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Distribution of Chlorides and Bicarbonates in Blood Following Addition of Hydrochloric Acid.

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A recent study of the distribution of chlorides between the plasma and red cells in cases of arterial hypertension (Apperly and Cary[1]) led us to question some of the usually accepted theories concerning electrolyte distribution in blood generally. Van Slyke and others,[2] as the result of experiments and considerations of thermo-dynamics, have formulated certain laws regarding the distribution of water and ions between red cells and plasma under various conditions. One of these laws can be expressed thus:

$$\frac{(Cl)_c}{(Cl)_s} = \frac{(HCO_3)_c}{(HCO_3)_s} = \frac{(H)_s}{(H)_c} = 1 - \frac{(B.Hb)_c}{2(A)_s}$$

where $(Cl)_c$, $(HCO_3)_c$, and $(Cl)_s$, $(HCO_3)_s$, etc., represent concentrations in millimols per liter of water in cells and in plasma respectively, (B.Hb) refers to concentration of base bound by hemoglobin and (A) to concentration of total monovalent ions (largely Cl and HCO_3). From the above it follows that these ratios rise or fall with increasing or decreasing H-ion concentration until finally the ratios equal unity at pH 6.6, the isoelectric point of hemoglobin.

Although these laws were worked out on horse's blood *in vitro*, they have since been confirmed, in the main, on the blood of man and other animals *in vitro*, after the use of varying pressures of CO_2 to produce changes in pH, and in dogs *in vivo*, following the intravenous injection of hydrochloric acid (Harkins and Hastings[3]). We have also found the $(Cl)_c:(Cl)_s$ and $(HCO_3)_c:(HCO_3)_s$ ratios to vary in a roughly parallel manner following a ketogenic diet for 18 days (Apperly and Norris[4]) and as the result of elevation to the moderate altitude of 6000 feet (Apperly[5]). In severe disease the laws also hold good (Muntwyler, *et al.*[6]).

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1 Apperly, F. L., and Cary, M. K., *Am. J. Med. Sci.*, 1937, **194**, 352.

2 Van Slyke, D. D., *Factors Affecting the Distribution of Electrolytes, Water and Gases in the Animal Body*, Lippincott Co., Philadelphia and London, 1926.

3 Harkins, H. N., and Hastings, A. B., *J. Biol. Chem.*, 1931, **90**, 565.

4 Apperly, F. L., and Norris, J. H., *Am. J. Med. Sci.*, 1933, **185**, 802.

5 Apperly, F. L., *Am. J. Physiol.*, 1938, **122**, 179.

6 Muntwyler, E., Myers, V. C., and Way, C. T., *J. Biol. Chem.*, 1931, **92**, 721.

The laws, however, are only approximations. Thus Van Slyke, *et al.*,[7] found by direct observation that:

$$\frac{(H)_s}{(H)_c} = 0.77\frac{(Cl)_c}{(Cl)_s} = 0.62\frac{(HCO_3)_c}{(HCO_3)_s}$$

Further, the ratios do not reach unity at the pH corresponding to the isoelectric point of hemoglobin. Various explanations, such as an incomplete dissociation of Cl, or the formation of carb-hemoglobin in the cells, with an apparent rise in the $(HCO_3)_c$: $(HCO_3)_s$ ratio, have been invoked to explain these discrepancies.

Methods. In an endeavor to avoid some of these possible sources of error we have introduced certain modifications.

(a) In all previous work the blood changes in pH, and consequently in redistribution of ions, have been brought about by the addition of H_2CO_3, HCl or other acid, all involving the addition of 2 different ions, and, generally, an altered total ionic concentration in the blood, so that the variations in the curves obtained were each the resultant of several factors. This has been avoided by comparing bloods in which only the kation was changed, *i. e.*, the Na ion was gradually replaced by H ions, all other ions remaining constant.

(b) The CO_2 combined with reduced hemoglobin as a carbamino compound (carb-hemoglobin) accounts for about 6 volumes % in an average blood sample. In oxygenated blood, however, the carbamino CO_2 is reduced to about one-third of this, and falls to even lower values with fall in pH (Ferguson and Roughton[8]). Since in our experiments all bloods were oxygenated, and in most cases acidified with HCl, the carbamino CO_2 held must therefore, according to these authors, have been very small indeed. The slight, though constant differences between our curves for cell CO_2 and plasma CO_2 correspond closely in magnitude to the carbamino CO_2 content found by these workers and, in further discussions, will be ignored.

Mixtures were made by adding 1.5 cc of 0.5 N chloride solution to 23 cc of oxalated or defibrinated dog's blood. The composition of the chloride solutions varied in the relative amounts of HCl and NaCl, so that the resultant mixtures with blood varied only in their H and Na concentrations. The Cl mixture was slowly added and well mixed. The blood was then transferred to a 400 cc separatory funnel, saturated with the alveolar air of one of us, and the following then determined, *viz.:* Cl (Eisenman's modification of Van Slyke's

[7] Van Slyke, D. D., Hastings, A. B., Murray, C. D., and Sendroy, J., Jr., *J. Biol. Chem.*, 1925, **65**, 701.

[8] Ferguson, J. K. W., and Roughton, F. J. W., *J. Physiol.*, 1934, **83**, 87.

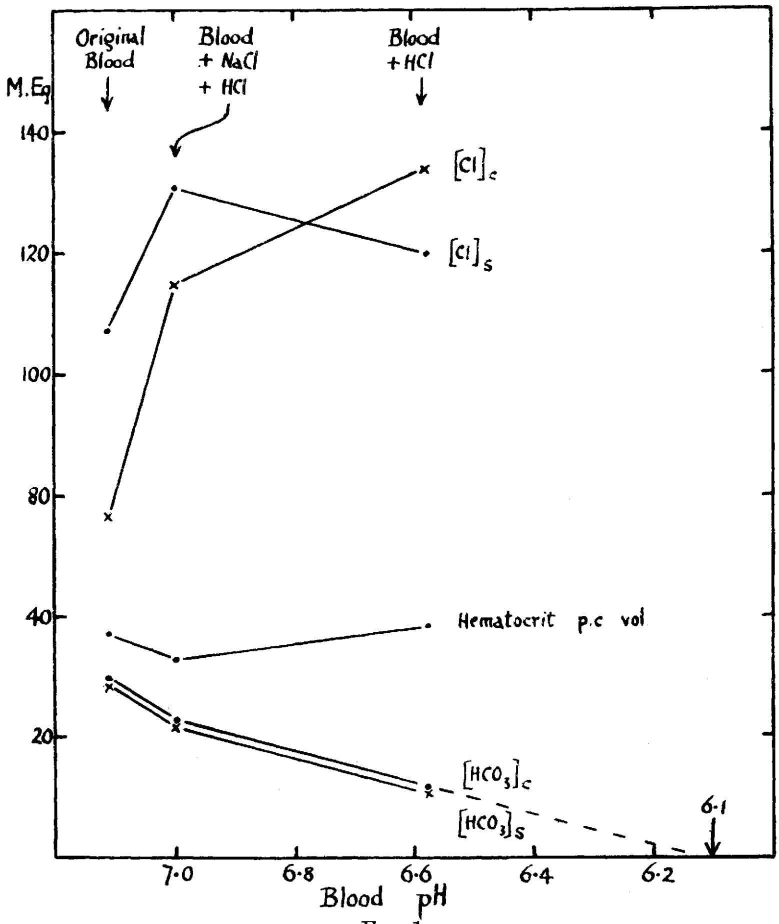

FIG. 1.

Showing alterations in Cl and HCO_3 concentrations in both plasma and red cells of blood following the addition of equal volumes of equi-molecular mixtures of NaCl and HCl.

method[9]) and CO_2 contents (Van Slyke and Cullen[10]) of plasma and whole blood; hematocrit volume of red cells: water content of plasma and red cells (by dry weight): and pH (Coleman glass electrode) of the various mixtures. From these figures the Cl and HCO_3 were calculated as mM per liter of plasma water and of red cell water. Where applicable, all results were corrected as for 38°C.

After many preliminary trials, 10 complete experiments were carried out. In each, (HCO_3) and (Cl) were plotted against pH since below pH 7.0 at any rate, the curves obtained were nearly

[9] Eisenman, A. J., *J. Biol. Chem.*, 1929, **82**, 411.

[10] Van Slyke, D. D., and Cullen, G. E., Jr., *J. Biol. Chem.*, 1917, **30**, 289.

straight lines (Van Slyke, *et al.*[11]). Fig. 1 is typical of the group.

Results. (a) The CO_2 content. The $(HCO_3)_c$ and $(HCO_3)_s$ curves nearly coincide, *i. e.*, the ratio of the former to the latter equals unity irrespective of pH. When these curves are traced to the right they reach their lowest values at about pH 6.1. If the blood is saturated with alveolar air as described above the CO_2 values are about 2 mM at pH 6.1, but if with atmospheric air this value falls to about 1 mM. These figures were confirmed in other experiments with plasma alone by adding sufficient acid to carry the mixture below pH 6.1. In these and other experiments we find a bend in both the plasma and red cell CO_2 curves somewhere between pH 7.0 and 7.2, below which the curves are almost straight lines.

(b) The Chlorides. With diminishing pH the red cell chloride curve rises and the plasma chloride curve falls, but the most careful work failed to give any constant pH point at which the curves crossed, *i. e.*, where $(Cl)_c/(Cl)_s = 1$. In general, the pH at which the crossing occurred seemed to depend upon and to vary roughly with the red cell content of the blood.

(c) The hematocrit volume shows a small but steady rise with falling pH, due, as is well known, to transfer of water from plasma to red cells.

Discussion. In general these results seem to be confirmed when we plot, in similar manner, some of the results of other workers in which acid other than CO_2 was added to blood *in vitro* or *in vivo* and when sufficient data were available for values below pH 7.0. In Harkins and Hastings' paper[3] experiments 2 and 3 and Fig. 7 all show practical disappearance of CO_2 at about pH 6.1. Again at normal blood pH, the average value given by several other authors for cell water is about 65% and for plasma water about 92%. Since the ratio 65/92 equals about 0.70 the same figure obtained by Apperly and Cary[1] for the value of the ratio $(HCO_3)_c/(HCO_3)_s$ for many bloods (when no allowance was made for cell and plasma proteins) it follows that in normal blood the ratio $(HCO_3)_c/(HCO_3)_s$ calculated as concentrations per unit of cell water, must approximate unity. Further, Peters[12] gives the value of $(HCO_3)_c/HCO_3)_s$ at normal blood pH as 0.027/0.028 which also, for practical purposes, equals unity. Since both the $(HCO_3)_c$ and $(HCO_3)_s$ curves are practically straight lines, and both reach very small values or zero at about pH 6.1, it follows that the 2 curves approximately coincide, and therefore that the values of the ratio at all pH levels less than

11 Van Slyke, D. D., Wu, H., and McLean, F. C., *J. Biol. Chem.*, 1923, **56**, 765.

12 Peters, J. P., *Body Water*, Bailliere, Tindall and Cox, London, 1935, p. 99.

normal approximately equal unity when the acid added is one other than CO_2. The HCO_3 ion would therefore appear to be freely diffusible into and out of the cell wall. The differing values of the ratio obtained by other observers, using different pressures of CO_2, suggest that the formation of carbhemoglobin has complicated the picture.

We have not yet further investigated the apparent dependence on the hematocrit value (or red cell content) of the pH at which $(Cl)_c/(Cl)_s = 1$. As already stated, this pH appears to vary directly with the hematocrit value. It would seem reasonable to suggest, however, other conditions being equal, that blood with high red cell content, *i. e.*, better buffered, would take up more CO_2 when saturated with alveolar air, and therefore result in a larger transference of Cl from plasma to red cells. This in turn would mean a more rapid rise in the $(Cl)_c$ curve and a correspondingly rapid fall in the $(Cl)_s$ curve, so that the crossing of the curves is shifted to left. Conversely, the pH at which $(Cl)_c/(Cl)_s = 1$ in anemic blood would be expected to shift to the right, *i. e.*, at a lower pH.

Summary. An investigation has been made of certain aspects of Van Slyke's laws concerning the distribution of ions between red cells and plasma at different pH values. When hydrochloric acid was used to bring about variations in blood pH, losses of CO_2 from plasma water and cell water were equal, so that the ratio $(HCO_3)_c/(HCO_3)_s$ remained unchanged and equal to unity at all pH values. The values of $(Cl)_c/(Cl)_s$ at different pH values did not parallel the values of $(HCO_3)_c/(HCO_3)_s$ but gradually rose to unity at values of pH which varied, the actual value appearing to depend upon and to vary with the red cell content of the blood.

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A New Method of Administering Heparin.*

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In the course of studies on experimental subacute bacterial endo-

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