Buffer Capacities of Human Blood and Plasma

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MANY CRITERIA are used to evaluate the acid-base status of a patient. In most instances the clinician must interpret and correlate the results of a number of independent chemical tests, such as the sodium chloride concentration, bicarbonate, etc. A single quantitative measure of the physiologic buffer systems as they function together might well be of value in judging the total severity of acid-base disturbance as well as the adequacy of the patient's buffer defenses. Such a test is one that measures the change in the hydrogen ion concentration following addition of acid or base to blood or plasma, and the information is expressed quantitatively as the buffer capacity ($\Delta B/\Delta pH$). Despite the potential usefulness of this concept, the buffer capacity of blood and plasma has received relatively little attention in the field of medicine. Singer and Hastings (1) calculated the relative concentration of buffer anions for oxygenated whole blood over varying partial pressures of carbon dioxide and *p*H values and constructed a useful nomogram that relates carbon dioxide content, hematocrit, plasma *p*H, whole blood buffer base, carbon dioxide pressure, and oxygen saturation. In 1952 Kilpi (2) reported the buffer capacities of the components of normal blood, with particular emphasis on the contribution of hemoglobin. Differences in buffer capacity as influenced by sex (3) and by various pathologic conditions (4, 5) have been observed by the Scandinavian workers. Recently, Salenius reported studies on the buffer capacity of whole blood, erythrocytes, and plasma, but his figures do not represent normal individuals because his data were taken from preoperative surgical patients (6).

The purpose of this investigation was to determine the normal

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buffer capacity of whole blood, plasma, and the relative contributions of the cells, bicarbonate, proteins, and other constitutents to the buffer capacity under conditions of strict anaerobiosis and controlled ionic strength. We have included the data from several pathologic specimens to illustrate the alterations in the titration curves and buffer capacities that result from disturbances in acid-base balance and blood protein levels.

EXPERIMENTAL

Venous blood, 25 ml., was obtained anaerobically from the patient using a 30 ml. syringe previously coated internally with a light film of heparin solution (10 mg./1 cc.). Aliquots for hematocrit and hemoglobin determination were removed immediately. A long needle, the tip of which was placed under a layer of mineral oil in a tube, was used to transfer 5.5 ml. of the whole blood to be titrated. The rest of the blood was transferred in like fashion to a 20 ml. Lusteroid centrifuge tube (International No. 657). When this sample reached room temperature, the plasma was obtained by centrifuging at 1000 g (3000 rpm) for 10 minutes. Samples covered with oil in these small-bore tubes suffered no loss of CO_2 or observable *p*H change during the centrifugation.

A discontinuous titration of the whole blood sample was undertaken immediately. Initial *p*H was determined by placing 1 ml. of the blood in the electrode* and allowing one minute for temperature equilibration before the readings were taken. Then 1 ml. of blood was drawn into a carefully calibrated 2 ml. nylon syringe ("Vim" Mac Gregor) from which air had been expelled with 0.93 per cent NaCl. One ml. of the appropriate acid-saline solution† was then drawn up and the syringe was sealed with mercury, some of which was drawn into the syringe to form a mixing bead. After the syringe was shaken for 30 seconds, the mercury was expelled, the remaining solution was introduced into the electrode, and the *p*H was measured. Between determinations the syringe was washed twice with distilled water and once with saline to remove the air and residual acid. The plasma was titrated in a manner similar to that used for whole blood.

The linearity and accuracy of the *p*H meter was checked with meas-

*Cambridge Model R with MacInnes-Belcher microelectrode coated with Desicote (Beckman).

†Solutions containing 0.0052, 0.0100, 0.0148, and 0.0254 milliequivalents of hydrochloric acid per ml. of 0.9 per cent sodium chloride solution, respectively, were used.

urements of phosphate buffers of *p*H 7.38 and *p*H 6.86 before and after each experiment.‡

All blood and plasma values were run at laboratory room temperature and corrected to 38° by the use of the Rosenthal correction factor (7). The buffer capacities were derived from the titration curves by the use of the Van Slyke equation for the calculation of buffer capacity, B.C. = (Δ acid/Δ *p*H) (8) and are expressed as *milliequivalents per liter per* p*H unit* (rather than milliequivalents per ml. per *p*H unit) so as to conform with modern units of expression for fluid electrolytes.

Total CO_2 content of the plasma was determined volumetrically according to Peters and Van Slyke (9). Hemoglobin was converted to acid hematin with 0.1 N HCl for colorimetric comparison with a standard.§ Total plasma proteins and albumin were determined by the biuret method (10). Hematocrit values were measured in Wintrobe tubes centrifuged at 1000 g until the surface of the erythrocyte layer became constant.

RESULTS

In Table 1 the average values of whole blood and of plasma at 38° as determined by the above methods are given. The whole blood values are the averages of 22 healthy student subjects. The plasma values represent 32 samples from 29 healthy subjects. The hematocrit and hemoglobin values were obtained from 20 of these same subjects. The total plasma CO_2 values were obtained from 31 of these same sub-

‡Buffer of *p*H 7.38 was prepared by adding 1.246 Gm. of potassium dihydrogen phosphate and 4.30 Gm. of disodium hydrogen phosphate to 1 liter of freshly boiled distilled water at 25°. The *p*H 6.86 buffer was made by adding 3.40 Gm. of potassium dihydrogen phosphate and 3.35 Gm. of disodium hydrogen phosphate to 1 liter of freshly boiled distilled water at 25°.

§Klett acid hematin standard 14.4 Gm. per cent.

Table 1. BUFFER CAPACITY OF BLOOD AND PLASMA AT PHYSIOLOGICAL *p*H VALUES

	*Present**	*Salenius (5)*	*Kûpi (3)*
Plasma *pH*, 38°	7.34 ± 0.04	7.42 ± 0.05	7.3
Hemoglobin, per cent	14.9 ± 1.1	11.7 ± 2.2	16
Hematocrit, per cent	49.6 ± 3.4	—	—
Plasma Protein, per cent	—	6.8 ± 0.7	—
Plasma HCO_3^- mEq/L	26.2 ± 1.6	19.8 ± 2.2	24.5
Blood BC	38.5	31.8 ± 3.5	36.1
Plasma BC	16.1 ± 0.9	15.2 ± 1.1	

*Average values ± one standard deviation.

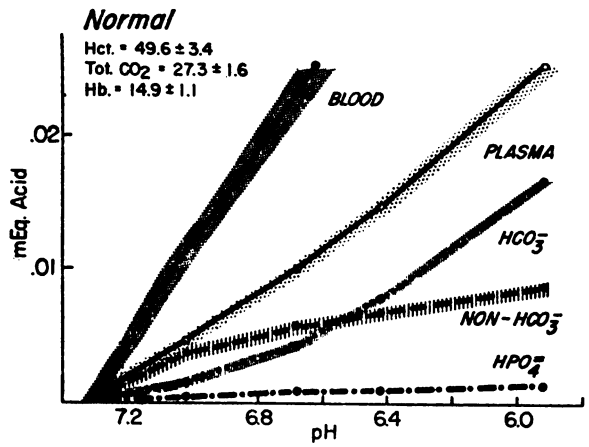

Fig. 1. Titration curves of blood, plasma, and plasma buffer constituents of normal human subjects. The buffer capacities are the slopes of the curves. The vertical axis on this and succeeding figures indicates the milliequivalents of acid added to 1 ml. of blood or plasma.

jects. The curves in Fig. 1, representing the contribution of the bicarbonate, nonbicarbonate, and phosphate buffers, were calculated from the data obtained by separate titration of the plasma using the Henderson-Hasselbalch equation

$$pH = pK_a + \log S/A$$

where S and A denote the salt and acid concentration respectively of the buffer pair. In the bicarbonate system, the pK' was derived from the Severinghaus (11) nomogram to correct for the effects of pH and temperature on the pK' of carbonic acid. The milliequivalents of carbonic acid produced from bicarbonate by each additional increment are calculated and plotted.

For example, in a plasma specimen of a measured pH 7.53 (corresponding to a pH of 7.34 at 38°) with a total CO_2 content of 27.3 mEq/L, the bicarbonate concentration (S) was 25.8 mEq/L and the carbonic acid concentration A was 1.5 mEq/L at 38°. After adding .00516 mEq of HCl to 1 ml. of this sample, the plasma pH fell to 7.21 (corresponding to pH 7.02 at 38° C). Thus S became 24.4 mEq/L and A became 2.9 mEq/L. Consequently 1.4 mEq of acid was produced over a pH change of 0.32. The buffer capacity (BC) was .0014/.32 or .0043 mEq/ml/pH or 4.3 mEq/L/pH.

To calculate the buffer action of the nonbicarbonate systems, the

mEq of carbonic acid formed during the titration were subtracted from the total mEq of acid added. An inorganic phosphate content of 2.1 mEq/L was assumed, and a pK_2 of 7.16 was used for the calculation of the phosphate contribution.

From Fig. 1 it is obvious that the BC of whole blood was about twice as great as that of plasma. The capacity of the nonbicarbonate buffers was greater than that of the bicarbonate buffer near the initial *p*H of the plasma, but after sufficient acid was added to lower the *p*H to 6.5, the bicarbonate buffers became relatively more important than the nonbicarbonate buffers. The net result was that the BC of plasma and blood remained fairly constant over a considerable range.

In Table 1 the values derived from the slopes of the curves after the addition of sufficient acid to lower the *p*H of blood and plasma from *p*H 7.34 to approximately *p*H 7.02 are given. They express the buffer capacities over the physiological range.

Table 2 shows the contribution of each component to the total BC of whole blood. Although the greatest contribution is that of hemoglobin, the buffering by plasma is considerable and is chiefly due to the protein components. The bicarbonate buffering *in vitro* is not as important as it is *in vivo*.

Blood and plasma samples from a number of patients suffering from disturbances in acid-base balance or blood protein content have been examined. The following typical cases were selected to illustrate the alterations of the titration curves observed.

As shown in Fig. 2, there was an evident decrease in the plasma bicarbonate and nonbicarbonate buffer capacities from a patient with a nephrotic syndrome. Since the total CO_2 content was 14.8 mEq/L

Table 2. FRACTIONAL CONTRIBUTION OF THE VARIOUS BLOOD CONSTITUENTS TO THE TOTAL BLOOD BUFFER CAPACITY

	*Present**		*Viikari (3)***	
	BC	*Per cent of total*	*BC*	*Per cent of total*
Blood	38.5	100	36.1	100
Cells	30.8	79	27.9	77
Plasma	8.1	21	8.2	23
Bicarbonate	2.4	6.1	4.8	13
Protein	5.2	13.6	2.8	8
Phosphate	0.6	1.5	0.6	2

*Calculated for the *p*H range 7.34—7.02, and assuming a hematocrit of 49.6.
**Restatement of the original data of Kilpi (2).

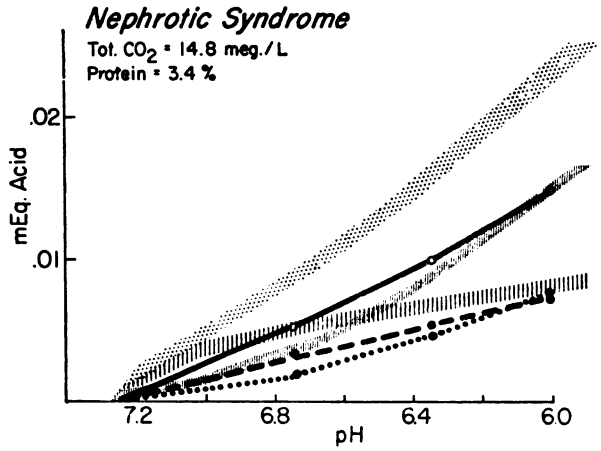

Fig. 2. The titration curves of plasma (solid line), plasma nonbicarbonate buffers (dashed line), and plasma bicarbonate (dotted line) from a patient with the nephrotic syndrome. Titration curve ranges of plasma (dotted band), plasma nonbicarbonate buffers (coarse vertically hatched band), and plasma bicarbonate (fine vertically hatched band) of normal subjects are included for comparison. (See Fig. 1.)

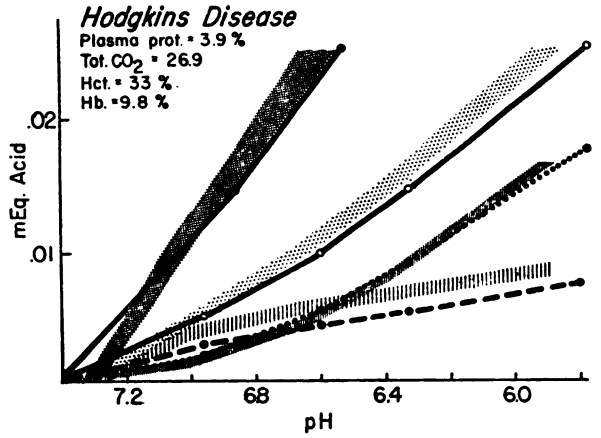

Fig. 3. Titration curves of blood (solid line, closed dots), plasma (solid line, open dots), plasma nonbicarbonate buffers (dashed line), and plasma bicarbonate (dotted line) from a patient with Hodgkin's disease. For comparative purposes are shown the titration curves of normal blood. (See Fig. 1 for symbols.)

and the total proteins were only 3.4 Gm. per cent, decreased buffer capacity was expected.

Variations in blood protein levels are reflected in the shape of the titration curves. The decreased buffer capacities (Fig. 3) of blood and plasma from a patient with Hodgkin's disease are consistent with the hematocrit of 33 per cent, hemoglobin of 9.8 per cent, and plasma protein level of 3.9 per cent, even though the total plasma CO_2 (26.9 mEq/L) was normal. Similarly, a hypochromic anemia patient with blood hemoglobin of 9.2 per cent, hematocrit of 40 per cent, and plasma protein level of 5.8 per cent showed a subnormal blood BC (Fig. 4) with less pronounced changes in plasma BC. The total plasma CO_2 level in this instance was 26.7 mEq/L.

In Fig. 5, the curves from a case of leukemia are shown. The depressed hemoglobin level (11.9 per cent) and hematocrit (44 per cent), together with a low plasma CO_2 (18.8 mEq/L), combined to bring about lowered blood and plasma BC, despite the essentially normal plasma protein level of 6.1 per cent.

DISCUSSION

The data presented here show hemoglobin to be the predominant buffer of blood at physiologic *p*H values, in agreement with Kilpi (2)

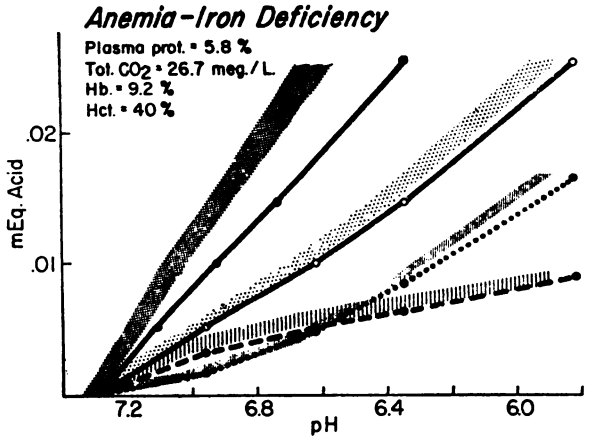

Fig. 4. Titration curves of blood (solid line, closed dots), plasma (solid line, open dots), plasma nonbicarbonate buffers (dashed line), and plasma bicarbonate (dotted line) from a patient with hypochromic anemia. Titration curves of normal blood and plasma are represented by the same symbols as are indicated in the legend of Fig. 3.

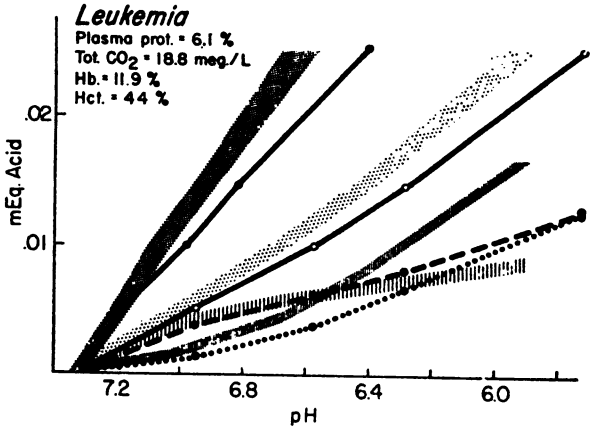

Fig. 5. Titration curves of blood (solid line, closed dots), plasma (solid line, open dots), plasma nonbicarbonate (dashed line), and plasma bicarbonate (dotted line) from a patient with leukemia. Titration curves of normal blood and plasma are represented by the same symbols as are indicated in the legend of Fig. 3.

and Viikari (3, 4). The blood BC values found by Salenius (6) for surgical patients with varying degrees of anemia and by Viikari for patients following surgery were lower than our values for normal subjects. The subnormal blood BC that we observed for various anemias (Figs. 3, 4, 5) also is in accord with this view.

The total plasma BC, which we found to be of the same magnitude as that reported by Salenius, was much smaller than that of whole blood, and allowing for the cell volume makes up only about one-fourth of the blood BC. Obviously the plasma BC is made up of the contributions of the bicarbonate, phosphate, and plasma protein buffers. Because of the small concentration of phosphates, we conclude that plasma proteins account for the bulk of the nonbicarbonate plasma BC. At physiologic *p*H, Kilpi ignored the buffering action of plasma proteins. Viikari concluded from recalculation of Kilpi's data that the BC of plasma bicarbonate was about three times as great as that of plasma proteins. Our findings show almost the exact opposite to be the case, namely, that the plasma proteins are about twice as effective as bicarbonate as buffers in the *p*H 7.34-7.02 range. Measurement of the buffer capacity of plasma in more alkaline ranges (12) might well serve as a basis for the direct titrimetric estimation of protein concentration. Since imidazole groups (with a pK of about

7) of constituent histidine molecules appear to be responsible for the buffering action of proteins over *p*H 7.6-6.0, the plasma protein BC decreases as the *p*H is lowered, while the bicarbonate BC increases rapidly. The net result is that the BC of plasma and of blood is relatively constant over a considerable *p*H range. Since proteins are the chief buffers of plasma at *p*H 7.3-7.0, and plasma bicarbonate is the chief buffer at *p*H 6.4-6.1, measurement of plasma BC over these *p*H values may afford a means of estimating the contribution of each of these buffer systems. Thus plasma with a normal CO_2 but low plasma protein content from a patient diagnosed as having Hodgkin's disease on titration showed a low initial BC that rose rapidly as the *p*H fell (Fig. 3). A sample of plasma from a nephrotic patient with metabolic acidosis and hypoproteinemia had a low BC that increased only slowly as the plasma became acid (Fig. 2).

Titration of blood and plasma may be a useful clinical tool to measure additional parameters in disturbances of acid-base balance and may be of particular value to distinguish between metabolic alkalosis and overcompensated acidosis. The total CO_2 or alkali reserve determination now used in the major determination in acid-base therapy does not adequately indicate the quantitative requirements of the therapy to be employed. Even though the blood *p*H is largely under respiratory control, these data suggest that the blood proteins are more important in acid-base balance than is usually supposed. They may be of critical importance if impairment of respiratory or renal function occurs.

At present the determination of buffer capacity is time-consuming; it would be of greater usefulness if a suitable means of measuring and recording continuous titration curves of blood under anaerobic conditions were developed.

SUMMARY

Normal values for buffer capacity of blood and plasma of a number of normal subjects over various intervals between *p*H 7.5 and 6.1 were determined. The buffer capacities of blood and plasma were 38.5 and 16.1 mEq/L/*p*H respectively at physiologic *p*H values. Normal blood and plasma buffer capacities were relatively constant from *p*H 7.4-6.6. The buffer capacity of plasma proteins was found to be greater than that of bicarbonate at physiologic *p*H range. Variations of buffer capacity caused by certain disease processes are reported and discussed.

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