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THE RELEVANCE OF PENTOSE PHOSPHATE PATHWAY STIMULATION IN RAT LUNG TO THE MECHANISM OF PARAQUAT TOXICITY

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Abstract—Paraquat and diquat have been shown to stimulate the production of $^{14}CO_2$ from [1-^{14}C]-glucose by slices of rat lung, but not the production of $^{14}CO_2$ from [6-^{14}C]glucose. This indicates stimulation of the pentose phosphate pathway. Paraquat was effective at concentrations as low as 7.5×10^{-7} M whilst a concentration of diquat of 10^{-5} M was required for comparable stimulation. Maximal stimulation occurred with approximately 10^{-5} M paraquat and approximately 10^{-4} M diquat. The stimulation of pentose phosphate pathway in lung slices by paraquat has been shown to be related to paraquat accumulation.

Lung slices from rats dosed intravenously with 65 μmoles of either paraquat or diquat/kg body wt had increased pentose phosphate pathway activity compared with slices from saline injected controls. At all times studied from 0.5 to 18 hr after injection, pentose phosphate pathway activity in slices from diquat poisoned rats was equal to or greater than that observed in slices from paraquat poisoned rats. Since only rats dosed intravenously with paraquat subsequently develop lung damage, it is concluded that there is no simple relationship between stimulation of the pentose phosphate pathway in lung and the production of lung damage.

Paraquat (1,1′-dimethyl-4,4′-bipyridilium) and diquat (1,1′-ethylene 2,2′-bipyridilium) are closely related bipyridylium herbicides which can be reduced to free radicals, stable in the absence of oxygen [1,2]. In the presence of oxygen these radicals are rapidly reoxidized to the parent cations with production of superoxide radical ion (O_2^-) and hydrogen peroxide [3, 4]. Paraquat and diquat are reduced by photosystems present in the green leaves of plants, and cyclic redox reactions then lead to production of reactive oxygen species which are thought to be the molecules responsible for herbicidal activity of the bipyridyls [5].

Paraquat and diquat are also toxic to mammals [6, 7]. Following paraquat administration to rats the organ most severely affected is the lung [6, 8], whereas following diquat administration the lung appears undamaged [7; Smith and Rose, unpublished work]. It has recently been suggested that the specificity of paraquat for the lung is due to selective accumulation of paraquat by this organ [9].

Both paraquat and diquat can be reduced to free radicals by homogenates of liver, kidney or lung [10, 11]. The reduction of paraquat or diquat by liver homogenates has been shown to be dependent on a cytoplasmic enzyme and NADPH, and marked stimulation of NADPH oxidation accompanies the cyclic oxidation and reduction of both bipyridyls [10]. Incubation of lung slices with paraquat has also been reported to result in increased metabolism of glucose by the pentose phosphate pathway [12]. It has therefore been suggested that, as in plants, the mammalian toxicity of paraquat is related to its cyclic oxidation and reduction within cells with resultant production of reactive species of oxygen [10, 12, 14, 15]. In the work reported here, the effect of both paraquat and diquat on the oxidation of glucose via the pentose phosphate pathway in lung has been examined in order to determine the relationship between redox reactions and accumulation of paraquat by lung and subsequent production of lung damage.

MATERIALS AND METHODS

Materials. Paraquat dichloride and diquat dichloride monohydrate were obtained from Plant Protection Division, Jealott's Hill Research Station, Berks. [Methyl-^{14}C]paraquat (30 mCi/m-mole), [ethylene-^{14}C]diquat (30 mCi/m-mole), [1-^{14}C]glucose (3 mCi/m-mole), [6-^{14}C]glucose (3 mCi/m-mole), [^{14}C]Na_2CO_3 (11 μCi/ml; ^{14}C reference solution), [hydroxymethyl-^{14}C]inulin (12 mCi/m-mole), [^{3}H]-inulin (690 mCi/m-mole) and [^{3}H]water (5 mCi/ml) were all purchased from the Radiochemical Centre, Amersham. Imipramine hydrochloride was obtained from Geigy Pharmaceuticals, Macclesfield, Cheshire and inulin (purified, 10% solution) was purchased from Thomas Kerfoot and Company Limited, Lancashire.

Animals. Male, Alderley Park (Wistar derived) specific pathogen-free rats (body wt 180–220 g) were used throughout.

Measurement of $^{14}CO_2$ production. Slices of rat lung were prepared as described previously [16] and incubated in 3 ml of a modified Krebs–Ringer phosphate medium [9] containing glucose (11 mM) and 1 μCi of either [1-^{14}C]glucose or [6-^{14}C]glucose. Incubation was carried out under air in respirometer flasks in a shaking water bath at 37°. $^{14}CO_2$ was trapped in 0.2 ml of KOH (20%, w/v) placed in the centre well of the flask together with a 2-cm square of hard filter paper (Whatman No. 542) to facilitate absorption. After incubation, the filter paper and KOH were

Table 1. The effect of paraquat and diquat *in vitro* on the oxidation of glucose by slices of rat lung

	Dpm of $^{14}CO_2$/100 mg slice	
	[1-^{14}C]glucose	[6-^{14}C]glucose
Control slices	4250 ± 428(3)	1900 ± 268(3)
Diquat (10^{-6} M)	4670‡ ± 653(3)	1810‡ ± 233(3)
Diquat (10^{-5} M)	8870** ± 565(3)	2130‡ ± 27(3)
Diquat (10^{-4} M)	16700** ± 2230(6)	1960‡ ± 370(5)
Paraquat (10^{-6} M)	7800* ± 1662(3)	2230‡ ± 211(3)
Paraquat (10^{-5} M)	14500** ± 2595(3)	1640‡ ± 357(3)
Paraquat (10^{-4} M)	12800** ± 1670(6)	2310‡ ± 380(6)

Slices of rat lung were incubated with the indicated concentration of bipyridyl and $^{14}CO_2$ production from either [1-^{14}C]glucose or [6-^{14}C]glucose measured for 1 hr.

* significantly different from control, $0.05 > P > 0.02$

** significantly different from control, $P < 0.01$

‡ not significantly different from control, $P > 0.05$

The values given are mean ± S.E.M. with the number of determination in parentheses.

transferred to plastic scintillation vials containing 1.0 ml of water, and 15 ml Instagel scintillator (Packard Instrument Company Limited) was added. The radioactivity was measured using a liquid scintillation spectrometer and the efficiency of counting determined by the use of standard [^{14}C]Na_2CO_3 placed on filter paper and processed in an identical way to the samples.

Measurement of paraquat accumulation in lung slices. Paraquat accumulation in lung slices was measured as described previously [16].

Measurement of spaces in rat lung slices. Slices of rat lung were incubated (with shaking at 37°) with 3 ml of Krebs–Ringer phosphate containing glucose. Combinations of 0.1 μCi of [^{3}H]water, [^{3}H]inulin, [hydroxymethyl-^{14}C]inulin and [ethylene-^{14}C]diquat were added to the medium such that the ratio in the slices of [^{3}H]inulin to [^{14}C]inulin, [^{3}H]water to [^{14}C]inulin, [ethylene-^{14}C]diquat to [^{3}H]water and [ethylene-^{14}C]diquat to [^{3}H]inulin could be measured. The inulin space was shown to remain constant over a range of inulin concentrations (approx $5 \times 10^{-7} - 4 \times 10^{-4}$ M). The concentration of diquat in the incubation medium was 10^{-5} M. After incubation, excess medium was removed from the slices by wiping on a clean glass plate, the slices dissolved in 1.0 ml soluene (Packard Instrument Company Limited) and 10 ml Dimilume Scintillator (Packard Instrument Company Limited) added. Medium (0.1 ml) was added to water (0.9 ml) and 10 ml Instagel scintillator added. ^{14}C and ^{3}H radioactivity were determined on the same sample using two channels on a liquid scintillation spectrometer. Efficiency of counting was determined by addition of internal ^{14}C and ^{3}H standards. Since the space found in slices for ^{3}H-labelled inulin was consistently higher (14 per cent) than that found for ^{14}C-labelled inulin, an appropriate correction factor was applied so that the results are expressed as those for ^{14}C-inulin.

RESULTS

Neither paraquat nor diquat significantly altered the oxidation of [6-^{14}C]glucose by rat lung slices at concentrations of between 10^{-6} and 10^{-4} M (Table 1). However, paraquat stimulated the production of $^{14}CO_2$ from [1-^{14}C]glucose at all concentrations in this range whereas diquat did not stimulate at 10^{-6} M (Table 1). Maximal stimulation of [1-^{14}C]-glucose oxidation occurred with 10^{-5}M paraquat and 10^{-4} M diquat (Table 1).

Slices were initially incubated with paraquat at 7.5×10^{-7} M. After different periods of incubation up to 3 hr, they were transferred to fresh media in the absence of paraquat, and the production of $^{14}CO_2$ from [1-^{14}C]glucose measured for 1 hr. Paraquat was accumulated linearly with time during the 3 hr of initial incubation and the amount of $^{14}CO_2$ produced in the second incubation also increased (Table 2A). The experiment was repeated using 10^{-5} M paraquat, a concentration which appeared to stimulate maximal $^{14}CO_2$ production from [1-^{14}C]glucose (Table 1). With this concentration of paraquat, the paraquat in the slices increased with time as before, whilst the

Table 2. The relationship between paraquat accumulation by rat lung slices and the stimulation of the pentose phosphate pathway

Time of initial incubation (hr)	Paraquat in slice (nmoles/g wet wt)	A* $^{14}CO_2$ Produced during 1 hr incubation (dpm/100 mg slice)
0.5	1.4(2)	10100(2)
1.0	2.8 ± 0.3(3)	13700 ± 1100(3)
1.5	3.7 ± 0.4(3)	11100 ± 600(3)
2.0	5.0 ± 0.7(3)	16000 ± 1400(3)
3.0	7.3 ± 0.8(3)	18000 ± 200(3)
		B‡
0.5	21.7(2)	28200(2)
1.0	38 ± 7(3)	31300 ± 5200(3)
1.5	53 ± 4(3)	29400 ± 2000(3)
2.0	68 ± 4(3)	31500 ± 1500(3)
3.0	92 ± 4(3)	28400 ± 6400(3)

Slices of rat lung were incubated either with 7.5×10^{-7} M paraquat (A) or 10^{-5} M paraquat (B) for up to 3 hr. Slices were removed at intervals and the paraquat present measured as described in the Methods section, or they were transferred to fresh medium and $^{14}CO_2$ production from [1-^{14}C]glucose measured for 1 hr.

* Concentration of paraquat in the incubation medium was 7.5×10^{-7} M

‡ Concentration of paraquat in the incubation medium was 10^{-5} M.

The values given are means ± S.E.M. with the number of determinations in parentheses.

Table 3. The effect of imipramine on the rate of [1-^{14}C]-glucose oxidation in the presence or absence of paraquat or diquat

	Rate of $^{14}CO_2$ production (dpm/100 mg slice/hr)	
	A (− imipramine)	B (+ imipramine)
Control	10.900	12.200
Diquat (10^{-5} M)	14.400	14.900
Paraquat (10^{-6} M)	(a) 19.200	11.700
	(b) 31.500	23.200

Slices of rat lung were incubated in the presence or absence of imipramine (10^{-4} M) and the production of $^{14}CO_2$ from [1-^{14}C]glucose measured after 0.5, 1 and 2 hr of incubation. Two slices were used at each time point. The values for the rate of $^{14}CO_2$ production were then derived from the fitting of best straight lines to these data. In separate experiments, the rate of paraquat accumulation under identical conditions was inhibited 45 and 44 per cent.

amount of $^{14}CO_2$ produced in the second incubation remained constant (Table 2B).

When imipramine (10^{-4}M) was included in the incubation medium with paraquat (10^{-6} M), the amount of $^{14}CO_2$ produced from [1-^{14}C]glucose was reduced, as was the accumulation of paraquat into the slices (Table 3). This concentration of imipramine had no effect on the $^{14}CO_2$ production from [1-^{14}C]glucose by control slices, or slices in the presence of diquat (10^{-5} M) (Table 3).

The penetration of diquat into slices of rat lung. The total water, inulin and diquat spaces in rat lung slices were measured during 4 hr of incubation in the presence of diquat. The total water and inulin spaces did not alter during the incubation but the amount of diquat associated with the slices increased with time (Table 4). If the difference between the total water space and inulin space is defined as "intracellular" water, and the difference between the total diquat space and inulin space is defined as "intracellular" diquat, then it can be seen that diquat takes more than 4 hr to equilibrate with the total tissue water (Table 4). A first order plot of the data of Table 4 gives an approximate half-time for equilibration of 80 min.

The effect of paraquat or diquat administered intravenously to rats on the oxidation of [6-^{14}C]*glucose and* [1-^{14}C]*glucose by lung.* Intravenously administered paraquat or diquat had no effect on the production of $^{14}CO_2$ from [6-^{14}C]glucose by rat lung slices taken at times between 0.5 and 18 hr after dosing (Table 5). However, the production of $^{14}CO_2$ from [1-^{14}C]-glucose was markedly increased at all time intervals studied with lung slices taken from both paraquat and diquat poisoned rats (Table 6).

The effect of paraquat or diquat administered orally to rats on the oxidation of [1-^{14}C]*glucose by lung.* As with intravenously administered bipyridyl, there was no observed effect on the production of $^{14}CO_2$ from [6-^{14}C]glucose by lung slices from poisoned animals at 4, 24 or 30 hr after dosing. However, the production of $^{14}CO_2$ from [1-^{14}C]glucose was very significantly elevated at all times following paraquat and somewhat elevated at 24 and 30 hr following diquat (Table 7).

DISCUSSION

The consequence of intracellular reduction of paraquat and diquat. Both paraquat and diquat can be

Table 4. Water, inulin and diquat spaces in slices of rat lung

Time of incubation (hr)	Total water space	Inulin space	Total diquat space (ml/g wet wt slice)	"Intracellular"* water space	"Intracellular"** diquat space
0.5	0.71 ± 0.01(12)	0.35 ± 0.01(12)	0.52 ± 0.02(18)	0.36	0.17
2.0	0.64 ± 0.01(6)	0.34 ± 0.01(6)	0.53 ± 0.01(12)	0.30	0.19
4.0	0.70 ± 0.01(12)	0.40 ± 0.02(12)	0.66 ± 0.03(12)	0.30	0.26

Slices of rat lung were incubated as described in the Methods section, for up to 4 hr. The values given in the table are the ratios of the amounts of tritiated water, inulin or diquat in the slice to the amount present in an equivalent wt of medium.

* The "intracellular" water space is defined as (total water space − inulin space).

** The "intracellular" diquat space is defined as (total diquat space − inulin space).

The values given are means ± S.E.M. with the number of determination in parentheses.

Table 5. The oxidation of [6-^{14}C]glucose by lung slices from rats injected intravenously with paraquat, diquat or saline

Time after dosing (hr)	Saline injected	Paraquat injected (dpm/100 mg lung slice)	Diquat injected
0.5	1750 ± 170(14)	1810* ± 256(6)	1300* ± 90(6)
4.0	2290 ± 127(12)	2150* ± 231(6)	2750* ± 270(5)
18.0	1780 ± 167(12)	2190* ± 594(6)	1710* ± 227(5)

Fed rats were injected intravenously with 65 μmoles/kg body wt of either paraquat or diquat, or injected with an equivalent volume of isotonic saline. They were killed at the intervals shown, the lungs removed and slices prepared as described in the Methods section. The production of $^{14}CO_2$ from [6-^{14}C]glucose by the slices was then measured for 1 hr.

* Not significantly different from saline injected ($P > 0.05$)

The values given are means ± S.E.M. with the number of determinations in parentheses.

Table 6. The oxidation of [1-^{14}C]glucose by lung slices from rats injected intravenously with paraquat, diquat or saline

Time after dosing (hr)	Saline injected	Paraquat injected (dpm/100 mg lung slice)	Diquat injected
0.5	3020 ± 362(15)	7700** ± 1000(6)	7620** ± 722(8)
4.0	4290 ± 353(11)	6510** ± 475(6)	10800** ± 179(5)
18.0	2490 ± 167(11)	3440* ± 457(6)	5100** ± 975(5)

Fed rats were injected intravenously with 65 μmoles/kg body wt of either paraquat or diquat, or injected with an equivalent volume of isotonic saline. They were killed at the intervals shown, the lungs removed and slices prepared as described in the Methods section. The production of $^{14}CO_2$ from [1-^{14}C]glucose by the slices was then measured for 1 hr.

* Significantly different from saline injected $0.05 > P > 0.02$

** Significantly different from saline injected $P < 0.01$

The values given are means ± S.E.M. with the number of determinations in parentheses.

reduced to their free radicals by enzymes present in the cytoplasm of cells [10, 11]. This reduction is coupled to the oxidation of NADPH [10]. The reduction of paraquat or diquat intracellularly would, therefore, be expected to raise the $NADP^+$ level and thus increase the activity of the pentose phosphate pathway [17] in order to generate more NADPH for vital cell processes such as protein synthesis [18].

The stimulation of the pentose phosphate pathway in rat lung slices by paraquat and diquat in vitro. Since the production of $^{14}CO_2$ from [6-^{14}C]glucose by slices of rat lung was unaffected by incubation with a range of concentrations of either paraquat or diquat (Table 1), the glycolytic pathway in the lung appears to be unaffected by the bipyridyls and the increased production of $^{14}CO_2$ from [1-^{14}C]glucose (Table 1) can, therefore, be taken as an indication of an increase in the activity of the pentose phosphate pathway. This increase in activity suggests that both paraquat and diquat penetrate the cells of lung slices and undergo cyclic oxidation–reduction reactions which result in oxidation of NADPH. These observations confirm previous findings [12] using relatively high concentrations of paraquat. In our experiments, maximal stimulation of CO_2 production occurred with 10^{-5} M paraquat or 10^{-4} M diquat but the maximal rates observed were not the same in different experiments (c.f. Tables 1 and 2B). This discrepancy was possibly due to differences in the nutritional states of the animals used, as the activity or both the glycolytic and pentose phosphate pathway in lung slices were affected markedly by starvation (c.f. Tables 5, 6, with 7).

Although previous work has shown that diquat is reduced more rapidly in broken cell preparations than paraquat [11] and that diquat stimulates NADPH oxidation in liver homogenates at concentrations an order of magnitude lower than paraquat [10], we have shown that paraquat stimulates the pentose phosphate pathway in lung slices *in vitro* at much lower concentrations in the incubation medium than diquat (Table 1). The explanation for this difference probably resides in the intracellular concentrations of the compounds achieved during the incubation. Paraquat is accumulated by an energy-dependent process into slices of rat lung whereas diquat is not [9, 16]. Diquat has been shown to diffuse into lung slices (Table 4) with a half-time of approximately 80 min and as a consequence, the concentration inside cells will only slowly approach that of the medium during the incubation. With low concentrations of diquat in the medium, therefore, (e.g. 10^{-6} M) there will be insufficient present inside cells to stimulate the pentose phosphate pathway significantly [10]. A similar diffusion process can be assumed to occur with paraquat at low concentrations (7.5×10^{-7} M) but the accumulation of paraquat can be seen to lead to a 4-fold increase in the amount present in the whole slice when compared with that of medium (Table 2A). However, it has been suggested that the type I and II alveolar epithelial cells are responsible for the accumulation of paraquat into lung [19]. These cells will only represent a small proportion of the wet weight of the lung slice. In consequence, the concentration of paraquat in these cells will be very much higher than that in the whole slice,

Table 7. The oxidation of [1-^{14}C]glucose by lung slices from rats given paraquat or diquat orally

Time after dosing (hr)	Control	Paraquat (d.p.m./100 mg lung slice)	Diquat
4.	6410 ± 240(12)	11.200** ± 1100(6)	6740† ± 225(6)
24	4140 ± 230(12)	11.300** ± 620(6)	5170* ± 240(3)
30	5100 ± 320(12)	13.000** ± 769(6)	8040** ± 818(6)

Rats were starved for 24 hr before being dosed orally with 680 μmoles of either paraquat or diquat/kg body wt. They were killed at the intervals shown, the lungs removed and slices prepared as described in the Methods section. The production of $^{14}CO_2$ from [1-^{14}C]glucose by the slices was then measured for 1 hr.

* Significantly different from control $0.05 > P > 0.02$

** Significantly different from control $P < 0.01$

† Not significantly different from control $P > 0.05$

Values given are means ± S.E.M. with the number of determinations in parentheses.

perhaps by a factor as large as 10, and will therefore be more than sufficient to stimulate the pentose phosphate pathway.

That accumulation of paraquat is related to stimulation of the pentose phosphate pathway is indicated (a) by the increase in $^{14}CO_2$ production from [1-^{14}C]glucose seen as paraquat is accumulated from a very low concentration in the medium (Table 2A) and (b) by the inhibition of stimulation seen when imipramine, an inhibitor of paraquat accumulation, is present in the incubation medium (Table 3). It is, therefore, possible to speculate that the compartment accumulating paraquat is cytoplasmic since once accumulated, the paraquat undergoes cyclic oxidation–reduction with resultant oxidation of cellular NADPH.

The relevance of stimulation of the pentose phosphate pathway to lung damage. The production of $^{14}CO_2$ from [6-^{14}C]glucose by lung slices taken from rats injected intravenously with 65 μmoles of either paraquat or diquat/kg body wt was not different from that of saline injected controls (Table 5), whereas the production of $^{14}CO_2$ from [1-^{14}C]glucose was significantly elevated (Table 6). Thus the pentose phosphate pathway in the lungs of both paraquat and diquat poisoned rats is stimulated. The stimulation was more marked in slices from diquat poisoned rats, particularly 4 and 18 hr after dosing (Table 6). This indicates that following intravenous dosing of 65 μmoles of either paraquat or diquat/kg body wt, sufficient paraquat or diquat has entered lung cells to have undergone cyclic redox reactions and to have led to oxidation of NADPH and consequent stimulation of the pentose phosphate pathway. However, whereas this dose of paraquat leads to extensive lung damage (Smith and Rose, unpublished work) this dose of diquat does not damage the lung (Smith and Rose, unpublished work). Thus there is no simple relationship between stimulation of the pentose phosphate pathway (and by implication, free radical generation) and lung damage.

When rats were dosed orally with paraquat or diquat, stimulation of the pentose phosphate pathway was shown to be more marked in the lungs of paraquat poisoned rats than in those of diquat poisoned rats (Table 7) which is the reverse of that observed following intravenous dosing. Since blood concentrations of the bipyridyls are very low after oral dosing [9], the diffusion-controlled concentration of diquat in lung will consequently be low. However, paraquat is accumulated into the lung under these conditions [9, 20], which would explain the observed stimulation of the pentose phosphate pathway.

It is clear, therefore, that both paraquat and diquat can enter lung cells and can stimulate the pentose phosphate pathway. This, in turn, can be taken to indicate that both are undergoing catalytic oxidation and reduction through their respective free radicals. This process results in the oxidation of NADPH and also the production of reactive species of oxygen [21]. However, since there is no damage to the lung following intravenous administration of diquat to rats, when there is clear evidence for free radical generation, it must be presumed that generation of those radicals *per se* is not sufficient to cause general cell damage. It is possible, however, that radical generation in the cell type which accumulates paraquat (i.e. alveolar epithelial type I and II cells) [19] is responsible for damage to those cells. Alternatively, the accumulation of paraquat by certain cells may lead to exceptionally high intracellular concentrations of paraquat which may lead to such fast rates of NADPH oxidation that cell death results from an inability of the cells to maintain normal levels of NADPH and ATP. This latter hypothesis, which does not require damage to the cell from superoxide, hydrogen peroxide or singlet oxygen, might explain why it has proven difficult to demonstrate lipid peroxidation in lung following paraquat [14].

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REFERENCES

1. L. Michaelis and E. S. Hill, *J. Am. Chem. Soc.* **55,** 1491 (1933).
2. R. F. Homer and T. E. Tomlinson, *Nature* **184,** 2012 (1959).
3. A. Calderbank, *Proc. 7th Br. Weed Control Conference*, p. 312 (1964).
4. J. A. Farrington, M. Ebert, E. J. Land and K. Fletcher, *Biochim. biophys. Acta* **314,** 372 (1973).
5. A. D. Dodge, N. Harris and B. C. Baldwin, *Biochem. J.* **118,** 43 (1970).
6. D. G. Clark, T. F. McElligott and E. W. Hurst, *Br. J. industr. Med.* **23,** 126 (1966).
7. D. G. Clark and E. W. Hurst, *Br. J. industr. Med.* **27,** 51 (1970).
8. G. S. Vijeyaratnam and B. Corrin, *J. Path.* **103,** 123 (1971).
9. M. S. Rose, E. A. Lock, L. L. Smith and I. Wyatt, *Biochem. Pharmac.* **25,** 419 (1976).
10. J. C. Gage, *Biochem. J.* **109,** 757 (1968).
11. R. C. Baldwin, A. Pasi, J. T. MacGregor and C. H. Hine, *Toxicol. appl. Pharmac.* **32,** 298 (1975).
12. H. K. Fisher, J. A. Clements, D. F. Tierney and R. R. Wright, *Am. J. Physiol.* **228,** 1217 (1975).
13. H. K. Fisher, J. A. Clements and R. R. Wright, *Am. Rev. resp. Disease* **107,** 246 (1973).
14. K. F. Ilett, B. Stripp, R. H. Menard, W. D. Reid and J. R. Gillette, *Toxicol. appl. Pharmac.* **28,** 216 (1974).
15. J. S. Bus and J. E. Gibson, *Pharmacologist* **16,** 230 (1974).
16. M. S. Rose, L. L. Smith and I. Wyatt, *Nature* **252,** 314 (1974).
17. H. Holzer, *A. Rev. Biochem.* **28,** 171 (1959).
18. D. Massaro, M. R. Simon & H. Steinkamp, *J. appl. Physiol.* **30,** 1 (1971).
19. I. F. H. Purchase, B. I. Sykes and L. L. Smith, *J. Path.* (in press) (1976).
20. L. L. Smith, A. Wright, I. Wyatt and M. S. Rose, *Br. med. J.* **4,** 569 (1974).
21. J. S. Bus, S. D. Aust and J. E. Gibson, *Biochem. biophys. Res. Commun.* **58,** 749 (1974).