The Effect of Oxygen and Paraquat on the ^{14}C-Glucose Oxidation of Rabbit Alveolar Macrophages and Lung Slices

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SUMMARY

In this study, we measured the effects of different concentrations of paraquat (0,01 mM and 1,0 mM) on the 1-$^{14}CO_2$ and 6-$^{14}CO_2$ production of rabbit lung slices and isolated alveolar macrophages, in 20% and 95% oxygen phases respectively.

A 95% oxygen phase induced an increase in the 6-^{14}C-glucose oxidation of control lung slices over a 3-hour period, while the increased activity of the pentose pathway over the first 2 hours started to decline during the third hour of incubation.

Paraquat (1,0 mM) in 20% oxygen caused a consistent increase in the 6-$^{14}CO_2$ production by lung slices, but in a 95% oxygen phase gradually inhibited the 6-^{14}C-glucose oxidation over a period of 3 hours. The pentose phosphate pathway was highly significantly stimulated by 1,0 mM paraquat in 20% and 95% oxygen over 3 hours. When isolated alveolar macrophages (viability 95%) were incubated in a 20% and a 95% oxygen phase respectively, both the 6-^{14}C-glucose and 1-^{14}C-glucose oxidation rates were significantly inhibited by 1,0 mM paraquat after 1 hour.

Our results confirmed the initial increase in glycolytic metabolism induced by paraquat, but also indicated that the 6-$^{14}CO_2$ production was significantly inhibited by paraquat when lung slices were incubated in a 95% oxygen phase. The fact that the glucose metabolism in alveolar macrophages is more sensitive to paraquat exposure than that of cells in lung slices may be related to the genesis of the intra-alveolar pulmonary lesions described in the literature.

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The metabolic functions of the lung are markedly affected by the bipyridylium herbicides.[1] Exposure to paraquat *in vitro* resulted in an initial increase in the oxygen consumption of lung slices, followed by an inhibition of aerobic metabolism.[2] Both these reactions of lung tissue to paraquat were more marked in a 95% oxygen phase than in an air phase. Furthermore, it has been shown that the cyanide-insensitive respiration of both lung homogenates and isolated alveolar macrophages were markedly stimulated by paraquat, in contrast to its inhibition of mitochondrial respiration.[3,4]

A cytoplasmic enzyme and NADPH have been shown to be necessary for the reduction of paraquat by liver homogenates.[1] On incubation with lung slices, paraquat enhanced glucose oxidation by the pentose phosphate pathway, but not the production of 6-$^{14}CO_2$ from ^{14}C-glucose.[5] It has therefore been suggested that, as in plants, the toxicity of paraquat in mammals is related to its cyclic oxidation and reduction within cells, in conjunction with the synthesis of NADPH and its subsequent oxidation.[6]

We therefore decided to study the effect of paraquat on the oxidation of ^{14}C-glucose via the pentose phosphate pathway and the classic Embden-Meyerhof pathway in a 20% and 95% oxygen phase, to determine the relationship between the effects of high oxygen tensions and paraquat in the subsequent production of lung damage.

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MATERIALS AND METHODS

Male New Zealand White rabbits weighing 1,5 - 2,0 kg were used. Paraquat dichloride was purchased from Aldrich Laboratories, Wisconsin, and ^{14}C-glucose (6-^{14}C-glucose (53,2 mCi/mmol) and 1-^{14}C-glucose (56,8 mCi/mmol)) from Radiochemical Centre, Amersham. Slices of rabbit lung and alveolar macrophages were prepared as described previously.[2,4]

The lung slices and macrophages were incubated in 4 ml Krebs-Ringer bicarbonate medium (pH 7,4) which was saturated with gas mixtures (95% oxygen and 5% carbon dioxide or 20% oxygen and 5% carbon dioxide) for 1 hour, with adjustment of the pH every 15 minutes. The concentrations of the gas mixtures were monitored by an O_2 and CO_2 gas analyser (Beckman Instruments). The medium contained 7,5 mM glucose and approximately 0,25 μCi of 1-^{14}C-glucose or 6-^{14}C-glucose was added to each flask, which contained either 300 mg of lung slices or 10×10^6 alveolar macrophages.

Incubation was carried out in a shaking waterbath (120 cycles/min) at 37°C for 1 - 3 hours. $^{14}CO_2$ was trapped in a polythene beem capsule containing 0,2 ml Carbosorb II (Packard Instrument Co. Ltd) which was fitted into the centre well of the flask. After 1, 2 and 3 hours, the reactions were stopped by the addition of 2 ml 6% perchloric acid through the rubber stopper and the flasks incubated for another hour to collect all the CO_2. The beem capsules were then transferred to glass scintillation vials each containing 10 ml Instagel (Packard Instrument Co. Ltd). The radioactivity was measured in a Beckman liquid scintillation counter until a counting error of 1% was obtained.

The results are expressed as nanomoles of $^{14}CO_2$ per 100 mg tissue or as milligrams of cell protein per hour. Each experiment (see Figs 1 and 2) was repeated 5 times in triplicate, and standard methods were used to compute the mean and standard error of mean. Pairwise comparisons (*P* values) were made, using a two-sided Student's *t* test.

RESULTS

The rate of $^{14}CO_2$ production of control lung slices in a 20% oxygen and 5% carbon dioxide phase remains practically constant over an incubation period of 3 hours (Fig. 1).

High oxygen tensions (95% oxygen and 5% carbon dioxide at 1 atmosphere) induced a significant increase in both the 1-^{14}C-glucose and 6-^{14}C-glucose oxidation by rabbit lung slices. The generation of 6-$^{14}CO_2$ in a 95% oxygen phase is progressively increased with time (Fig. 1B), whereas the activity of the pentose pathway reached a maximum at 2 hours of incubation ($P<0,01$), followed by a decline during the third hour (Fig. 1A).

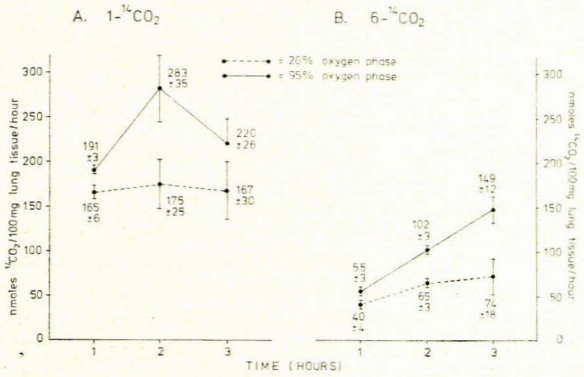

Fig. 1. Graphs to illustrate the effect of 20% and 95% oxygen on the 1-^{14}C-glucose and 6-^{14}C-glucose oxidation of rabbit lung slices. Each value represents the mean ± SEM of 5 triplicate determinations.

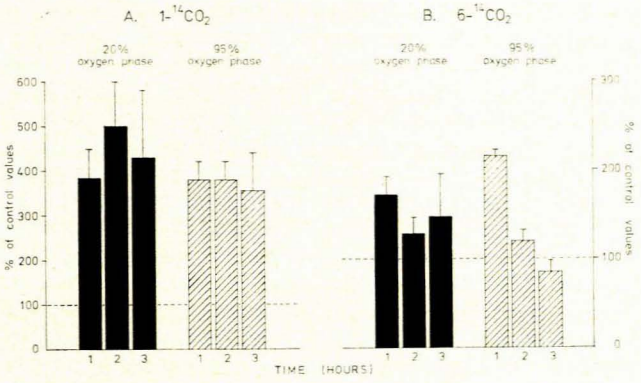

Fig. 2. Histograms to illustrate the effect of 1mM paraquat on the 1-$^{14}CO_2$ and 6-$^{14}CO_2$ production of rabbit lung slices in 20% and 95% oxygen phases. Each value represents the mean ± SEM of 5 triplicate determinations (see Fig. 1 for the corresponding control values).

Fig. 2 summarizes the effect of paraquat (1 mM) on the ^{14}C-glucose oxidation of lung slices incubated in a 20% and a 95% oxygen phase. The generation of 1-$^{14}CO_2$ in the pentose pathway is markedly increased by paraquat in both 20% and 95% oxygen phases. Although the stimulation of the pentose pathway by paraquat is smaller in the presence of a 95% oxygen phase than in a 20% oxygen phase (Fig. 2A), the differences between the responses are insignificant.

In a 20% oxygen phase, paraquat stimulates the 6-$^{14}CO_2$ production of lung slices over a period of 3 hours. In 95% oxygen its stimulatory effect is even more marked over the first hour than in a 20% oxygen phase (Fig. 2B). Although the rate of 6-$^{14}CO_2$ production induced by paraquat in the presence of 95% oxygen remains almost constant over a period of 3 hours (118 ± 7; 121 ± 3 and 125 ± 22 nmol $^{14}CO_2$ per 100 mg lung tissue per hour, during the first, second and third hour respectively) the glycolytic activity is progressively decreased after 1 hour, when the rate of $^{14}CO_2$ generation was expressed as a percentage of the control rate obtained with a 95% oxygen phase alone.

Rabbit alveolar macrophages were incubated for 1 hour in a 20% and a 95% oxygen phase, in the presence of 0,01 mM or 1,0 mM paraquat, in a medium containing 1-^{14}C-glucose or 6-^{14}C-glucose respectively (Table I). In 20% oxygen and 5% carbon dioxide the effect of 0,01 mM paraquat is insignificant, but 1,0 mM inhibits both the pentose pathway and glycolytic activity of resting macrophages significantly. High oxygen tensions *per se* decreased both the 1-$^{14}CO_2$ and 6-$^{14}CO_2$ production of alveolar macrophages. Paraquat 1,0 mM in combination with a high concentration of oxygen markedly decreased the oxidation of ^{14}C-glucose still further. In contrast, 0,01 mM paraquat seems to stimulate the production of 1-$^{14}CO_2$ in a 95% oxygen phase ($P>0,05$), while the amount of 6-$^{14}CO_2$ produced by macrophages did not significantly differ from that of the control values.

TABLE I. EFFECT OF PARAQUAT (0,01 mM AND 1,0 mM) ON THE 1-$^{14}CO_2$ AND 6-$^{14}CO_2$ PRODUCTION IN RABBIT ALVEOLAR MACROPHAGES IN A 20% AND A 95% OXYGEN PHASE*

	$^{14}CO_2$ (nmol/mg cell protein/h)			
	1-$^{14}CO_2$		6-$^{14}CO_2$	
	20% O_2	95% O_2	20% O_2	95% O_2
Control	36,4 ± 7,3 (5)	26,3 ± 2,7 (18)	13,5 ± 1,4 (5)	8,9 ± 1,4 (15)
Paraquat (0,01 mM)	39,3 ± 6,8 (5)	32,2 ± 4,4 (9)	13,9 ± 1,8 (5)	6,9 ± 1,9 (8)
Paraquat (1,0 mM)	9,8 ± 2,5 (5)	8,4 ± 1,4 (9)	1,2 ± 0,3 (5)	2,6 ± 0,5 (7)

* The values given are mean ± SEM with the number of determinations in parentheses.

Comparing the effects of paraquat on the ^{14}C-glucose oxidation of lung slices and alveolar macrophages, it seems reasonable to conclude that alveolar macrophages are more sensitive to damage by paraquat than lung slices.

DISCUSSION

When patients or experimental animals inhale 95% oxygen at 1 atmosphere (760 mmHg), the alveolar cells are exposed to a markedly higher P_{O_2} than cells of other organs. The lung may therefore develop mechanisms to protect itself against the deleterious effects of high oxygen tensions. Oxygen-induced pulmonary changes have been demonstrated, and evidence from both *in vivo* and *in vitro* experiments confirms that hyperoxia produces a broad spectrum of pathology.[2,7-11] However, the mechanism by which oxygen causes injury to tissues is not understood.

Pratt[7] found that type I alveolar epithelium and pul-

monary capillary endothelium are severely damaged by hyperoxia, whereas type II alveolar cells are relatively resistant and proliferate on continued exposure to high oxygen tensions. In hyperoxia-tolerant animals, an increase in the activity of superoxide dismutase, elevated levels of glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase (G-6-PD) and an increase in the concentration of non-protein sulphydryl compounds were found.[8,9] It was suggested that hyperoxia increases the lung anti-oxidant defence mechanisms in order to decrease its susceptibility to oxygen toxicity. Hyperoxia also inhibited DNA synthesis in lung tissue and retarded lung growth in the newborn mouse by inhibiting cell replication.[10] Various authors[2,11] showed that *in vitro* hyperoxia for about 2 hours decreased the oxygen consumption of lung tissue, which was more pronounced in the presence of exogenous glucose in the medium.

Our present *in vitro* investigations showed that glycolysis in rabbit lung slices is progressively increased in a 95% oxygen phase, and this increase correlates well with the initial enhanced oxygen utilization of lung slices.[2] However, the pentose pathway, under similar experimental conditions, is increased only over the first 2 hours, followed by a decline during the next hour (Fig. 1). This initial increase in 1-^{14}C-glucose oxidation correlates well with the higher concentrations of G-6-PD found in oxygen-tolerant animals,[8] and the accompanying increase in NADPH and its subsequent oxidation via the microsomal respiratory chain may contribute to the initial increase of the *in vitro* oxygen consumption of lung slices.[2,11]

Certain similarities exist between the metabolic responses of tissue to hyperoxia and those induced by paraquat.[12] The increase in 1-$^{14}CO_2$ production caused by a 95% oxygen phase (Fig. 1), and by paraquat in a 20% and a 95% oxygen phase (Fig. 2), confirmed that both hyperoxia and paraquat stimulate the pentose pathway in lung slices. Paraquat might somehow increase the utilization of NADPH, probably by stimulating its microsomal oxidation[1,3] and thereby produce a constant stimulation of the pentose pathway. Although oxygen is not directly used in the pentose pathway, the microsomal oxidation of the increased amounts of NADPH may be reflected by an increase in oxygen consumption. It therefore seems obvious that the previously observed progressive inhibition of oxygen consumption induced by paraquat in lung slices[2] is certainly not directly associated with decreased pentose pathway activity.

It has been shown that the inhibition of aerobic metabolism by paraquat could at least in part be explained by an inhibition of mitochondrial respiration.[3] Whereas Rose *et al.*[5] found no change in the glycolytic activity of lung slices due to paraquat, our findings indicate that paraquat in a 95% oxygen phase initially stimulates 6-$^{14}CO_2$ production, followed by a progressive inhibition over the last 2 hours. This observation may indicate a suppression of one or other glycolytic enzyme or a blockage of the citric acid cycle where most of the CO_2 is generated.

The delayed bacterial clearance from lungs of oxygen-exposed animals[13] suggested possible interference with alveolar macrophage function, although Bowden *et al.*[14] failed to demonstrate any changes in the turnover rate and morphology of alveolar macrophages in mice exposed to 90% oxygen for up to 10 days. After exposure of BCG-treated rabbits to 95% oxygen for 48 and 72 hours, Fisher *et al.*[15] found that the pentose pathway activity in alveolar macrophages was not altered, and concluded that a shift toward glycolytic pathways occurred as a result of oxygen exposure exceeding 48 hours. In mouse alveolar macrophages maintained in tissue culture under hyperoxic conditions (Po_2 ± 640 mmHg for 24 hours), Simon *et al.*[16] demonstrated a significant increase in superoxide dismutase activity compared with normoxic conditions, but agreed that their results did not unequivocally establish an important role for superoxide dismutase in protecting against cellular oxygen toxicity.

In the present investigation we found that both the pentose pathway and glycolytic pathway activity (Table I) were significantly inhibited ($P<0,05$) when normal resting alveolar macrophages were exposed *in vitro* to 95% oxygen at 1 atmosphere for 1 hour. It therefore seems as if the metabolism of BCG-activated cells, which are characterized, among other factors, by an increase in pentose pathway activity,[17] rendered it more resistant to oxygen toxicity compared with normal resting alveolar macrophages.

Paraquat is known to inhibit the oxygen consumption and viability of alveolar macrophages.[4] Its inhibitory effect on the glucose metabolism of resting alveolar macrophages supports our findings on the aerobic metabolism on lung tissue.[2-4] However, compared with lung slices the effects of paraquat on macrophages are far more pronounced (significant inhibition already after 1 hour). The observed vulnerability of macrophages might be due to their pinocytotic activity, establishing a high intracellular concentration of paraquat within a relatively short time. In view of its highly significant inhibition of the pentose pathway in alveolar macrophages, which is linked with the oxidative systems for the removal of NADPH, the apparent contradictory stimulation of the cyanide-insensitive respiration of macrophages by paraquat[4] should be further explored. Experiments are now under way in our laboratory to investigate the effects of paraquat on both the NADH- and NADPH-dependent microsomal systems.

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REFERENCES

1. Gage, J. C. (1968): Biochem. J., **109**, 757.
2. Rossouw, D. J. and Engelbrecht, F. M. (1978): S. Afr. med. J., **54**, 199.
3. *Idem* (1978): *Ibid.*, **54**, 1101.
4. *Idem* (1979): *Ibid.*, **55**, 20.
5. Rose, M. S., Smith, L. S. and Wyatt, I. (1976): Biochem. Pharmacol., **25**, 1763.
6. Ilett, K. F., Stripp, B., Menard, R. H. *et al.* (1974): Toxicol. appl. Pharmacol., **22**, 241.
7. Pratt, P. C. (1974): Amer. Rev. resp. Dis., **110**, 51.
8. Kimball, R. E., Reddy, K., Peirce, T. H. *et al.* (1976): Amer. J. Physiol., **230**, 1425.
9. Crapo, J. D. and Tierney, D. F. (1974): *Ibid.*, **226**, 1401.
10. Northway, W. H., Petriceks, B. and Shahinian, L. (1972): Pediatrics, **30**, 67.
11. Gail, D. B. and Massaro, D. (1976): Amer. Rev. resp. Dis., **113**, 889.
12. Tierney, D. F., Ayers, L. and Kasuyama, R. S. (1977): *Ibid.*, **115**, 59.
13. Huber, G. L. and La Force, F. M. (1970): Ann. intern. Med., **72**, 808.
14. Bowden, D. H., Adamson, I. Y. R. and Wyatt, J. P. (1968): Arch. Path., **86**, 671.
15. Fisher, A. B., Diamond, S. and Mellen, S. (1974): J. appl. Physiol., **37**, 341.
16. Simon, L. M., Liu, J., Theodore, J. *et al.* (1977): Amer. Rev. Resp. Dis., 115, 279.
17. Stubbs, M., Kühner, A. V., Glass, E. A. *et al.* (1973): J. exp. Med., **137**, 537.