A New Hemolytic Agent for the Manometric Determination of the Oxygen Content of Blood

Marvin J. Yiengst[1]

National Heart Institute, National Institutes of Health, Bethesda, Maryland, and *Gerontology Section, Baltimore City Hospitals, Baltimore, Maryland*

It has been the experience of a number of investigators that some samples of saponin currently available are inadequate as hemolytic agents for the estimation of the oxygen content of whole blood by the manometric method (*2, 3*). It has been found desirable to substitute a solution of 0.25% Aerosol[2] for the saponin. The working reagent is prepared daily by diluting one vol of 1% Aerosol with 3 vol of 0.8% potassium ferricyanide. The stock Aerosol is stable, as satisfactory results have been obtained from solutions stored for a year. Duplicate analyses with a variation of less than 0.05 vol % oxygen on samples of 1 ml of blood are more easily obtained with the Aerosol reagent than with satisfactory grades of saponin because of the cleaner-working and more thorough emulsifying properties of the Aerosol.

TABLE 1

BLOOD OXYGEN VALUES OBTAINED WITH 0.25% AEROSOL AND OTHER REAGENTS

Sample No.	Oxygen content (vol %) 0.25% Aerosol	Urea-albumin reagent	% difference
	Section A		
1	16.00	15.08	−5.8
2	14.39	13.82	−4.0
3	14.27	13.42	−6.0
4	9.36	8.78	−6.2
5	15.13	14.31	−5.4
	Section B		
	0.25% Aerosol	5% Aerosol	
1	15.48	15.28	−1.5
2	15.23	14.81	−2.8
	0.25% Aerosol	2.5% Aerosol	
1	10.36	9.17	−11.5
2	16.00	15.53	−2.9
	0.25% Aerosol	0.5% Aerosol	
1	13.46	13.39	−0.6
2	12.56	12.40	−1.3
	0.25% Aerosol	0.1% Aerosol	
1	17.02	17.03	+0.1
2	18.60	18.65	+0.3
3	11.13	11.09	−0.4
4	6.78	6.82	+0.6
5	6.30	6.28	−0.3

Determinations of the oxygen content of blood samples were made as shown in Table 1. Lack of a supply of satisfactory saponin has made it impossible to compare results obtained with Aerosol and saponin reagents. Aerosol, however, has given consistently higher values than were obtained with the urea-albumin reagent (*2*) (Table 1, *A*). The blank obtained with the Aerosol reagent was uniformly low and stable (0.8–1.0 mm at 2.0 ml gas volume), indicating that no oxygen was liberated from the reagents. Reduced blood prepared as in the carbon-monoxide method of gas analysis (*1*) gave blank values of 0.22 vol % oxygen in three experiments, indicating that no significant amount of oxygen was liberated from blood components other than oxyhemoglobin.

Various concentrations of Aerosol were tried and satisfactory results obtained with concentrations of 0.10% and 0.25% Aerosol (Table 1, *B*). Higher concentrations (5% and 2.5%) produced a gel with blood which interfered with the reading of the meniscus.

[1] The technical assistance of W. I. Jones is gratefully acknowledged.

[2] Aerosol OT, 100% pellets, is dioctyl sodium sulfosuccinate, a product of the American Cyanamid and Chemical Corporation.

References

1. ARNOLD, H. R., *et al.* *Amer. J. Physiol.*, 1921, **56**, 313.
2. HILLER, A., PLAZIN, J., and VAN SLYKE, D. D. *J. biol. Chem.*, 1948, **176**, 1431.
3. VAN SLYKE, D. D., and NEILL, J. M. *J. biol. Chem.*, 1934, **61**, 523.

Radioautographs of Frog Membrane[1]

Russell E. Lux,[2] Paul M. Scott, and John E. Christian

Laboratory of Bio-Nucleonics, Pharmacy Department, Purdue University, Lafayette, Indiana

In connection with problems arising from previous work (*1*) on the permeability of frog membrane, it became desirable to study the mode of ion passage through this membrane. In carrying out this investigation, a reliable method for making radioautographs of frog membrane was developed and is given in the following procedure: Highly radioactive sections of whole frog skin were prepared by exposing the morphological inner surface of a freshly excised membrane to solutions of labeled NaI (I^{131}) for 4 hr. The radioactive solution assayed approximately 10^6 cpm/ml, as determined by depositing aliquots on filter strips 1 cm × 5 cm. The filter strips were counted by placing them lengthwise in closest proximity to an Eck and Krebs counter tube. The active membrane was fixed in Bouin's solution 2 hr, and was then treated according to the following schedule:

70% ethyl alcohol	60° C	30 min
96% ethyl alcohol	60° C	30 min
100% ethyl alcohol	60° C	30 min
50% ethyl alcohol–50% acetone	25° C	5 min
100% acetone	25° C	15 min
100% xylol	25° C	15 min
100% paraffin	60° C	3 hr

[1] The isotope used in this research was obtained on allocation by the U. S. Atomic Energy Commission.

[2] Now with Whitmoyer Laboratories, Myerstown, Pa.