Oxygen Tension Measurements in Bone Marrow Fleck Cultures

By J. G. M. Davis and H. J. Woodliff

In this paper we describe the methods we have used for measuring oxygen tension in the fluid phase of serum agar cultures of bone marrow in Rose chambers, present the results obtained from a small series of normal marrows and discuss the possibilities of the technic.

Materials and Methods

Cultures.—Marrow fluid was aspirated from the sternum, ilium or vertebral spine and placed in a tube containing 1 mg. of heparin dissolved in 1 ml. of normal serum. After pouring it into a petri dish, the separated flecks of marrow were cultured as described elsewhere.1

Fifty marrow flecks from 7 individuals were cultured, and measurements of oxygen tension in the fluid medium were taken at intervals. Cytologically the marrows were within normal limits.

Method of measuring oxygen tension.—Oxygen tension measurements were made by an electrochemical method based on that described by Cater, Silver and Wilson2 using flush-ended electrodes in conjunction with a transistor amplifier. The oxygen-cathode electrodes were made from platinum wire (0.33 mm. diameter), insulated with 8 coats of "araldite 985e" resin (kindly supplied by Aero Research Ltd., Duxford, Cambridge, England). The reference electrode of silver/silver chloride was in electrical contact with the culture fluid through an agar bridge. The bridge was made from thin glass tubing pulled to a capillary of about 1 mm. bore at one end and bent through 90 degrees to facilitate the accommodation of the chambers in the incubator. Both the platinum electrodes and the bridge were inserted through guide holes in the rubber gaskets of the Rose chambers (fig. 1), until they protruded several millimeters into the interior. A molten 2 per cent agar in balanced salt solution was then introduced into the bridge and the end plugged with drawn-out polythene rod. The culture fluid used was 20 per cent normal serum and 80 per cent medium 199; after equilibration with air containing 5 per cent carbon dioxide it was introduced into the chambers by means of a syringe. The gasket holes were oversealed on the outside with high vacuum grease to prevent any diffusion of air into the chamber. The platinum electrode was left sealed into the chamber for the duration of each experiment, but for ease of handling the reference electrode was inserted into the agar only when required for reading. A potential of 600 mV was placed across the electrodes and the current passing was measured in terms of amps. $\times 10^{-6}$ using a transistor amplifier.

Correlation of oxygen tension and electrical resistance.—Cater and colleagues3 found an approximately linear relationship between current and oxygen tension over the range 7.2 to 143 mm. of mercury with their earlier apparatus and a similar relationship with the apparatus used here.5

We have confirmed this using our own tissue culture fluid over a range from 0 to 680 mm.
Fig. 1.—Culture chamber and electrodes. A potential of 600 mV is supplied by the constant output box via lead D to the reference electrode B. Oxygen diffusing onto the end of the platinum electrode E is reduced and a current proportional to its tension flows from B through the fluid medium to E, which is connected to the current measuring box. The marrow fleck on top of the agar pillar is diagramatically represented (A); oxygen from the medium diffuses to the cell where it is utilized, and as a result the oxygen tension diminishes.

The range in which our readings were made, that is up to 150 mm. of mercury, is illustrated on a larger scale in figure 3b. To make these readings a Rose chamber fitted with electrodes was perfused with culture fluid with the aid of a peristaltic pump (American Instrument Company, Silver Spring, Md., Cat. No. 5-8950). Alterations in the oxygen tension of the fluid were made by bubbling various gas mixtures through the special equilibration apparatus (fig. 2), which was connected to the pump and the chamber by nylon tubing.

Readings were taken with the pump switched off to avoid mechanical fluid disturbance at the ends of the electrodes. The fact that the calibration curve does not go exactly through the origin may be due to the diffusion of air through the nylon tubing after equilibration with 95 per cent nitrogen and five per cent carbon dioxide.

Readings of oxygen tension in the cultures. Readings were taken immediately after removal of the chambers from the incubator, while they were maintained at body temperature on a warm plate. An even oxygen tension in the fluid was obtained by inverting the chamber by hand several times; this procedure in no way damaged the culture which was held in position by the agar pillar.

RESULTS

Figure 4 shows the oxygen tension curves obtained with a control containing no cells (top line) and with cultures of one fleck of bone marrow from each of seven individuals. The 50 curves obtained, which are represented by those illustrated, followed a general pattern. The oxygen tension fell moderately at first and then more steeply for about two days before falling off with the decline in activity of the culture. The utilization of oxygen measured by the fall in oxygen tension, varied considerably with the size of the fleck and between different individuals. Curves obtained from the flecks from a single specimen showed a closer resemblance to one another than to those from a different specimen.
Fig. 2.—Gas equilibration apparatus. The filtered gas is lead into A and then by controlling tap B into either chamber C or E where it equilibrates with the culture fluid before being discharged through the water seal F. Culture fluid is placed in the reserve medium chamber C and by controlling tap H some of it allowed to flow into chamber E. After equilibration the fluid flows via I to the Rose chamber and then through a peristaltic pump back to the apparatus via J.

**Discussion**

Measurements of oxygen uptake in vitro have long been used as tools for the investigation of intermediary metabolism. Usually the Warburg or Cartesian diver techniques have been used, and although electrochemical methods have been known since the end of the last century it is only recently that they have been employed in biologic research. The reason for this has probably been the difficulties in developing the technic quantitatively. Davies and Brink made absolute measurements of oxygen tension but could only make one observation every 5 to 20 minutes; they also found that after one hour the current readings fell gradually when they made in vitro measurements. Montgomery and Horwitz used a similar method to measure oxygen tension in skin, but there was considerable variation in the day to day readings of their electrodes. Cater, Phillips and Silver considered that their apparatus could be
used to give an approximate measurement of absolute oxygen tension in addition to its useful role of measuring changes in oxygen tension. Cater, Silver and Wilson have now developed the technic further and consider that the results are reliable; this is further borne out by the work presented here.

The only cell culture studies in which electrochemical methods of oxygen tension measurements have been employed that we have been able to trace have been those of Harris and Barclay. The apparatus they used to study the respiration of rabbit macrophages differed in detail from the one used in the studies; for example, they used a potassium chloride agar bridge containing a reference electrode of lead. They reported a logarithmic relationship between current flow and oxygen tension, but later Harris when studying the respiration of the rat connective tissue cells in vitro found a curvilinear relationship when the glass bridges were replaced by nylon. This he attributed to external resistance of the circuit and to the fact that the potential developed by the lead half cell was not constant but fell with diminishing oxygen tension. From the practical point of view the important factor is that the relationship between oxygen tension and current flow should be constant.

Harris in his studies was able to count the number of cells in his preparations and so measure both the rate of cell multiplication and the oxygen uptake. Because of the nature of our explant we could not do this and have been content to record only the general pattern of oxygen utilization by marrow flecks. We have commenced preliminary studies on the effects of antileukemic drugs on the oxygen uptake of normal marrow flecks, and we hope to extend the study to see if the pattern differs in different diseases affecting the bone marrow. We hope to develop an in vitro sensitivity test of leukemic marrows.
Fig. 4.—Oxygen tension measurements of the fluid phase of 7 bone marrow fleck serum agar cultures from 7 different individuals. The top line is a control containing no cells but otherwise exactly comparable to the cultures.

based on the hypothesis that the oxygen uptake of resistant cells would not be affected by concentrations of the same chemotherapeutic agent causing inhibition of oxygen uptake by sensitive cells.

SUMMARY

An electrochemical method of measuring the oxygen tension in the fluid phase of serum agar cultures of bone marrow flecks is described. The normal pattern of oxygen uptake by the flecks is presented and the possible applications of the technic discussed.

SUMMARIO IN INTERLINGUA

Es describite un methodo electrochimic pro le measuration del tension de oxygeno in le phase fluid de culturas de medulla ossee in agar-sero. Le modo normal del acception de oxygeno per le particulas de medulla ossee es presentate, e le applicationes possibile del technica es discutite.

REFERENCES

3. —, Phillips, A. F., and Silver, I. A.: Apparatus and techniques for the measurement-
Oxygen Tension Measurements in Bone Marrow Fleck Cultures

J. G. M. DAVIS and H. J. WOODLIFF