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

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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

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Radioautographs of Frog Membrane[1]

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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.

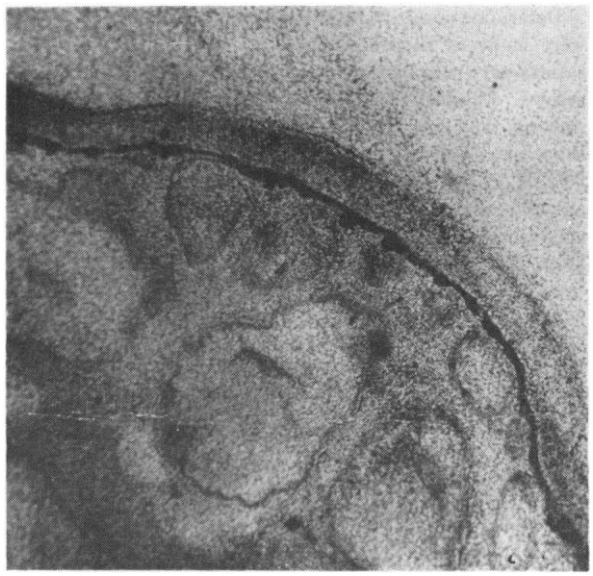

FIG. 1. Radioautograph of frog membrane obtained using I^{131}, showing sievelike penetration of the membrane by I. Magnification 100×. Staining was with methylene blue.

The embedded section was blocked in paraffin, and sectioned to a 10-μ thickness on a Bausch and Lomb microtome. The sections were floated onto microscope slides from warm water placed in Petri dishes. After deparaffinizing 5 min in each of three successive baths of xylol, xylol, and absolute alcohol, the sections were stabilized by dipping in a 1% solution of Mallinckrodt's Parlodion in alcohol ether. The slides so prepared were coated in the darkroom (under a Wratten safelight) by spreading on melted lantern slide emulsion. Slides that counted 930 cpm when placed in closest proximity to a thin-window (2.9 mg/cm²) counter tube gave relatively dense exposures in 8 hr. Generally, however, the optimum exposure time had to be determined empirically. The exposed slides were developed according to procedures recommended by Eastman Kodak for their emulsion. The developed slides were dried, stained with methylene blue when desired, and then mounted as usual.

These investigations revealed that there was no selective avenue of penetration of frog membrane by iodide ion. Reference to Fig. 1 shows that the membrane is penetrated as though it were a sieve. While resolution could be improved by suitably modifying the general procedure, it can be seen that there is no evidence that the glands, for example, are a route taken preferentially by iodide ion.[3] Fig. 2 is a typical radioautograph, which shows unusual concentration of iodine at the keratinized layer of cells (upper surface of section). This was a general finding, and was unexpected, since in every case the opposite membrane surface was the one exposed to the highly radioactive solution. This localization has been tentatively interpreted as indicating that either iodide ion is selectively adsorbed by the keratinized cells, e.g., the constituent proteins of these cells are more easily iodinated than other cells, or that the inert layer of cells acts as a barrier to the passage of iodide ion.

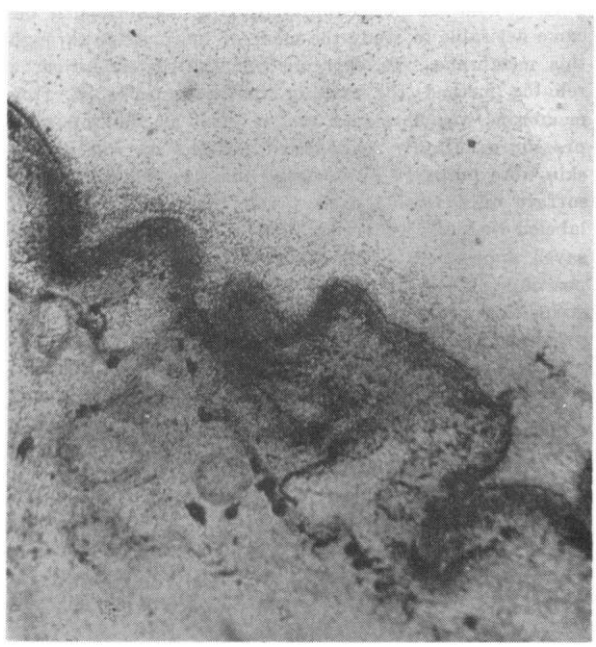

FIG. 2. Radioautograph of frog membrane obtained using I^{131}, showing concentration of iodine at the keratinized cells. Magnification 100×. Staining was with methylene blue.

Reference

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[3] It is worthy of suggestion that the sievelike penetration is due to the relatively few layers of cells of each type which comprise the membrane.

Action of Bacterial Toxins on the "Fragility" of Chicken Erythrocytes[1]

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The effect of a number of toxins on the oxygen consumption of chicken erythrocytes has been reported in a preceding paper (*8*). As the next step in an initial survey of the action of bacterial toxins on cells, measurements were made of the osmotic behavior of chicken erythrocytes exposed to these same toxins. Although it is well known that many pathogenic bacteria produce hemolytic toxins, few studies have been made of the prehemolytic changes brought about by them.

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