Catches of male *Sesamia cretica* Led. in traps baited with Z-9-TDOL and Z-9-TDA at 3 locations in Gezira

| Bait (µg) Z-9-TDOL | Z-9-TDA | Mean No. males/trap* |
|---|---|---|
| 1000 | 0 | 4 |
| 750 | 250 | 48 |
| 500 | 500 | 15 |
| 250 | 750 | 8 |
| 0 | 1000 | 2 |

*4 replicates.

In the experiments conducted in Gezira region, the traps were placed in the field on 16 November and collected on 20 December. During this time they were inspected at 2-day-intervals. In all experiments each lure was tested in 4 replicates.

To point out the effect of the combination of the 2 compounds, various mixtures of Z-9-TDAL and Z-9-TDA were field tested. The table shows the average numbers of males *S. cretica* captured per trap during the period of the experiment.

It is evident from the results that the mixture Z-9-TDOL and Z-9-TDA is a potent attractant for male dura stemborer. More field trials with various release rates, compounds of greater purity, and different ratios of the 2 components are needed to optimize male attractancy.

## The role of oxygen diffusivity in biochemical reactions

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*Summary.* It has been shown that increasing protein concentrations can decrease oxygen diffusion in 3 in vitro systems. We postulate that it is possible, and in some circumstances probable, that diffusion might be a rate limiting step in both in vitro and in vivo biological systems.

In almost every biological system, substances which react inside a cell must first diffuse to and then into that cell. Diffusion, the process by which molecules move through quiescent liquids or boundary layers is a very slow process. It may be the controlling factor in any diffusion plus reaction sequence unless the reaction rate is extremely slow. The diffusion step is generally ignored in determining the rate of metabolism in biological systems. This is done by assuming that the thickness of any quiescent liquid film or boundary layer surrounding the cell is quite thin and thus offers negligible resistance to transport. Although such boundary layers may be quite thin, the transport rate across them may govern the rate of cell division[4].

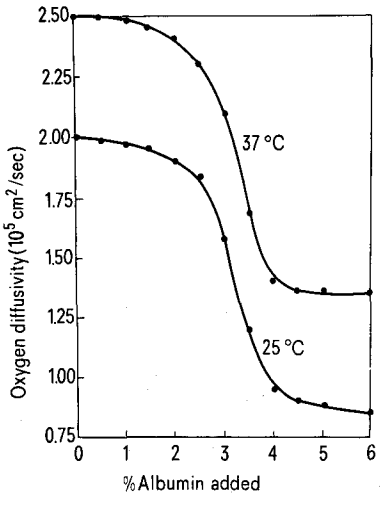


Fig. 1. Variation of oxygen diffusivity with albumin concentration in plasma.

In this study we have attempted to determine the role of diffusion across the boundary layer in the metabolism of L1210 mouse leukemia cells. Specifically we have examined the effect of altering diffusion rates on the supply of oxygen available for aerobic metabolism. Diffusion rates can be altered by adding many types of compounds to the liquid medium[5,6]. A substance which appears to have a substantial effect on the rate of diffusion is protein[7]. As the protein level increases, even over physiological ranges, the diffusivity of oxygen decreases.

*Materials and methods.* The effect of protein levels on the availability of oxygen was studied for L1210 cells. The rate of oxygen consumption by L1210 cells was determined as a function of protein concentration. The cells were grown in a medium consisting of RPMI 1640 plus 10% fetal calf serum. They were then harvested and resuspended in 3 ml of the growth medium in a final concentration of $9 \times 10^5$ cells/ml. This suspension was then placed in the chamber of an oxygen electrode (maintained at 37 °C by a constant temperature bath) which was connected to an oxygen monitor (Yellow Spring Instruments) and a strip chart recorder. The suspension was continuously stirred by means of a magnetic stirring bar.

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The rate of oxygen utilization was estimated from the slope of the oxygen utilization curve on the strip chart recorder. The sample was exposed to air to allow oxygenation of the medium and an initial slope for the oxygen consumption was determined. The system was then reoxygenated, and lyophylized human plasma was added to the medium. This process was repeated so that one sample would be exposed to concentrations of lyophylized plasma proteins ranging from 0 to 8% (weight to volume). The total protein concentration in the sample was actually higher for each point (thus shifting the curve to the right) because the growth medium itself contained approximately 0.4% protein. Total time for an experiment was approximately 30 min.

*Results and discussion.* Figure 1 shows the variation of oxygen diffusivity in plasma with albumin concentration. Figure 2 shows the effect of increasing protein concentration on the rate of oxygen consumption of L1210 cells. It is apparent from the configuration of the curves shown in figures 1 and 2 that diffusion may be a factor in limiting the rate of metabolism. Figure 1 represents a measurement of diffusion only, whereas figure 2 represents a measurement of diffusion plus the process of oxygen uptake by L1210 cells. The data in figure 1 were obtained by estimating the oxygen diffusivity in plasma using a standard diaphragm cell method[8]. No biochemical reactions are taking place in this system, so that the only process being measured is the diffusion of oxygen from points of high concentration to those of lesser concentration. In order to determine if diffusion would still be a rate-limiting factor in a nonbiological system containing both diffusion and a chemical reaction, further experiments were performed.

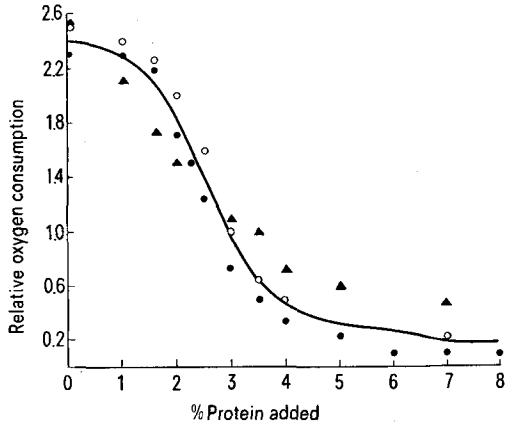


Fig. 2. Relative oxygen consumption as a function of added lyophilized human plasma at 37 °C. The different symbols represent different experiments.

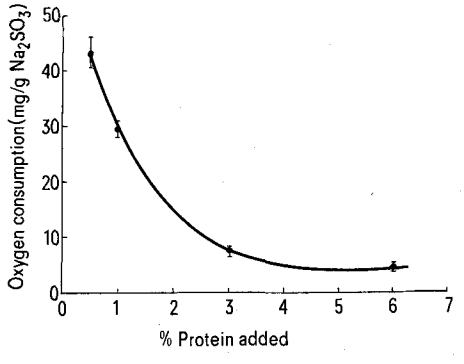


Fig. 3. Oxygen consumption in $Na_2$ $SO_3$ reaction as a function of added lyophilized bovine serum at 25 °C.

These experiments were similar to the experiments described using L1210 cells. The L1210 cells were replaced in these experiments by sodium sulfite which reacts with oxygen to produce sodium sulfate. The sulfate ion concentration was quantitated in order to determine the magnitude of oxygen utilization at various time points. These experiments were conducted to control for the possibility that high protein levels might affect metabolism of L1210 cells, and are frequently done to determine oxygen consumption[9].

Figure 3 shows data obtained in this experiment. The curve again has a configuration and relative magnitude similar to those shown in figures 1 and 2. Because of limitations imposed by the methods employed, these experiments were carried out at either 25 °C (figures 1 and 3) or 37 °C (figures 1 and 2). As can be seen from figure 1, temperature has a substantial effect on the magnitude of oxygen diffusivity but very little effect on the concentration of protein required to decrease oxygen diffusivity to half-maximum (3.3% protein at 37 °C and 3.2% protein at 25 °C). For figure 2 the apparent half-maximal protein concentration is 2.8% added protein but since the L1210 cell growth medium contains 0.4% protein the actual half-maximum is at approximately 3.2% total protein. It is significant that the curve in figure 3 has approximately the same configuration as those in figures 1 and 2. The half-maximal protein concentration for this experiment is approximately 1.3%. The differences in half-maximal effect between the experiments may be due to some difference in the chemical composition of the protein solutions used. Alternatively, endogenous antioxidants present in serum (e.g. ascorbic acid and glutathione) may have caused the entire curve in figure 3 to be shifted to the left. Because of the methods used to generate the data in figures 1 and 2, no effect of endogenous antioxidants would be expected in these experiments.

It would thus appear from these 3 sets of experiments that, under certain circumstances, oxygen diffusivity might well be a rate-limiting factor in the metabolism of living cells. Since diffusion is frequently assumed to be negligible in metabolism studies, such studies might be reflecting some rate-limiting effects of diffusion and not solely metabolic rate. These experiments have been concerned with the effects of protein levels on oxygen diffusion; however, diffusivity is also greatly affected by other compounds such as glucose[5].

We would postulate that it is possible and in some circumstances probable, that diffusion may be a rate-limiting step in biological systems. We have shown this to be the case in several in vitro systems, but it may also be true for in vivo systems. Since diffusion may be a more important factor in biochemical reactions that previously recognized, diffusivity should not be ignored as a potential rate-limiting step in biological systems.

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