Continuous Recording of Blood Oxygen Tensions by Polarography[1]

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POLAROGRAPHIC PROCEDURES using solid microelectrodes to determine the oxygen content of electrolyte solutions and of various tissues, such as brain and skin, have found application in many biological problems (1–19). The theoretical and historical background of polarographic analysis can be obtained from the excellent monographs available (20, 21). Suffice to say that if a platinum cathode is maintained at about 0.6 v. with respect to a nonpolarizable anode, the current which flows as the oxygen is reduced is proportional, under proper conditions, to the oxygen content of the solution. The smaller the exposed platinum surface, the smaller the current and the more rapidly the electrode responds to changing oxygen tensions. One can obtain stable readings in a solution having a given oxygen tension only if the solution is completely quiescent or if it is stirred to the point of maximum response. Use of the electrodes *in vivo* may be influenced by movement of the tissue, the rate of oxygen diffusion through the tissue, and by ischemia produced by pressure of the electrode.

The presence of red cells on the platinum surface interferes with the operation of the cathode. This fact, not completely understood as yet, is recognized as a problem in determining tissue oxygen tensions (5) and has, of course, complicated the application of polarography to the direct determination of oxygen tensions in whole blood (22, 23). Morgan and Nahas (24, 25) reported that covering a rotating platinum cathode with a film of silicone delayed the loss of reactivity in blood, but they reported that the sensitivity and reproducibility were not satisfactory. Brink (23) studied a recessed electrode covered with agar for determining oxygen tension in whole blood but found that the electrode was not stable. Drenckhahn (26) prevented the undesirable effect of erythrocytes by coating the platinum wire cathode with a collodion coating 0.3 mm thick; under these conditions he reported that $2\frac{1}{2}$ min. were required to reach equilibrium between readings, that the electrodes were stable for several days, and that not all electrodes made were useful.

After many unsuccessful attempts to obtain a stable, reproducible electrode for use in whole blood, an electrode consisting of a platinum surface covered with a cellophane membrane was devised and studied under various conditions. Although the work is not yet complete, it is thought desirable to publish the results obtained so far in view of its potentially wide usefulness. The present report describes the construction, standardization and operation of such oxygen cathodes in the determination and continuous recording of the oxygen tension of whole blood *in vitro* and *in vivo*.

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Apparatus

The circuit used was similar to that described by Tobias (11) and Horwitz *et al.* (7). A potassium chloride-calomel electrode (Beckman) was used as the anode and a shiny platinum button covered with cellophane as the cathode. Wrapping the anode tip with saline-saturated cotton was found to improve the electrical contact with bubbling solutions and with tissue. A variable shunt was used to compensate for variations in current from the cathode. The current was measured with a Rubicon galvanometer[2], or, for continuous recording, with a General Electric suspension type photoelectric galvanometer[3].

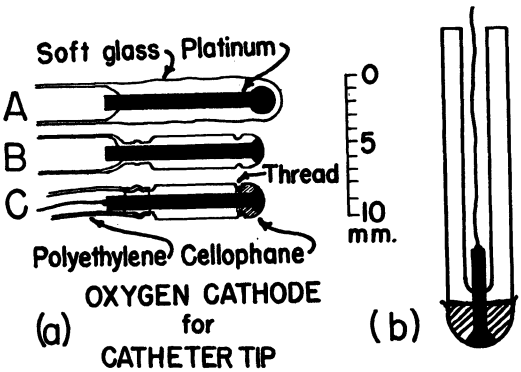

Fig. 1. Construction of cathodes.
(*a*). *A*, 20 gauge platinum wire with bead 2 mm diam. sealed into soft glass tubing. *B*, glass is ground on small revolving stone to reduce diameter, provide recess for thread to hold cellophane, and reduce diameter for fastening catheter tubing. *C*, unit carefully reheated in soft flame to fire polish. Copper lead is soldered in place and catheter tubing and cellophane fastened in place.
(*b*). 20 gauge platinum wire with 2 mm bead is sealed in heavy walled capillary tubing. Surface of bead is ground flush with end of glass using a fine stone or emery paper. In all cases platinum is polished by rubbing with fine pumice.

Table 1. Effect of membranes on cathode response

Membrane	Layers	Thickness, mg/cm²	Galvanometer Stirred	Galvanometer Quiet	Time for Steady Reading, sec.
none	0	0	70	50	10
300 PUT71 cellophane	1	3.17	42	38	20
300 PUT71 cellophane	2	6.34	28	23	30
Dialysis tubing, 1 in.	1	3.52	33	28	45
Dialysis tubing, 1 in.	2	7.04	24	22	45
Dialysis tubing, 1½ in.	1	3.67	36	32	50
Dialysis tubing, 1½ in.	2	7.34	23	20	45
Dialysis tubing, 6 in.	1	16.6	12	12	60
Condom rubber	1	5.31	26	24	90
Condom skin	1	3.21	46	44	60
Polyethylene	1	9.99	18	16	45

After covering the electrode it was placed for 1 min. in saline continuously stirred with tiny streams of oxygen bubbles. Voltage was then applied and time recorded for the galvanometer to reach a steady reading. Time of response of the cathode under usual conditions would be considerably less, since the potential is continuously maintained. Temperature, 38.5°C, Cathode potential, −0.60 v. G. E. Galvanometer. Membranes were dried before weighing.

In figure 1*A* is shown the construction of an electrode for measurement of oxygen tension in saline and blood *in vitro*, while in figure 1*B* is a small electrode suitable for measurement inside the chambers of the heart and in arteries and veins.

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[2] Rubicon galvanometer No. 3403H: 0.007 microamp/mm, 975 ohms resistance, 2.3 sec. period. 7700 ohms CDR.

[3] General Electric Photoelectric Recorder Model 8CE 3CK21B2: 0.96 microamp. full scale, 3.97 sec. response time, 1900 ohm coil resistance, 41, 170 external damping resistance. Chart speed, 1 in./min. and 1 in./hr.

Experimental

Effect of Cellophane Covering. In table 1 is a comparison of the response of a bare platinum electrode with the response of the same electrode covered with various membranes. It can be seen that, while the cellophane covering lowers the absolute current flowing from the cathode, it nearly abolishes the 'stirring effect.' As the membrane becomes thicker the time required for a steady reading, or equilibrium point, increases so that the cathode, in this sense, becomes less sensitive. The time of response of the cathode to varying oxygen tensions after the potential has been on for several minutes is, as will be seen later, measured in terms of a few seconds at most. Selection of the membrane will depend upon the relative importance of these

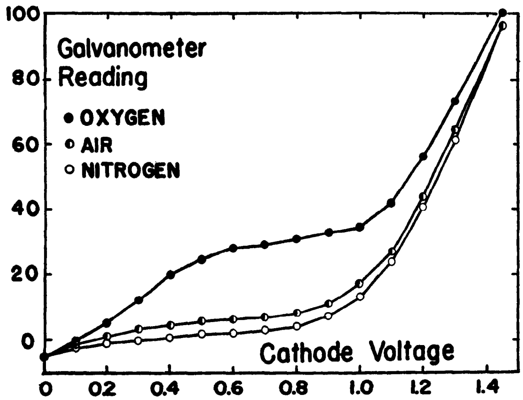

Fig. 2. Cathode voltage vs. galvanometer reading. A solution of 0.9% saline at 40°C was continuously bubbled with the gas mixtures shown.

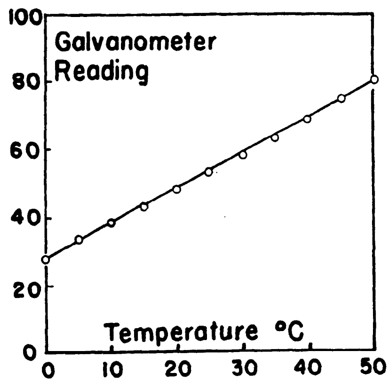

Fig. 3. Effect of temperature. Galvanometer was adjusted to read 100 (full scale) at 50°C by means of a variable shunt. Cathode was immersed in 0.9% saline continuously bubbled with O_2 and slowly cooled to 0°C.

variables for a particular experimental condition. The results to be reported here were obtained using a double layer of 1½ in. dialysis cellophane stretched tightly, while wet, over the platinum tip and secured in place by fine silk thread. Preliminary measurements showed that other films, both wettable and non-wettable, give promise of application as well.

Cathode Voltage. Figure 2 shows the relation between the applied cathode voltage, with respect to the anode, and the current flowing from the cathode as measured by the galvanometer. The potentials across the electrodes were checked with an accurate vacuum-tube voltmeter. The measurements were made in saline solutions vigorously bubbled with nitrogen, air and oxygen. Although the range of −0.6 to −0.8 v. is suitable for these electrodes, the lower value was chosen in order to minimize any undesirable effects such as blood clotting or irritation of the myocardium when the electrode is used *in vivo*. The reversal of current flow, seen in the figure, which occurs at the very low voltages may be due to cation accumulation in the cellophane or perhaps to a capacitance effect, but it has no practical significance since it is independent of the oxygen tension of the solution.

Temperature. Figure 3 shows that the cellophane-covered electrode responds to temperature in a linear fashion. Temperature corrections are easily made, then, after determining the calibration factor from measurements at 3 or 4 temperatures in saline continuously bubbled with either air or oxygen. Such calibrations cannot be made using blood since the pO_2 of blood itself varies with temperature.

Calibration. The cathodes have been calibrated by placing them in saline solutions continuously bubbled with gas mixtures of known oxygen tensions and kept at constant temperature. Perhaps more accurate calibration may be obtained by using plasma equilibrated at known oxygen tensions.

Measurements in Blood. The calculated pO_2 of whole blood, from the CO_2 tension and oxygen saturation, agrees, within the variability of this calculation,

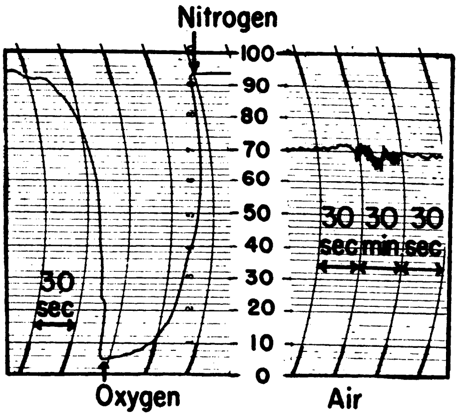

FIG. 4. Oxygen tension in aorta. *Right*, galvanometer tracing (30 min.) obtained while dog was breathing air. Identical readings were obtained 3 hr. previously. *Left*, rapid fall in pO_2 occasioned by the dog breathing nitrogen, followed by a prompt return while oxygen breathing was started. (Heparinized dog, endotracheal tube with cuff and breathing bag). Sensitivity of the galvanometer was reduced for the oxygen-nitrogen breathing experiment. Galvanometer reading of 74 represents a pO_2 of 95 mm on the right, while on the left a reading of 96 represents a pO_2 of 700 mm.

with the pO_2 as measured with the cellophane covered platinum cathode. Blood equilibrated with 5% CO_2, 95% N_2 for 60 min. in a tonometer showed 2% saturation by Van Slyke analysis, 53 vol % CO_2, an oxygen tension by polarography of 1 mm, and an oxygen tension of 2 mm from Barcroft's tables. Another sample, equilibrated with 4.4% CO_2, 6.6% oxygen, and 89.0% nitrogen, showed 90% saturation, 29 vol % CO_2, an oxygen tension by polarography of 52 mm, and of 60 mm from Barcroft's tables. Samples of arterial blood from normal dogs have shown oxygen tensions by polarography of 85–95 mm while after breathing oxygen, tensions up to 700 mm have been measured.

Several cathodes similar to that shown in figure 1*B* have been constructed and used for measurements *in vivo*. Figure 4 shows the galvanometer tracing from an electrode inserted into the aorta via the femoral artery of a heparinized, Nembutalized dog. The sensitivity of the electrode can be judged from the fact that in the air-breathing-record (*right*, fig. 4), the variations in pO_2 related to respiration can be seen. Breathing 100% nitrous oxide produced a curve almost identical with the nitrogen breathing.

Cellophane covered platinum cathodes are now being used to regulate the oxygen tension in the arterial line of the dispersion oxygenator during total by-pass of the heart and lungs (27–29). A similar cathode mounted on a glass probe is being used to explore the oxygen tensions in the normal and fibrillating heart and in other tissues, during total perfusion of the animal while perfusing the coronary arteries at varying rates.

Summary

A shiny platinum cathode covered with a layer of cellophane has been found to be suitable for the direct measurement of oxygen tension in whole blood by polarographic procedures. The cellophane covering prevents the undesirable effects of the red cells which interfere with oxygen tension measurements using a bare platinum electrode, and in addition nearly abolishes the 'stirring effect.' A tiny cathode of this type (2 mm diameter and 8 mm long) mounted on a catheter can be passed into the major blood vessels and the chambers of the heart, for continuous recording of oxygen tension. Such cathodes are easily calibrated and are stable, reproducible and sensitive.

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REFERENCES

1. Davis, P. W. and F. Brink, Jr. *Rev. Sci. Instruments* 13: 524, 1942.
2. Carlson, F. D., F. Brink, Jr. and D. W. Bronk. *Rev. Sci. Instruments* 21: 923, 1950.
3. Olsen, R. A., F. S. Brackett and R. G. Crickard. *J. Gen. Physiol.* 32: 681–703, 1949.
4. Mochizuki, M. *Researches Biophysic*, Monograph Series, Research Inst. Applied Elect. (Japan), No. 2: 39–56, 1951.
5. Montgomery, H. and O. Horwitz. *J. Clin. Investigation* 29: 1120, 1950.
6. Sayen, J. J., W. F. Sheldon, O. Horwitz, P. T. Kuo, G. Peirce, H. F. Zinsser and J. Mead, Jr. *J. Clin. Investigation*, 30: 923, 1950.
7. Horwitz, O., J. J. Sayen, W. F. Sheldon and P. T. Kuo. *J. Clin. Investigation* 29: 823, 1950.
8. Penneys, R. *J. Clin. Investigation* 31: 204, 1952.
9. Behrmann, V. G., W. D. Griest, G. H. Mangum and F. W. Hartmann. *Federation Proc.* 9: 10, 1950.
10. Penneys, R. and H. Montgomery. *J. Clin. Investigation* 31: 1042, 1952.
11. Tobias, J. M. *Rev. Sci. Instruments* 20: 519, 1949.
12. Roseman, E., C. W. Goodwin and W. S. McCulloch. *J. Neurophysiol.* 9: 33, 1946.
13. Davies, E. W., W. S. McCulloch and E. Roseman. *Am. J. Psychiat.* 100: 825, 1944.
14. Davis, P. W. and F. Brink, Jr. *Federation Proc.* 1: 19, 1942.
15. Bronk, D. W., M. G. Larrabee, P. W. Davies and F. Brink, Jr. *Federation Proc.* 5: 11, 1946.
16. Posternak, J. M., M. G. Larrabee and D. W. Bronk. *Federation Proc.* 6: 182, 1947.
17. Bronk, D. W., F. Brink, Jr. and M. G. Larrabee. *Federation Proc.* 7: 14, 1948.
18. Davies, P. W., R. G. Grenell and D. W. Bronk. *Federation Proc.* 7: 25, 1948.
19. Davies, P. W. *Federation Proc.* 5: 21, 1946.
20. Kolthoff, I. M. and J. J. Lingane. *Polarography, vol. 1.* New York: Interscience Publishing Co., 1952.
21. Heyrovsky, J. *Polarographisches Praktikum.* Berlin: Springer, 1948.
22. Brown, L. M. and J. H. Comroe, Jr. *Methods in Medical Research, vol. 2.* Chicago: Yearbook Publishers, 1950.
23. Brink, F., Jr. *Methods in Medical Research, vol. 2.* Chicago: Yearbook Publishers, 1950.
24. Morgan, E. H. and G. G. Nahas. *Federation Proc.* 9: 91, 1950.
25. Morgan, E. H. and G. G. Nahas. *Am. J. Physiol.* 163: 736, 1950.
26. Drenckhahn, F. O. *Naturwissenschaften* 38: 455, 1951.
27. Clark, L. C., F. Gollan and V. Gupta. *Science* 111: 85, 1950.
28. Clark, L. C., F. Hooven and F. Gollan. *Rev. Sc. Instruments* 23: 748, 1952.
29. Helmsworth, J. A., L. C. Clark, S. Kaplan, R. T. Sherman and T. Largen. *J. Thoracic Surg.* 24: 117, 1952.