control of hydrogen ion concentration is absolutely essential before valid conclusions can be drawn as to the influence of electrolytes, alone or in combination.

Furthermore, it is essential to follow with care the changes which go on in a suspension of living and dead cells as well as to determine the initial conditions which are provided. We find that a bacterial suspension in 5 isotonic NaCl solution quickly reverts to a pH of about 7.2 whether its initial hydrogen ion concentration be above or below this value. A similar change takes place in a balanced solution of 5 isotonic NaCl + isotonic $CaCl_2$ but at a much slower rate as indicated by the table below.

HYDROGEN ION CONCENTRATION OF SUSPENSIONS OF *Bact. coli* IN THE PRESENCE OF ELECTROLYTES.

Initial.	5 Isotonic NaCl.			Initial.	5 Isotonic NaCl + Isotonic $CaCl_2$.		
	4½ Hrs.	9 Hrs.	30 Hrs.		4½ Hrs.	9 Hrs.	24 Hrs.
4.0	7.0	7.0	7.2	4.0	4.8	4.8	5.8
5.0	7.3	7.2	7.2	5.0	6.5	6.5	7.2
6.0	7.5	7.2	7.2	6.0	7.2	7.2	7.1
7.0	7.2	7.0	7.4	7.0	7.2	7.2	7.5
8.0	7.2	7.2	7.2	8.0	8.0	8.0	8.0

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Discrepancies in blood oxygen analyses by the methods of Van Slyke and Henderson-Smith.[1]

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Loosely bound oxygen is liberated from the hemoglobin in blood by the addition of potassium ferricyanide. In the Van Slyke method,[2] all the gases are exhausted by means of a Toricellian vacuum from a laked blood-ferricyanide mixture and measured directly. In the Henderson-Smith method,[3] the oxygen is evolved

[1] This work was initiated in the Laboratory of Intermediary Metabolism, Chemical Warfare Service, Yale Station, under Lt.-Col. F. P. Underhill.

[2] Van Slyke, D. D., *Jour. Biol. Chem.*, 1918, XXXIII, 127.

[3] Henderson, Y., and Smith, A. H., *Jour. Biol. Chem.*, 1918, XXXIII, 39.

into a fixed volume of air, a portion of which is analyzed directly for oxygen by absorption with alkaline pyrogallate. After application of all the corrections suggested by the authors of these methods, the results are not identical,—analyses by the Van Slyke method yielding 4 to 10 volumes per cent. more oxygen than those by the Henderson-Smith method. The divergence between the results from each method may represent a variation of 17 to 64 per cent. A few typical figures are cited in Table I.

TABLE I.

Sample.	Van Slyke Vol. Per Cent. O_2.	Henderson-Smith Vol. Per Cent. O_2.	Difference.	
			Amount.	Per Cent.
18*a*. Venous content	12.9	10.4	2.5	19.4
18*b*. Venous capacity	18.5	12.8	5.7	30.8
18*c*. Arterial content	20.1	14.6	5.5	27.4
18*d*. Arterial capacity	20.0	15.5	4.5	22.6

It is clear that there is a constant factor or factors at play, inherent in the methods of analysis employed, which ought to account for this discrepancy.

The gas evacuated by the Van Slyke procedure is not all oxygen but probably contains in addition to nitrogen, minute amounts of carbon monoxide, hydrogen, methane and the rare atmospheric gases. Bohr[1] states that blood contains 1.23 volumes per cent. of nitrogen (incorrectly quoted by Van Slyke as 0.9 vol. per cent.) and 0.22 volumes per cent. of the other gases—a total of about 1.45 volumes per cent. of gas not oxygen. We have absorbed with alkaline pyrogallate, the oxygen from the gas extracted in the Van Slyke procedure and have found in all cases a residue of 0.055 to 0.082 c.c. from 2 c.c. of blood—an average of about 3.3 volumes per cent. This residue does not contain CO_2, and we have reason to believe that it is practically all nitrogen. It occurs to the same extent in aerated blood as in venous. Since this residue is almost constant within narrow limits, its value may be subtracted from the total Van Slyke gas volume as an average correction. We have applied this correction in Table II, and have thereby reduced the level of the Van Slyke figures by about two volumes per cent. A further refinement would be to deduct

[1] Bohr, C., in Nagel, W., "Handb. Physiol. d. Mensch.," 1909, I, 117.

the amount of oxygen physically held in the blood if the analysis is intended to show the amount of oxygen loosely held in the oxyhemoglobin.

In the Henderson-Smith method a small correction should be applied for water vapor pressure. This is however of theoretical interest for it is only 0.1 to 0.3 volumes per cent. in amount. Much more important is the fact that the oxygen content of the air in the diffusion tube has been considered without regard to the amount physically held in the 3 c.c. of blood-ferricyanide mixture. The volume of air in the diffusion tube is constant at about 10 c.c. When 0.5 c.c. potassium ferricyanide is injected through the rubber stopper, this volume is reduced, and the pressure within the tube correspondingly increased by about 5 per cent. As a result of the chemical reaction oxygen is liberated, and the pressure further increased by one per cent. Consequently, the 3 c.c. of fluid within the tube will absorb oxygen in proportion to its increased partial pressure. This physically held oxygen should be added to that determined analytically to give the total contained in the sample of blood.

We have calculated the amount of oxygen that the blood-ferricyanide mixture would hold on the basis of the gas laws (assuming that the mixture has 90 per cent. the absorption capacity of water), and it will be seen from Table II that the level of the Henderson-Smith figures has been raised about 1.5 volumes per cent.

TABLE II.

Sample.	Van Slyke Method.		Henderson-Smith Method.		Difference Between VS and H-S Corrected Values.
	Volumes Old Value.	Per Cent. O_2 Corrected Value.	Volumes Old Value,	Per Cent. O_2 Corrected Value.	
18*a*.	12.9	10.7	10.4	11.7	—1.0
18*b*.	18.5	16.7	12.8	14.1	2.6
18*c*.	20.1	18.0	14.6	16.0	2.0
18*d*.	20.0	18.4	15.5	16.9	1.5

From Table II it is evident that our corrections have brought the Van Slyke and Henderson-Smith values for blood oxygen much closer together than they were originally. The figures are however not identical, and we are now engaged in testing out other factors that may be involved.