6515

Oxygen Physically Dissolved in Blood Cell Suspensions and Hemoglobin Solution.

EN FU YANG. (Introduced by A. Carruthers.)

From the Department of Biochemistry, Peiping Union Medical College.

In Bohr's method[1, 2] for calculating the oxygen physically dissolved in blood or hemoglobin solution the assumption is made that the solubility of the gas in these fluids is as it is in water. In actual practice nitrogen is determined in blood or hemoglobin solution and from this and the solubility of oxygen in water the amount of oxygen physically dissolved in blood or hemoglobin solution is calculated. Direct determination of the oxygen in suspensions of cells or solutions of hemoglobin which have previously been treated with sodium nitrite, after which treatment it is supposed[3] hemoglobin no longer combines with oxygen, shows that the oxygen is greater than is accounted for in Bohr's method of calculation. Assuming the same physical properties for hemoglobin after treatment with nitrite it would appear that a correction should be made in the usual determinations of oxygen chemically combined with hemoglobin.

Treatment of cells or hemoglobin. (1) *Cells:* A mixture of 2 volumes of 0.9% sodium chloride solution and 8 volumes of 1.05% sodium nitrite was added to 10 volumes of fresh sheep's blood cells. The mixture was allowed to stand in the cold for half an hour and centrifuged. The cells were washed 3 times with cold van Dyke-Hastings solution[4] containing 0.60 gm. sodium cyanide per litre. (2) *Hemoglobin:* Cells prepared as above were electrodialyzed and the concentrated hemoglobin solution treated with alumina cream to remove the stroma. Solutions containing 20% hemoglobin could be obtained.

To show that oxidation in the cell preparation was complete, (1) nitrite treated cells were suspended in an equal volume of normal saline mixture saturated with air and 5 cc. were introduced into the van Slyke gas chamber. Five cc. of air saturated ferricyanide solution[5] were added and the oxygen determined. The oxygen from this determination was the same as the oxygen found in a sample

[1] Bohr, C., *Compt. rend. Acad.*, 1897, **124**, 414.

[2] Van Slyke, D. D., and Stadie, W. C., *J. Biol. Chem.*, 1921, **49**, 1.

[3] Haldane, J., Makgill, R. A., and Mavrogordato, *J. Physiol.*, 1897, **21**, 160.

[4] Miller, C. P., Jr., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 748.

[5] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.

of cells mixed with ferricyanide outside of the chamber. (2) Samples of cells were treated with equal volumes of isotonic solutions of various nitrite concentrations and allowed to stand at room temperature for half an hour. When the nitrite concentration was higher than 0.5% the oxygen content showed no change.

Equilibration and analysis: Samples of cells or hemoglobin were equilibrated with various tensions of oxygen at 38°C. in a pair of double tonometers, the gas constantly released to atmospheric pressure and after the pressure became constant equilibration was continued for half an hour. Gas and fluid were separated in the usual manner. Oxygen in the fluid was determined with van Slyke's manometric apparatus,[5] 5 cc. samples being used for each determination, and oxygen in the gas phase was determined with Haldane's gas apparatus.[6] The total solid in the cell or hemoglobin preparations was determined by drying 5 cc. samples over night at 110°C.

Results. The relationship between concentration of cells or

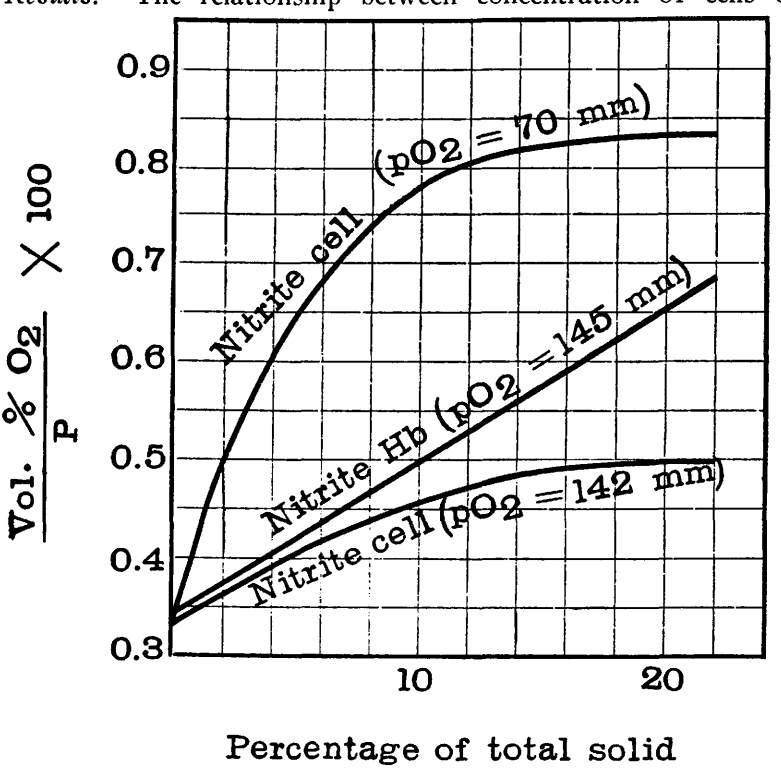

Fig. 1

[6] Henderson, Y., *J. Biol. Chem.*, 1918, **33**, 21.

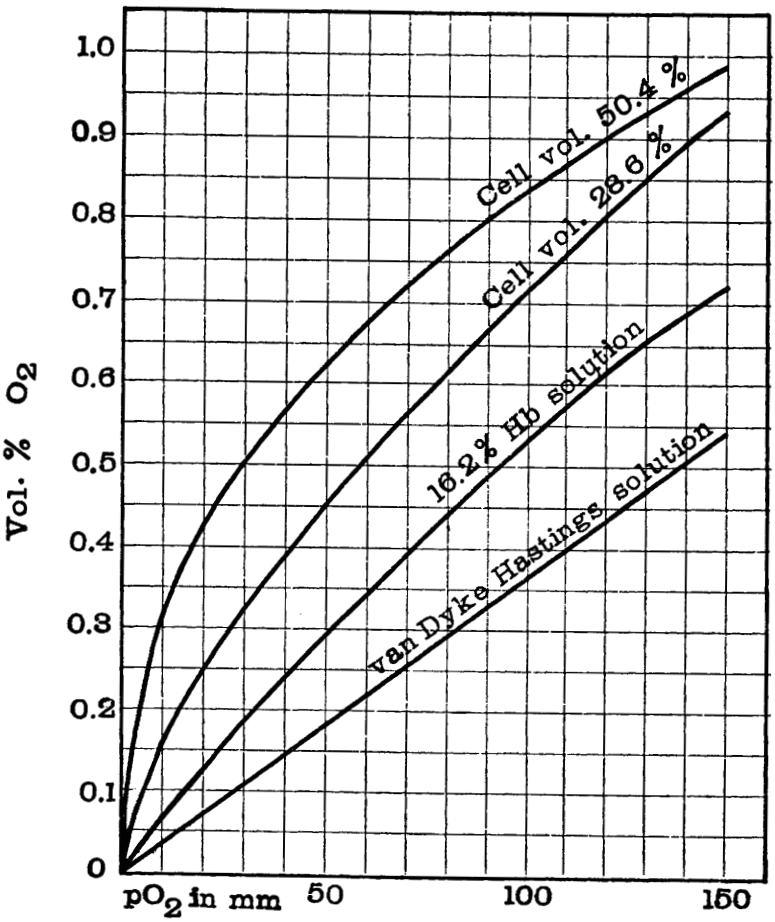

Fig. 2

hemoglobin and oxygen is shown in Fig. 1. For nitrite cells there is an increase in oxygen content up to 50% of cells by volume (*i. e.*, total solid of 16%) but above this level there appears to be no further increase. With nitrite hemoglobin solutions there is an increase in oxygen proportional to the increase of solid matter up to 20%.

Effect of gas tension: Fig. 2 shows that when cells are suspended in an equal volume of van Dyke-Hastings solution and equilibrated with gas of various oxygen tensions the ratio of oxygen in the gas phase to that in the liquid phase decreases with increase in partial pressure of oxygen. Individual preparations of cells give detectable variations, but the shape of the curve is unchanged. With nitrite hemoglobin solution the change in ratio at varying oxygen tensions is slight.