unit when standardized within ±1.0 pH unit of test solution. To facilitate reading of pH, the output of this meter was recorded with a Texas Instrument Company recorder.

The glass electrode was filled from below without opening the thermostatically controlled chamber. The top of the electrode tube was fitted with a glass cap which had only a small orifice open to the air in the chamber. This cap was intended to minimize errors resulting from the escape of CO2 during pH measurement upon poorly buffered samples such as cerebrospinal fluid and bicarbonate buffers. Immediately before pH determination, samples were brought to approximately the desired temperature.

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1 pK' represents the negative logarithm of the first dissociation step of carbonic acid, K'. K' is defined as K+·M[CO3]/M[HCO3], where K+ is the hydrogen ion activity and M is the molar concentra-
by warming them in a water bath controlled within one degree of the experimental temperature. The temperature of solutions in the glass electrode was measured at the time of each determination by means of a thermistor mounted within the electrode. In a series of 84 unselected pH determinations, the mean difference between electrode temperature and tonometer temperature was 0.04° with a standard error of less than ±0.01. The mean difference between duplicate measurements of pH on 18 specimens of cerebrospinal fluid and 24 samples of the sodium chloride-sodium bicarbonate solution was found to be ±0.009 pH unit, with a standard error less than ±0.001.

Each pH determination upon an unknown sample was bracketed by measurement of a commercial buffer solution the pH value of which had been standardized with freshly prepared National Bureau of Standards 0.025 M equimolal phosphate buffer (9). A subsequent restandardization of the commercial buffer against National Bureau of Standards 0.025 M equimolal phosphate and 0.01 M borate buffers (9) led to the surprising finding that the value used for the original equimolal phosphate buffer was 0.04 pH unit too high. This discrepancy was traced to the presence of ammonia (about 0.5 mmoles per liter) and excess carbon dioxide (0.1 mmoles per liter) in the glass-distilled water used to make up the phosphate buffer. After the glass-distilled water was boiled to remove volatile agents, the equimolal phosphate buffer was found to have the correct pH value when compared with the 0.01 M borate buffer. The commercial buffers employed for the tonometric determination of pH were therefore restandardized with the second, more reliably prepared National Bureau of Standards buffer. This permitted the previously measured pH and pH values to be properly adjusted for referral to an accurate buffer standard, as originally intended.

The gross defect in the standard phosphate buffer would have been undetected had the borate buffer also been employed. The latter is apparently not appreciably affected by the presence of ammonia. Certainly it is important that the distilled water employed in preparing National Bureau of Standards buffers should be boiled just before its use.

Within 2 hours of sampling, the concentration of carbon dioxide in the equilibrated cerebrospinal fluid was measured on 1-ml aliquots, by the manometric method of Van Slyke and Neil (10). The mean difference between duplicate measurements of carbon dioxide concentration on 18 separate samples was 0.15 volume per cent with a standard error of ±0.02 volume per cent. Carbon dioxide concentration of the sodium chloride-sodium bicarbonate solution was established by the gravimetric procedure used in preparing the solutions.

**Derivation of Carbon Dioxide Solubility Factor**—The solubility of carbon dioxide in cerebrospinal fluid (S = mmoles per liter per mm Hg of pCO₂) appears not to have been experimentally determined at any temperature. A solubility factor, S, of 0.0316, used by Shohl and Karelitz (4) in calculating pHK at 38°C was derived from data obtained by Hastings and Sendroy on NaHCO₃-NaCl solutions (11). Table I indicates for various temperatures the values for S employed in the present study. These relationships were derived for a 0.16 M solution from the data of Harned and Davis (2) by means of the interpolation procedure outlined by Markham and Kobe (12). The former workers studied the solubility of carbon dioxide in sodium chloride solutions of different ionic strengths over a temperature range of 0 to 50°C. Harned and Bonner (3) have shown that, for computations of hydrogen ion concentration, solutions of sodium bicarbonate with sodium chloride may be considered to have the same carbon dioxide solubility factors as a solution of sodium chloride at comparable ionic strength, providing that the [HCO₃⁻]/[Na⁺] is not greater than 0.05 M and the ratio [Cl⁻]/[HCO₃⁻] is not less than 5. These conditions are met by the true and synthetic cerebrospinal fluid. Therefore, the solubility factors shown in Table I are considered reasonable.

**Calculation of pK'**—Values of pK' at various temperatures were calculated from the Henderson-Hasselbalch equation, using the values of S of Table I and experimental observations, as follows. For cerebrospinal fluid:

\[
pK' = pH - \log (\frac{CO_2 \text{ content}}{S \times pCO_2} - 1)
\]

For NaHCO₃-NaCl solutions:

\[
pK' = pH - \log (\frac{[NaHCO_3]}{S \times pCO_2})
\]

where CO₂ content and NaHCO₃ are each expressed in mmoles per liter, pCO₂ in mm Hg, and S in mmoles per liter per mm Hg.

**RESULTS**

Values obtained in 18 determinations of the pK' of carbonic acid in human cerebrospinal fluid are shown in Table II; the average values found in 24 similar studies on 0.025 M NaHCO₃-0.135 M NaCl solutions are presented in Table III. The data of each study were evaluated by multiple regression analysis (13) to determine the manner of interaction of pK', pH, and temperature. The slopes of change in pK' against change in pH and temperature are significantly different from zero (p < 0.01 in

**Table I**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>S</th>
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<tr>
<td>°C</td>
<td>(mmol/liter/mm Hg)</td>
</tr>
<tr>
<td>25</td>
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</tr>
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<tr>
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<td>35</td>
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<tr>
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</tr>
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</tr>
<tr>
<td>39</td>
<td>0.0310</td>
</tr>
<tr>
<td>40</td>
<td>0.0302</td>
</tr>
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</table>

with pH was not found in the prepared solution (p > 0.5). In contrast with cerebrospinal fluid, a statistically significant variation of pK' was found to be about 0.001 unit for a fall of 0.0917 pH - 0.0022 units for a fall of 1° and about 0.092 unit for a fall of 1.0 pH unit. Over the range of temperature and pH of this study, the pK' of carbonic acid in cerebrospinal fluid is therefore defined by the expression: pK' = -0.0917 pH - 0.0022 T + 6.898, where T is temperature in °C.

This equation, which was derived from the data shown in Table II, was then used to construct the nomogram shown in Fig. 1, which expresses the relationship of pK' to temperature and pH over the range 26 to 40° and from 7.22 to 7.51 pH units. A straight line between the known pH and temperature intersects the center line at the applicable pK'.

The establishment of dependable values for the pK' of carbonic acid in cerebrospinal fluid is of importance in determination of absolute pH of cerebrospinal fluid under various conditions, of the changes of pH in cerebrospinal fluid induced by alterations of its pCO2 or fixed base, and in comparisons of the pH in blood and cerebrospinal fluid. Each of these appears to have significance in studies of the chemical control of respiration (14–17).

The results of this study indicate that true cerebrospinal fluid, which is similar to the NaHCO3–NaCl solution in electrolyte composition and in the influence of temperature upon pK', differs from the synthetic cerebrospinal fluid in showing a small, but significant variation of pK' with change in pH.

With a normal body temperature of 37° and a pH of 7.3 to 7.4, the pK' of cerebrospinal fluid is about 0.001 unit lower than the current scale of the National Bureau of Standards. The small discrepancy in pK' for carbonic acid in the two studies on true cerebrospinal fluid is actually less than it appears from the above values, since the pK' reported by Shohl and Karelitz was determined on a pH scale which is 0.02 unit lower than the current scale of the National Bureau of Standards (4, 18, 19) which formed the basis for the present experiments. Correction of the previously derived pK' to agree with the pH scale of the National Bureau of Standards yields a value of 6.15, virtually identical to that found in these studies for the same temperature.
The values of pK' for cerebrospinal fluid differ from those presented for blood by Severinghaus et al. (5) under the same conditions of pH and temperature (Fig. 1). Thus, at pH 7.4 and a temperature of 37°, the pK' values shown by the nomogram for blood (5) and for cerebrospinal fluid (Fig. 1) are 0.022 and 6.138, respectively. As pointed out by Severinghaus et al., the S values used in constructing the nomogram for pK' in blood were slightly low (20). When the recommended, more reliable values for S (21, 22) are employed, the pK' for blood becomes 6.10 at pH 7.4 and at 37°.

The influence of change in temperature upon pK' of cerebrospinal fluid also differs from that upon pK' in blood. From the nomograms for blood (5) and cerebrospinal fluid (Fig. 1) it can be seen that a fall in temperature from 37 to 20° results in a change of pK' in blood of 0.059 and in cerebrospinal fluid of 0.024. The data of Severinghaus et al. (5) indicate that the relationship of pK' to pH in blood varies with temperature. The small range of pH and the limited data at any one temperature in the present series prevent any accurate assessment of the relationship of pK' to pH in cerebrospinal fluid at different temperatures.

Limitations This study has shown the variation of carbonic acid pK' in cerebrospinal fluid with changes in temperature and pH. The regression equation and the corresponding nomogram expressing pK' as a function of these two variables aids in the determination of pCO₂ or bicarbonate concentrations in cerebrospinal fluid at various temperatures. However, certain limitations in the usefulness of these data deserve emphasis.

It is pointed out elsewhere (23, 24) that it is not feasible to make precise, quantitative comparison of pH values determined or calculated at different temperatures. The pH scale of the National Bureau of Standards was established by Bates et al., by determination of an “activity pH” (\(-\log aH^+\)) for several standard buffer solutions (25). The pH of these standards was calculated by a method requiring values for single ionic activity coefficients which, being unmeasurable by direct means, were estimated. The assumptions for this estimation included the necessarily arbitrary selection of a value for chloride “ion size parameter” (25). Since the estimations of ion size parameter and its change with temperature are not precisely definable, the conventional pH scale cannot be considered to have an absolute or physical definition. As stated by Bates (23), the scale for pH is different for each temperature. Although the differences in pH scales may be small, the exact magnitude of their separation is not known. Thus, quantitative comparisons of the pH of fluids sampled at different equilibration temperatures is not justified, in vivo or in vitro, if pH is to be interpreted as a measure of hydrogen ion activity.

The limitation arising from the nature of the pH scales cannot be surmounted by use of the observed relationships of pK' and temperature to calculate pH from known values of pCO₂ and carbon dioxide content. The thermodynamic ionization constant of carbonic acid, in contrast with its pK’, varies with temperature but not with pH (2). The pK’ derived tonometrically is, of course, not a thermodynamic entity, but the expression of a composite quantity which includes implicit corrections for at least four factors in addition to the true ionization constant upon which it is based. These additional factors include the effect of temperature and pH upon protein ionization, error in the values for physical solubility of carbon dioxide, fundamental limitations of the conventional pH scale, and the difference in liquid junction potentials arising at the interface between the standard buffer and the salt bridge and between the test solution and the salt bridge.

In spite of these obstacles to study of the effects of temperature change upon pH in body fluids, there are special situations in which empirically measured pH-temperature coefficients can be of considerable usefulness (26). When, for example, it is not practical to maintain the pH electrode assembly exactly at the body temperature of the experimental animal or subject, the pH-temperature coefficient determined for blood by Rosenthal (26) may be employed to convert a pH number measured at one temperature to a value representative of the experimental temperature at which the specimen was obtained. As has been pointed out elsewhere (27, 28), variations in this coefficient among subjects and a partial dependence of the coefficient upon pH (29) limit somewhat the utility of this approach. Ideally, pH should be measured at the body temperature of the individual subject. In practice, the temperature coefficient for blood may be employed over a small temperature range without gross error when suitable conditions of anaerobic sampling, storage, and pH measurement are met. At present, no pH-temperature coefficient is available for use with cerebrospinal fluid and, for this reason, measurements of cerebrospinal fluid pH must be carried out at temperatures very close to that of the animal or experimental condition under study.

This discussion has re-emphasized that pH, and thus pK', are defined on arbitrary, but reproducible, scales different at each temperature; these numbers can therefore be strictly compared only at a constant experimental temperature. Within this limitation, experimentally determined pH values are useful in assessing relative changes in acidity under many conditions and, together with pK’ values, may be utilized for unequivocal determinations of pCO₂ and bicarbonate concentration of cerebrospinal fluid in experiments conducted over the temperature range for which the values are available.

SUMMARY

The pK' of carbonic acid in human cerebrospinal fluid has been determined at 39, 34, and 26°, and at various pH levels from 7.22 to 7.51. pK' was found to vary inversely with temperature and pH in the manner, pK' = \(-0.0917 \text{ pH} - 0.0022 T + 6.898\), where T is the temperature in °C. These relationships are also presented as a nomogram. Similar studies of the pK' of carbonic acid in a protein-free, 0.025 M NaHCO₃-0.135 M NaCl solution showed variation of pK' with temperature but not with pH. Limitations in the use of pK'-temperature relationships and in comparison of pH values obtained at different temperatures are discussed.

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REFERENCES

The $pK'$ of Carbonic Acid in Cerebrospinal Fluid
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