A COLORIMETRIC METHOD FOR THE DETERMINATION OF THE pH OF CEREBROSPINAL FLUID.*

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Recently the pH of the blood has been exhaustively studied but that of the cerebrospinal fluid has received little attention. Because the composition of spinal fluid is less complex, the determination of pH and interpretation of the results should be simpler than for blood. Cerebrospinal fluid is not only free from hemoglobin but also contains so little protein as to be, for practical purposes, protein-free. Is spinal fluid a transudate or a secretion? Is its relation to the blood such that it fulfills the laws of the Donnan equilibrium? The simultaneous determination of the reaction of blood and of spinal fluid give data applicable to such questions.

In the course of an investigation on the ionization of calcium it became evident that test-tube experiments, involving a simple water system, could not be applied directly to the body fluids in which the protein effect is at present not subject to quantitation. Therefore, an effort was made to apply such consideration to a body fluid free from protein, namely, cerebrospinal fluid. One of the necessary determinations is an accurate estimation of pH.

HISTORICAL.

Foa (1), in 1906, was the first to use the electrometric method for measuring pH of spinal fluid. He found a pH of 7.22 for a dog. In 1911 Polányi (2), working in Tangl's laboratory, found values approximating pH 10. Bisgaard (3), reports values determined for him in the Carlsberg laboratory by

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Paltiszch. After passing H₂ through spinal fluid all night, he found the reaction to be more alkaline than pH 9.0. He also used Sörensen's buffer standards and the colorimetric method on fresh fluid dropping directly from the needle and found the fluid acid to phenolphthalein. In 1916, using phenol red as an indicator and reading the pH values colorimetrically either directly or on the dialysate through collodion membranes according to the method of Levy, Rowntree, and Marriott, Hurwitz and Tranter (4) obtained values of pH 8.11 on the dialysate and 8.26 directly; and Weston (5) found pH 7.9 to 8.3 on the dialysate. Levinson (6), employing both the gas chain and phenol red in open and stoppered tubes and at various intervals after lumbar puncture, obtained values of pH 7.7 to 7.9 on fresh material. Felton, Hussey, and Bayne-Jones (7), who used phenol red, record the value of spinal fluid immediately after removal as 7.4 to 7.6. Levinson and Felton, Hussey, and Bayne-Jones came to the conclusion that allowing spinal fluid to stand unprotected had permitted the escape of CO₂, hence, previous studies had given values much too alkaline. Ylppö (8), used the electrometric method with the refinements of Hasselbalch and Michaelis, but recognized the error in his determinations through loss of CO₂; Ylppö obtained values of pH 8.4. Milroy (9) made an electrometric determination at 37.5°C. of a single spinal fluid and found a value of pH 7.24 at 29.3 mm. CO₂ tension and concluded that spinal fluid approximates a solution of 0.02 N NaHCO₃ in 0.18 N NaCl. McClendon (10) introduced a method of titrating the alkaline reserve and then calculating the pH. Parsons and Shearer (11) studied three cases by electrometric method at various CO₂ tensions on two specimens obtained without contact with air. They calculated that under body conditions the pH was 7.32 and 7.12 at CO₂ tensions of 48 mm. and 64 mm. respectively. In a second paper, Shearer and Parsons (12) report pH values of 7.4, 7.4, and 7.3 for three normal individuals. The colorimetric method was employed and all readings were made at room temperature. Klothilde Meier (13), at approximately the same time, made a similar colorimetric pH measurement on fluid received under albolene without contact with air. She also plotted absorption curves and thus calculated the tension of CO₂ in the body. On two normal cases she obtained pH 7.38 and 7.33 and, by calculation, in the second case pH 7.35 under a tension of 39 mm. CO₂. This is the first time that values so closely approaching those of normal blood were obtained. For fluids from pathological cases Meier found extreme variations of pH 7.11 to 7.58. Using Michaelis' bufferless standards of nitrophenol, Brock (14) measured the pH of spinal fluid, obtained under paraffin oil at room temperature, and obtained values of 7.50 to 7.55 with a CO₂ tension of 24 to 27 mm. He tested his standards against Sörensen's phosphate buffer standards and found his values too alkaline by 0.025 pH. He was not unmindful of the difference of temperature and determined the pH at 38°C. but was unable to detect differences of more than 0.02 pH to the acid side. After saturation with expired air at 40 mm. tension he obtained values of pH 7.28 to 7.30. Behrendt (15) and Waltner (16) (1924) used Brock's method and confirmed his results.
Principle of Method.

The earlier work does not take cognizance of the importance of the CO₂ tension. Loss of CO₂ resulted in a pH value much too alkaline. At first, values of pH 9.0 to 10.0 were found, but with increasing precaution to prevent the escape of CO₂, the values have gradually approached that of the blood.

The principles of the method that we have used are, first, to prevent loss of CO₂, and second, to determine the pH colorimetrically at body temperature, 38°C.

1. Loss of CO₂.—The investigations of Van Slyke and his collaborators have shown that the precautions necessary for the handling of blood or plasma are not adequate in dealing with bicarbonate solutions. Spinal fluid approximates a solution of sodium bicarbonate in physiological saline. Therefore, we attempted to fulfill the following conditions: first, the spinal fluid must never come in contact with air; second, removal under mineral oil should be replaced by collection over mercury; third, all transfer should be avoided. By following the principle of the Van Slyke technique the apparatus shown in Fig. 1 was devised.

The apparatus was made from one of the tubes used for the pH standards so as to insure uniform size, color, thickness, and material. To one end a three-way stop-cock and to the other a straight tube is fused. These are suitably calibrated, the narrow portion between the tube and stop-cock at 1 cc. and the remainder at 1 cc. marks. A rubber tube with pinch-cock and a small mercury leveling bulb complete the apparatus.¹

2. Colorimetric Comparison at Body Temperature.—The necessary data for the temperature factors have been furnished in the able paper of Hastings and Sendroy (17). The essential point is that a solution of phosphates prepared according to the technique of either Sörensen or Clark and containing phenol red apparently becomes more alkaline when heated. If a standard at pH 7.20, containing phenol red, is heated to 38°C and compared with an identical standard at 20°C, the warmer tube appears redder. In order to match the standard at 38°C which formerly was pH 7.20 the standard used must be at pH 7.28 at 20°C.

¹ The apparatus and comparison tubes were supplied by the Empire Laboratory Supply Company, New York City.
The change is due to two factors. First, the phosphates really become more acid by 0.03 pH; in other words, the pK₁ of the phosphates becomes less or the acid becomes stronger by 0.03; second, the indicator, which is also a weak acid, has become stronger and binds more base. Therefore, more of the indicator appears in its alkaline or red form. If both the solution and the standard are observed at 38° the effect due to the action of the increased temperature on the dye is equal in the two tubes and hence requires no correction. The only alteration is that of the actual pH value of the standard. By calling a standard which is pH 7.20 at 20°, pH 7.17 at 38°, solutions may be compared with the Sörensen standards when both are at 38°.

The method devised by Hastings and Sendroy employs bicolorimetric standards suitable for 38°. These are prepared by dividing
a constant quantity of indicator into varying amounts. One fraction is put into a tube containing 0.001 N HCl; the other tube contains 0.01 N NaOH. These two tubes replace the single buffer standard. The color which in the buffered standards depends upon mixtures of acid and alkali salts in the same tube is here produced by the use of two. This method has three advantages; the standards are easier to make, are more permanent, and are independent of the effect of temperature. Only the apparatus containing the spinal fluid plus the indicator solution need be heated to 38°. The solutions and standards are then compared in a suitable colorimeter such as described in principle by Bjerrum or Gillespie. Since the protein in spinal fluid is absent, or present in small amounts, no protein error results. The effect on the indicator due to the salt concentration has been studied by Hastings. This is constant and is 0.02 of a pH.

Procedure.

Measure into the apparatus as many tenths of cc. of Hastings' indicator solution (0.0075 per cent phenol red), as of cc. of spinal fluid which it is expected to use. Connect the upper capillary tube directly to the lumbar puncture needle by means of a rubber tube bearing a glass Luer adapter previously sterilized, observing sterile precautions the while. Allow the fluid to escape under its own pressure through the side of the three-way stop-cock which has been turned so as not to be connected with the apparatus. When all air has been removed from the capillary tube, allow the spinal fluid to enter the apparatus by turning the stop-cock and holding the bulb in such a position that its mercury level is at or very slightly below that in the sampling tube. The flow of spinal fluid is stopped when the desired amount has entered. Place a pinch-cock at the lower end of the apparatus. Immerse in a water bath at 38° for 5 minutes and compare with the bicolorimetric standards.

Results.

In Table I comparisons of the blood pH and spinal fluid pH are given. These results are selected from over 50 determinations. The one specimen from Dog 4 whose blood showed a pH 7.20 is included because it is our most discordant determination. It
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shows possible but not probable error. In cases with infection of the meninges the spinal fluid is more acid than the blood. It will be seen that normal spinal fluid pH and blood plasma pH are the same within the sum of the errors of the two methods. The measurement of the pH of spinal fluid has equalled in accuracy that of the blood, and its relation to the equilibrium of the body can therefore be evaluated.

TABLE I
Comparison of the pH of Blood and Cerebrospinal Fluid.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Blood (arterial)</th>
<th>Spinal fluid</th>
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<tbody>
<tr>
<td>Scarlet fever</td>
<td>7.35</td>
<td>7.35</td>
</tr>
<tr>
<td>Normal</td>
<td>7.32</td>
<td>7.32</td>
</tr>
<tr>
<td>Syphilis</td>
<td>7.34</td>
<td>7.34</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>7.38</td>
<td>7.32</td>
</tr>
<tr>
<td>Acidosis</td>
<td>7.42</td>
<td>7.29</td>
</tr>
<tr>
<td>Meningitis</td>
<td>7.17</td>
<td>7.26</td>
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<tr>
<td></td>
<td>7.38</td>
<td>7.30</td>
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DISCUSSION.

To analyze the results even of the latest and best investigations of the pH of the spinal fluid is difficult because experimental errors are not considered in the calculations. Parsons and Shearer place the normal pH at 7.32 and 7.12 with CO₂ tensions of 48 and 64 mm., Meier at pH 7.33 and 7.38 with CO₂ tensions of 30 and 39 mm., Waltner and Brock at pH 7.50 and 7.55 with CO₂ tensions of 24 and 27 mm. The most alkaline values are associated with the lowest CO₂ tension and vice versa. The question
of absorption curve of spinal fluid offers many difficulties involving the temperature, the absorption coefficient, the total salt concentration, and the values of pK_i used. The values given are consistent, but it is obvious that in three sets of experiments, all on normal individuals, the spinal fluid cannot be 50 per cent above that of the alveolar air in one case, equal to it in another, and 50 per cent below it in a third. There must be an error either in the CO_2 tension or in the pH. If the spinal fluid is in equilibrium with the blood a constant relationship must exist between the pH of both. A difference of 10 mm. in CO_2 tension causes a change in reaction of roughly 0.10 pH.

The difficulty of applying electrometric method to dilute carbonate solutions without other buffer under varying CO_2 tensions is great for several reasons. The absorption curve is so flat that small difference in tension causes large difference in pH and but small difference in the total CO_2. Second, the equilibrium is established so slowly as to make electrometric determinations either with solutions saturated with known mixtures or by refill method extremely difficult. If the colorimetric principle can be applied correctly, it should give more accurate and more nearly constant results. The temperature coefficient of the solutions, as worked out by Hastings and Sendroy, is important. Our tests have likewise shown a difference of about 0.06 pH between the measurements at 20° and 38°. Brock was probably in error because Austin and his coworkers have shown that CO_2 solutions, when heated, cannot be protected against loss of CO_2, unless paraffin and not paraffin oil is used. It seems much easier and simpler to heat the solution to the desired temperature over mercury.

It is interesting that normally the acidity of the blood and spinal fluid, simultaneously determined, should be so nearly equal. As the error of each reading is 0.02 pH, the total difference should be not 0.04 pH but 0.02 pH. If the determinations are made at the same time, the two tubes can be compared directly. The advance in accuracy is due to improved technique with regard to (1) prevention of loss of CO_2; (2) determination of pH at body temperature with improved colorimetric standards.
A comparison of blood and cerebrospinal fluid shows that the two normally have the same pH, 7.35 to 7.40 ± 0.02.

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