Toxicity of oxygen at amospheric concentration for newly explanted cancer cells

Our experiments [1, 2] on the destruction of blood-borne cancer cells by oxygen when present at concentrations found in arterial blood (i.e. close to equilibration with air) may be relevant to the cytoxicity of unreduced misonidazole. They also suggest that extrapolation from results obtained with established tissue culture cell lines to the response of tumours *in vivo* may be misleading when studying agents the toxicity of which depends on the level of intra-cellular glutathione.

In this symposium Biaglow *et al*. [3] reported that inhibition of growth by misonidazole *in vitro* under aerobic conditions of long-established tissue culture cells lines is greatly augmented (and indeed often only becomes apparent) if the cells are depleted of glutathione by being grown in the presence of 1-buthionine sulphoximine (BSO). We have found a similar phenomenon for the toxicity of dissolved oxygen and find that cells which grow equally well in cultures equilibrated with air or an atmosphere containing only 5% oxygen will after BSO treatment grow only in cultures having low amounts of dissolved oxygen. However, we frequently find that cancer cells derived by direct explant from tumours grown *in vivo* will not grown *in vitro* unless the partial pressure of oxygen in medium is reduced to some 40 mm or so either by equilibrating the culture with an atmosphere low in oxygen or by working at high cell densities when the rate of consumption of oxygen exceeds the rate of diffusion of oxygen into the culture and hypoxia results.

We were led to carry out these experiments in attempts to explain the inefficiency of blood-borne metastasis [1]. Only a very small fraction of cancer cells which gain access to the blood give rise to metastases and the vast majority die. *In vivo* all normal cells, except those lining blood vessels, as well as the majority of cancer cells divide in a milieu provided by extra-cellular fluid in which the concentration of dissolved oxygen is approximately one quarter of that in arterial blood (i.e. that attained by equilibration with air). Cancer emboli after being arrested in a capillary bed will be exposed until they have succeeded in extravasating to concentrations of oxygen that can be much higher than those found extravascularly. The precise oxygen concentration encountered will depend on the position within the micro-vasculature where the emboli are trapped. Thus in the lung the oxygen concentration will be at its lowest on the arterial side of the capillary and at its highest at the venous end, whereas in other organs (except those provided with a portal blood supply) the oxygen concentration will be higher for cells stopped in small arteries or precapillary sphincters than in the actual capillaries.

Recently explanted cells and cells adapted to culture

The hypothesis that oxygen at a concentration approaching that in equlibrium with air is harmful to cancer cells and that oxygen toxicity may contribute to the death of cancer cells trapped in capillary beds is not at variance with general tissue culture experience. While there can be no doubt that long established cell lines which can proliferate *in vitro* from low cell numbers (or indeed as single cells) are not inhibited by oxygen at normal concentrations, this does not apply to the *in vitro* growth of cells recently explanted, which are not adapted to standard *in vitro* conditions and which usually require large cell inocula if they are to proliferate. When the cell concentration is high, the actual oxygen concentration to which the cells are exposed *in vitro* can be very much lower than that which corresponds to the atmosphere to which the cultures are exposed because of consumption of oxygen which is not compensated by the slow diffusion in the tissue culture vessels. The magnitude of this effect depends on the seeding density and the metabolic rate of the cells [4].

To determine the effect of oxygen tension on growth therefore, cells need to be seeded at low densities so as to minimize depletion of oxygen. Indeed, at high *in vitro* cell densities, the oxygen concentration may be below that needed for optimum growth even when the culture is exposed to the atmosphere. There have been several reports [5] that cells isolated directly from animals grow at low seeding densities better in cultures exposed to atmospheres containing 5% oxygen than in cultures equilibrated with air. We [1, 2] have investigated the effect of oxygen concentration on the rate of proliferation of sarcoma and carcinoma cells taken from mice and rats with tumours, using the procedure outlined in Fig. 1. Most, but not all, of the cancer cells so far studied grow from low cell numbers only in atmospheres with low oxygen concentrations. The toxic effect of atmospheres rich in oxygen is lost as the initial seeding density is increaséd (see Fig. 2). Indeed, at the highest seeding densities cells grow less well or not at all when exposed to atmospheres low in oxygen. At high cell densities the rate of depletion by metabolism of oxygen in the medium is so great that the oxygen concentration with which the cells are in contact and measured with an oxygen microelectrode is now insufficient for optimal growth. The general pattern for many cancer cells prior to adaptation to tissue culture is that they proliferate optimally at oxygen concentrations equivalent to atmospheres of 2% to 5% (as found in extracellular fluid) and die at concentrations of oxygen that correspond to an atmosphere with more than 10% of oxygen.

We have noted that cancer cells rapidly adapt to oxygen when grown *in vitro* and that sometimes after as few as two passages *in vitro* and usually after five passages, the cells grow equally well when exposed to 18% as to 5% oxygen, even at low cell densities when measurements with oxygen micro-electrodes show that high oxygen concentrations are achieved at the point of contact of the medium with the cells.

Role of intra-cellular glutathione and susceptibility to oxygen

The toxicity of an atmosphere containing 20% of oxygen for freshly explanted sarcoma cells from the FS19 mouse tumour when cultured at 4×10^4 cells/ml was not abolished by the addition to the culture medium of 0.1% catalase, 0.1% superoxide dismutase or 1 mg/ml of mannitol. We thus have no evidence which suggests a direct role of hydrogen peroxide, peroxy radicals or OH radical in the oxygen toxicity studied here. I believe it would be useful to explore the idea that the oxygen toxicity is the result of a direct effect on enzymes involved in intermediate metabolism. A lactic dehydrogenase which is poisoned by oxygen at aerial concentrations has been identified in cancer cells [6] and the inability of spirochetes to grow aerobically has been attributed [7] to inhibition of its pyruvate oxidase by oxygen at partial pressures greater than 50 mm of Hg.

The level of intra-cellular glutathione is a key factor in determining sensitivity to oxygen at physiological concentrations. Thus cells that usually grown equally well in atmospheres of 20% oxygen as of 5% oxygen die in an atmosphere containing 20% of oxygen, when the culture medium contains BSO yet grow normally in equilibrium with 5% oxygen. We have observed this both with sarcoma cells referred to as FS19V which acquired resistance to oxygen after prolonged growth *in vitro* and with those tumour cells like the MT1 mammary carcinoma which grows in 20% oxygen even when obtained directly from the animal. With the oxygen-resistant fibrosarcoma line addition of 0.2 mM BSO did not affect growth at 5% oxygen, but caused lysis in 20% oxygen. The parent FS19 cells when taken directly from the animal fail to grow in the presence of 0.2 mM even under hypoxic conditions and
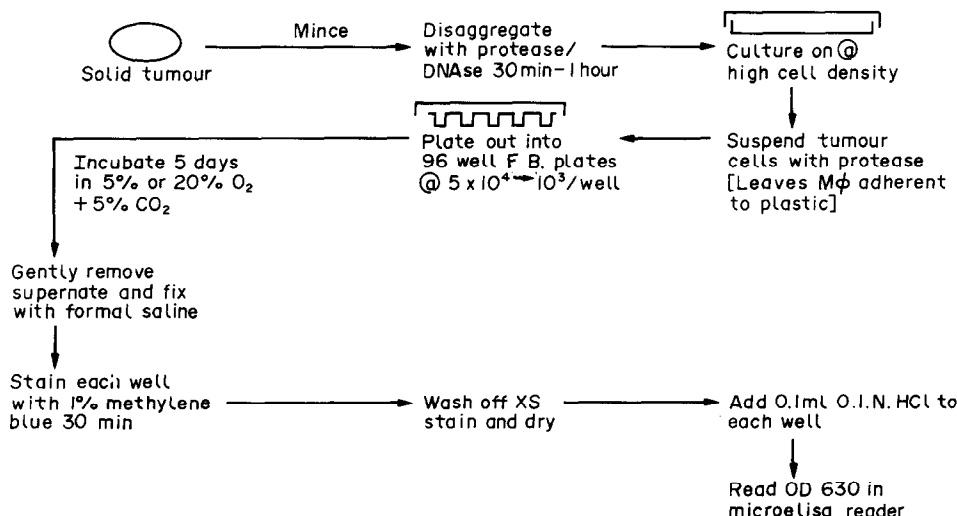
Fig. 1. Outline of "proliferation assay" to determine growth of freshly explanted tumour cells at different concentrations of oxygen.
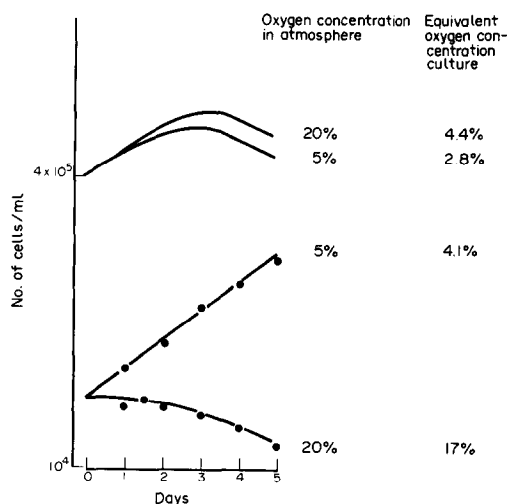

Fig. 2. Proliferation of FS19 murine sarcoma cells derived directly from a tumour plated at two different cell densities in an atmosphere of either 20% or 5% of oxygen. The oxygen concentration in the culture at the level of the cells (the steady-state concentration) was determined 16 hours after plating with an oxygen micro-electrode and expressed as the equivalent atmospheric oxygen concentration with which it is in equilibrium.

die *in vitro* if the oxygen concentration in the medium is in equilibrium with air but can proliferate in cultures at lower oxygen concentrations. This sensitivity to oxygen is lost after cells have been grown for some time *in vitro*, but such cells again become sensitive to oxygen if glutathione synthesis is inhibited by the presence of 1-buthionine sulphoximine (BSO) in the culture.

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the maxima concentration tolerated is 0.025 mM BSO. With 0.025 mM BSO added MT1 carcinoma cells grow normally at 5% O_2, but die in an atmosphere of 20% oxygen.

We conclude that frequently cancer cells, when taken directly from *in vivo* growing tumours, fail to grow, and

REFERENCES

1. P. Alexander and S. A. Eccles, *Cancer Invasion and Metastasis* (Eds. S. Nicolson and L. Milas), p. 293. Raven Press, New York (1984).
2. R. Clarke, P. V. Senior and P. Alexander, *Treatment of Metastasis: Problems and Prospects* (Eds. K. Hellman and S. A. Eccles) p. 223. Taylor & Francis, London (1985).
3. J. E. Biaglow. M. E. Varnes, L. Roizen-Towle, E. P. Clark, E. R. Epp, M. B. Astor and E. J. Hall, *Biochem. Pharmac.* **35**, 77 (1986).
4. R. J. Werrlein and A. D. Glinos, *Nature, Lond.*, **251**, 317 (1974).
5. A. Richter, K. K. Sanford and V. J. Evans, *J. nat. Cancer Inst.* **49**, 1705 (1972).
6. G. R. Anderson, W. P. Kovacik and K. R. Mariotti, *J. biol. Chem.* **256**, 10583 (1981).
7. J. T. Barbieri and C. D. Cox, *Infection & Immunity* **31**, 992 (1981).