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Paraquat Toxicity

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Paraquat toxicity

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Paraquat (1,1′-dimethyl-4,4′-bipyridylium dichloride) is marketed as a contact herbicide. Although it has proved safe in use there have been a number of cases of poisoning after the intentional swallowing of the commercial product. The most characteristic feature of poisoning is lung damage, which causes severe anoxia and may lead to death. The specific toxicity to the lung can be explained in part by the accumulation of paraquat into the alveolar type I and type II epithelial cells by a process that has been shown to accumulate endogenous diamines and polyamines. When accumulated, paraquat undergoes an NADPH-dependent, one-electron reduction to form its free radical, which then reacts avidly with molecular oxygen to reform the cation and produce superoxide anion, which in turn will dismutate to form H_2O_2. This may lead to the formation of more reactive (and hence toxic) radicals which have the potential to cause lipid peroxidation and lead to cell death.

Biochemical changes provoked by paraquat in the lung suggest that it causes a rapid, pronounced and prolonged oxidation of NADPH that initiates compensatory biochemical processes in the lung. NADPH may be further depleted as it is consumed in an attempt to detoxify H_2O_2 or lipid hydroperoxides. Thus it is possible that with toxic levels of paraquat in the cell, compensatory biochemical processes are insufficient to maintain levels of NADPH consistent either with cell survival or with the ability to detoxify H_2O_2 or prevent lipid peroxidation.

Introduction

Paraquat (1,1′-dimethyl-4,4′-bipyridylium dichloride) was first described in the literature in the latter part of the nineteenth century by Weidel & Rosso (1882). In 1933 its redox properties were discovered by Michaelis & Hill (1933) and since then it has been used as a redox indicator, known by its trivial name methyl viologen. Its herbicidal properties were discovered in the mid-1950s and it has been marketed as a herbicide for approximately 25 years. Although it has proved remarkably safe in use there have been a number of fatalities largely as a consequence of the intentional swallowing of the concentrated commercial product for suicidal purpose (Fletcher 1974).

When paraquat is swallowed the symptoms of poisoning depend largely on the amount consumed. Those who die from paraquat poisoning can be divided into two broad categories; (i) those who die within one to five days of its ingestion and (ii) those who die later than five days and as long as several weeks after ingestion. In the cases of death which occur within a few days of poisoning, extremely large amounts of paraquat have been ingested. Death, when it occurs, results from multi-organ failure associated with damage to the adrenal gland (Nagi 1970), liver (Fennelly *et al.* 1971), kidney (Oreopoulos *et al.* 1968), lung (Bronkhurst *et al.* 1968) and brain (Nienhaus & Ehrenfeld 1971). In these cases of poisoning the precise cause of death is difficult to diagnose because so many of the vital organs have been affected. In patients who

survive five days or longer but eventually die from paraquat poisoning, the most characteristic features of poisoning are damage to the lung and kidney (Fairshter *et al.* 1976), with the cause of death usually attributed to anoxia as a consequence of extensive lung damage.

In this paper the mechanism of toxicity of paraquat in the lung will be described. While it is generally agreed that it is the cyclical reduction and reoxidation of paraquat that represents its primary mechanism of toxicity, consideration will also be given to the delivery of paraquat to the lung, the biochemical consequences of redox cycling and the pathology that results.

Pathology in the lung

The toxic effects of paraquat in experimental animals was first reported by Clark *et al.* (1966) who showed that the histological effects of paraquat in rats, mice and dogs are similar. The most extensive studies on the pathogenesis of paraquat-induced lung damage have been performed on rats. Within 24 h of the administration of an approximate LD_{50} dose of paraquat the alveolar type I and type II epithelial cells are damaged (Vijeyaratnam & Corrin 1971). The destruction of the alveolar epithelium continues such that by two to four days after dosing, areas of the alveolar epithelium are destroyed. During this time an alveolitis develops with concomitant infiltration of inflammatory cells into the lung (Vijeyaratnam & Corrin 1971). Considerable oedema is produced during this phase and many animals will die within the first few days of dosing (Smith & Rose 1977). A few rats that develop this severe alveolitis survive for many days or weeks after dosing but then develop an extensive hypercellular lesion characterized by the proliferation of fibroblasts (Smith & Heath 1976). This phase of the lesion, along with residual oedema and alveolar collapse, results in death due to anoxia. Thus it appears that the initial lesion in the lung is damage to the alveolar epithelium and as a consequence of this a proliferative fibrosis develops (probably as part of the repair process) that, if extensive enough, will cause death.

Delivery of paraquat to the lung

Most authors agree that paraquat is not metabolized in experimental animals. Sharp *et al.* (1972) first demonstrated that the lungs of rats intravenously dosed with paraquat had the highest concentration and selectively retained the compound in comparison with other organs. When rats are dosed orally with paraquat the concentration in the plasma remains relatively constant over a period of 30 h, whereas that in the lung increases with time such that by 30 h there is six to seven times more in the lung than in the plasma (figure 1). Of the organs studied, no other tissue shows a similar time-dependent increase in paraquat concentration (Rose *et al.* 1976). Thus the lung, which is the organ most severely damaged by paraquat, has the ability to accumulate the bipyridyl from the plasma after oral dosing (Smith *et al.* 1974) and retains paraquat independent of the fall in the plasma concentration (Sharp *et al.* 1972). This selective accumulation–retention of paraquat in the lung provides a convincing explanation why this organ is selectively damaged by paraquat.

Experiments using lung slices have shown that the lung accumulates paraquat in a time-dependent manner by a process that is energy dependent (Rose *et al.* 1974). As with the *in vivo* situation, slices of organs taken from control rats were unable to accumulate paraquat,

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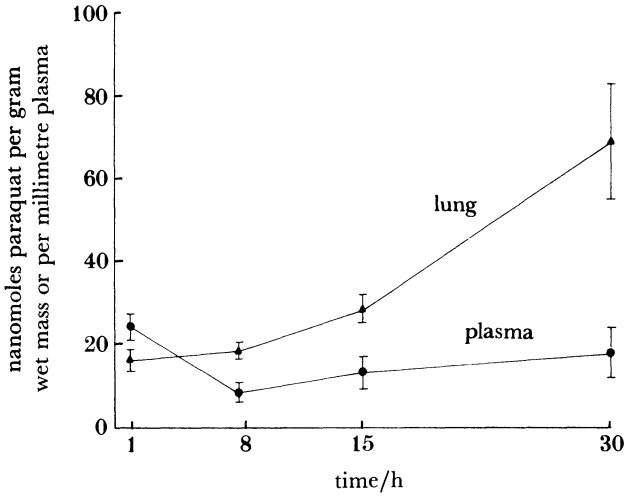

FIGURE 1. The level of paraquat in the lung and plasma of rats given 680 μmol of paraquat per kilogram of body mass orally. Points on the graph represent the mean ± s.e. (standard error) with at least five rats per time point. Rate of uptake: 4–6 nmol per gram wet mass per hour.

with the exception of brain slices, which could accumulate paraquat, but to a much lesser extent than lung slices (Rose *et al.* 1976).

The efflux of paraquat from lung slices has been shown to be biphasic, having a very fast component and a slow component (Smith *et al.* 1981). The fast component is thought to represent the diffusion of paraquat from the extracellular space or from cells damaged during slicing of the lung (Smith *et al.* 1981). The slow component is first order, being characterized by a $t_{\frac{1}{2}}$ of 17 h, which is similar to that found *in vivo* (Smith *et al.* 1981).

The discovery that paraquat was accumulated into the lung led to the suggestion that its uptake was the consequence of its ability to be mistaken for an endogenous compound (or compounds) that is normally accumulated by the lung. A number of compounds were examined for their ability to inhibit paraquat accumulation into the lung (Lock *et al.* 1976). Among these inhibitors was the diamine putrescine, which competitively blocks paraquat accumulation and is itself accumulated by an energy-dependent process in the lung (Smith & Wyatt 1981). The uptake of both paraquat and putrescine in the lung obeys saturation kinetics and the apparent K_m for the accumulation of paraquat is 70 μM with a V_{max} of 300 nmol per gram wet mass per hour (Rose *et al.* 1974) whereas that of putrescine is 7 μM with a V_{max} of 330 nmol per gram wet mass per hour (Smith 1982). The polyamines spermidine and spermine are also accumulated into the lung by a process that obeys saturation kinetics (Smith *et al.* 1982). The apparent K_m and V_{max} values for the accumulation of these oligoamines are similar (table 1). It seems reasonable to conclude that a single process is responsible for the accumulation of diamines and polyamines into the lung and that the reason paraquat is accumulated is that it is transported by this process in mistake for these or other endogenous substrates.

The most probable reason for the pulmonary uptake of paraquat is its structural similarity to the diamines and polyamines with respect to the separation of the quaternary nitrogen atoms of paraquat and the amino groups of the oligoamines. A characterization of the structural requirements of this process has been made by Gordonsmith *et al.* (1983).

Definitive evidence for the cellular compartments into which paraquat is accumulated is not

Table 1. The kinetic constants for the accumulation of diamines and polyamines by slices of rat lung

compound	method of preparing lung slice	K_m/μM	V_{max}/(nmol g h^{-1})
spermine	tissue chopper	8.8	639 (527–811)
spermidine	tissue chopper	15.7	606 (452–917)
cadaverine	tissue chopper	15.9	832 (601–1351)
putrescine	tissue chopper	11.4	635 (399–1546)
putrescine	hand slice	7.0	330

Slices of rat lung were incubated at 37 °C in K.R.P. glucose medium containing 1, 3, 10, 30 or 100 μM of ^{14}C-labelled spermine, spermidine, cadaverine or putrescine as appropriate. The rate of accumulation of label into the slice was determined for each concentration of each compound by using a least-squares regression on four slices at three time points. By using the rate of accumulation for each concentration, estimates of the Lineweaver–Burke relation were determined with the use of unweighted least-square linear regression and the apparent K_m and V_{max} determined for each compound. The figures in parentheses are the 95% confidence limits.

yet available. Waddell & Marlowe (1980) concluded that paraquat is accumulated almost entirely into cells having the typical distribution of alveolar type II epithelial cells. There is also indirect evidence that paraquat and putrescine are accumulated into the same cell types (Smith & Wyatt 1981). In a series of as yet unreported studies with the use of autoradiographic techniques the distribution of paraquat, putrescine and the polyamines spermidine and spermine in lung slices has been found to be confined to the alveolar type I and type II cells and Clara cells of the lung (Soames & Smith, unpublished results). Thus, it appears from both direct and indirect evidence that the alveolar epithelium is at least partly a site for the accumulation of paraquat.

The realization that paraquat is accumulated into specific cell types in the lung is of considerable importance in attempting to understand its mechanism of toxicity. It is claimed that there are more than 40 cell types in the lung (Sorokin 1970) and therefore if paraquat is accumulated into only a small proportion of the total cell population then the intracellular concentration of paraquat in those cells it damages (alveolar type I and type II epithelial cells) could be seriously underestimated. This is because the data describing the amount of paraquat in the lung is usually expressed as a function of the wet weight of tissue. Therefore the concentration, when expressed in this way, may underestimate by as much as two orders of magnitude the intracellular concentration of paraquat in those cells into which it has accumulated.

Primary mechanism of toxicity

Paraquat has the ability to undergo a one-electron reduction from the cation to form a stable blue coloured free radical in the absence of oxygen (Michaelis & Hill 1933). In the presence of oxygen the radical will immediately reform the cation with the concomitant production of superoxide anion (O_2^-). This reaction between paraquat radical and oxygen is so rapid that it is diffusion-limited (Farrington *et al.* 1973). Thus, provided there is a continuous supply of electrons to paraquat, and oxygen is present, paraquat will rapidly cycle from its oxidized to reduced form with the continuous production of O_2^-.

Gage (1968) first reported that under anaerobic conditions, NADPH together with a flavoprotein could reduce paraquat from its cation to radical. Under aerobic conditions the radical is reoxidized and this redox cycling continues until the available NADPH is consumed.

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The studies of Gage (1968) were extended by Baldwin *et al.* (1975) who demonstrated that microsomal preparations from liver, lung and kidney were all able to generate radicals of paraquat and eventually produce H_2O_2. The production of O_2^- and H_2O_2 may then lead to the formation of more reactive oxygen radicals, which in turn may be more toxic to the cell (Bus & Gibson 1984). Thus the cycling of paraquat from its reduced to reoxidized form provides a plausible primary mechanism of its toxicity that is entirely analogous with the proposed mechanism of the phytotoxicity of paraquat (Dodge 1971).

Biochemical changes associated with toxicity

The mechanism by which the redox cycling of paraquat leads to lung damage is still subject to considerable speculation. Emphasis has been placed on the ability of radical species of oxygen to react with lipid membranes and cause lipid peroxidation (Bus *et al.* 1974, 1975, 1976). Peroxidation of membranes is considered to lead to their dysfunction and hence damage to the cell. Both direct and indirect evidence for this mechanism has been produced. However, the *in vitro* evidence for the production of lipid peroxides is contradictory (Shu *et al.* 1979; Steffen & Netter 1979). The system to measure *in vitro* lipid peroxidation is usually one that uses microsomal preparations from either the liver or lung together with NADPH or an NADPH-generating system. Malondialdehyde production or diene conjugation is often used as a measure of lipid peroxidation in these systems. Several studies reported that paraquat does not cause lipid peroxidation *in vitro* (Illett *et al.* 1974; Steffen & Netter 1979; Kornbrust & Mavis 1980). However more recent studies by Trush *et al.* (1981) indicate that there is considerable *in vitro* stimulation of lipid peroxidation by paraquat in rat microsomes. It may be that in those studies where the *in vitro* evidence for lipid peroxidation was not obtained, NADPH became a limiting factor in the production of paraquat radical and hence superoxide anion.

Bus *et al.* (1976) have provided indirect evidence that lipid peroxidation is involved with the mechanism of paraquat toxicity *in vivo*. A mechanistic scheme incorporating their proposal is shown in figure 2 and using this scheme they have predicted the biochemical parameters that should be altered. Also, by interfering with some of the defence mechanisms shown in this scheme, the authors tested whether the toxicity of paraquat was altered. For example, the detoxification of lipid hydroperoxides, or indeed H_2O_2, via the enzyme systems glutathione peroxidase and glutathione reductase is known to require reduced glutathione and selenium as essential cofactors. Bus *et al.* (1975) demonstrated that paraquat toxicity was significantly enhanced in mice fed selenium-deficient diets. The studies with selenium, however, indicate the difficulty of this approach because selenium deficiency increases the toxic effect of paraquat to mouse liver, an organ that in mice is not usually damaged by paraquat.

In contrast to the evidence *in vitro*, there is little direct evidence that shows the generation of lipid peroxidation in the lungs of paraquat-treated animals. The problem of demonstrating lipid peroxidation *in vivo* is in many respects analogous to defining the amount of paraquat present in individual cell types. The inability to detect malondialdehyde or diene conjugates in the lungs of animals treated with paraquat may reflect the fact that only a relatively small proportion of the cell types are damaged. Thus, even if peroxidation does occur and is extensive enough to cause cellular damage, it may still not be large enough to be detected when the results are expressed per gram wet weight of whole lung. This problem is applicable to all investigations that attempt to demonstrate a critical biochemical event in a particular cell type which represents a small proportion of the total cell population. It is certainly no less applicable to

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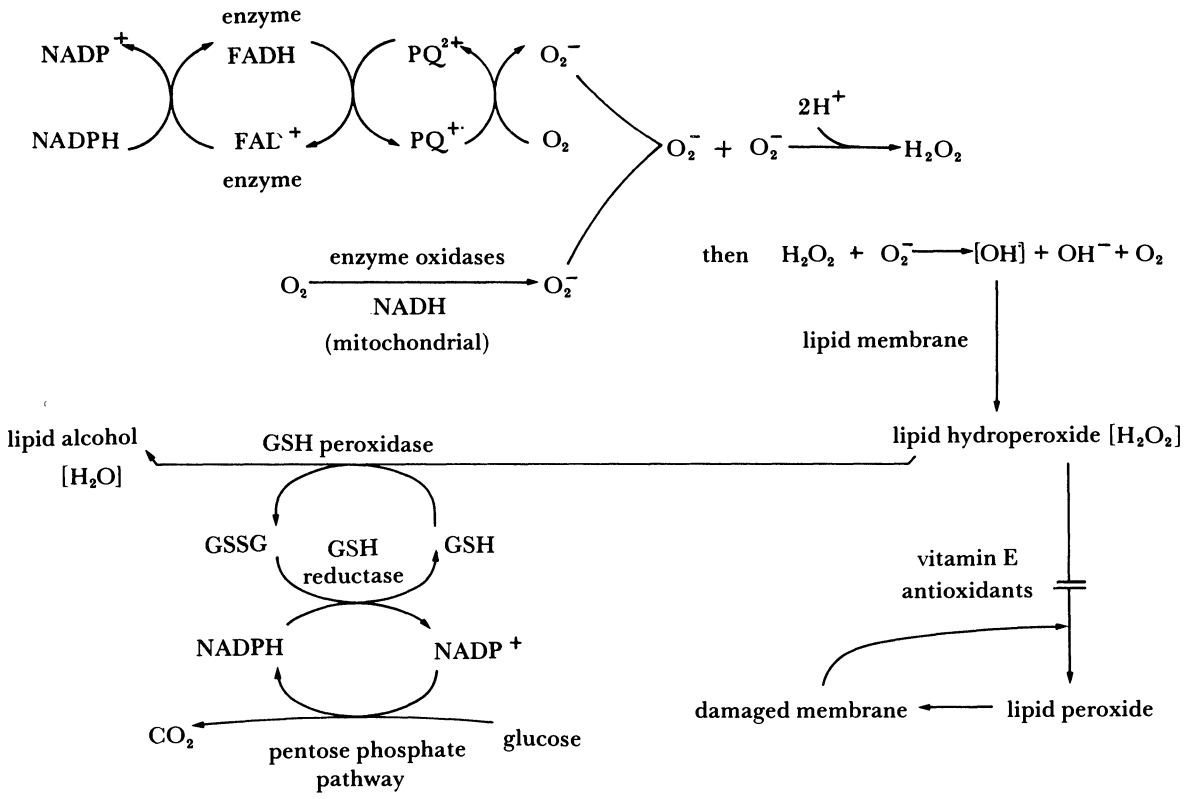

FIGURE 2. Mechanism of toxicity.

the suggestion that a critical biochemical event in the toxicity of paraquat is the depletion of NADPH levels in those cells in which paraquat is accumulated. (The sites of oxidation of NADPH are shown in figure 2.) This depletion would render the cells unable to perform essential physiological and biochemical functions. The first studies to indicate that this may be a possible explanation for the toxicity of paraquat were those of Fisher *et al.* (1975). They suggested that paraquat may shift the redox potential by oxidation of pyridine nucleotides and that the resulting scarcity of NADPH may then interfere with the synthesis of proteins and lipids. This hypothesis is supported by the evidence of Illett *et al.* (1974), who showed that NADPH oxidation was markedly increased in rat lung microsomes by paraquat. Fisher *et al.* (1975) also demonstrated that the oxidation of [1-^{14}C]glucose in lung slices was markedly enhanced in the presence of paraquat. They suggested this was consistent with a depletion in the levels of NADPH in the lung. They also demonstrated that lipid synthesis was inhibited in lung slices in the presence of paraquat, indicating that NADPH levels were reduced.

Witschi *et al.* (1977) measured the NADPH:NADP$^+$ ratio in the lungs of rats intravenously dosed with paraquat. They demonstrated that paraquat causes an oxidation of NADPH *in vivo*, although the relation of this effect to lung damage is not clear. More recently it has been shown that the pentose phosphate pathway (as measured by [1-^{14}C]glucose oxidation) is stimulated directly in proportion to the dose of paraquat given to rats and therefore the amount of paraquat present in the lung (figure 3). Also fatty-acid synthesis was inhibited in a dose-dependent manner in the lungs of treated rats (figure 4) and the degree of stimulation of the pentose phosphate pathway was directly related to the degree of inhibition of fatty-acid

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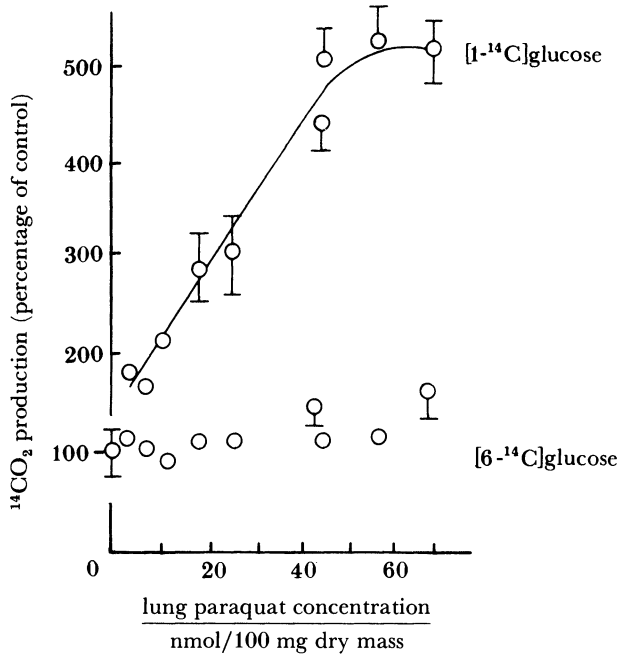

FIGURE 3. Relation between the production of $^{14}CO_2$ from [1-^{14}C]- or [6-^{14}C]glucose and lung paraquat concentration 2 h after dosing. Lung slices were prepared (0.6 mm thick) and $^{14}CO_2$ production from [1-^{14}C]- or [6-^{14}C]glucose was measured for 1 h. Results are expressed as a percentage of control. Points on the graph represent the mean ± s.e. with five rats per determination. Control values were 10102 ± 1304 d.p.m. (disintegrations per minute) $^{14}CO_2$ per 100 mg wet mass [1-^{14}C]glucose and 2851 ± 287 d.p.m. $^{14}CO_2$ per 100 mg wet mass [6-^{14}C]glucose. (From Keeling *et al.* 1982.)

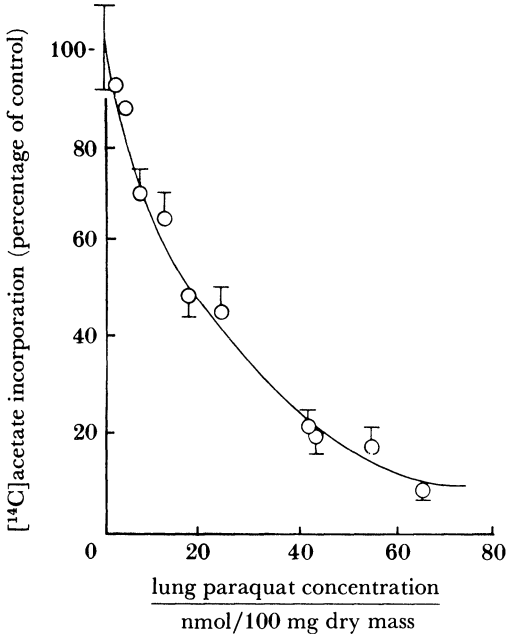

FIGURE 4. Relation between [^{14}C]acetate incorporation into fatty acids and lung paraquat concentration 2 h after dosing. Lung slices were prepared (0.6 mm thick) and [^{14}C]acetate incorporation into fatty acids was measured for 1.5 h. Results are expressed as a percentage of control. Points on graph represent the mean ± s.e. with five rats per determination. Control values were 10222 ± 580 d.p.m. ^{14}C per mg fatty acid. (From Keeling *et al.* 1982.)

synthesis (figure 5). Because both pathways appear to be equally affected (figure 5), this indicates that a single factor (probably NADPH) is affected, which in turn controls the activity of both pathways. In addition the direct measure of NADPH levels in the lung showed that this cofactor is reduced following the administration of paraquat (Keeling & Smith 1982). These data are consistent with the results of Witschi *et al.* (1975) in that the ratio of NADPH to $NADP^+$

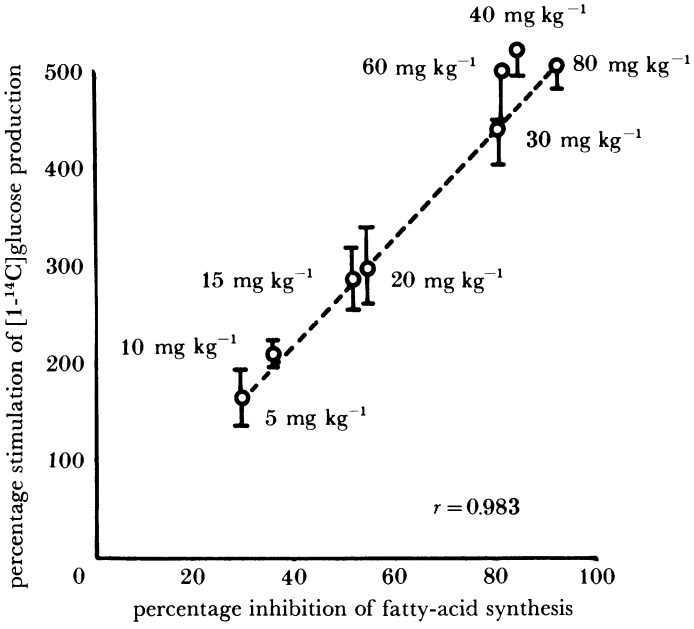

FIGURE 5. Relation between reduced [^{14}C]acetate incorporation into fatty acids and stimulated $^{14}CO_2$ production from [1-^{14}C]glucose in lung slices taken from rats 2 h after dosing subcutaneously with paraquat. Lung slices were prepared (0.6 mm thick) and $^{14}CO_2$ production from [1-^{14}C]glucose was measured for 1 h as d.p.m. $^{14}CO_2$ per 100 mg wet mass. [^{14}C]acetate incorporation into fatty acids was measured for 1.5 h as d.p.m. ^{14}C per milligram fatty acid. Results are expressed as percentage of control. Points on graph represent the mean of five rats per determination. (From Keeling *et al.* 1982.)

in the lung was reduced by paraquat. However Keeling & Smith (1982) demonstrated that it was a loss of NADPH that caused the change in the ratio rather than an alteration in both the NADPH and $NADP^+$ levels. The significance of these effects to an understanding of the mechanism of paraquat toxicity can best be demonstrated if they are considered in the context of the time course of events. The stimulation in pentose phosphate pathway activity and inhibition in fatty acid synthesis in the rat lung occurs within 2 h of administering an LD_{50} of paraquat (Keeling *et al.* 1982). These biochemical changes occurred at the time when it is not possible to demonstrate changes in the alveolar epithelium of the lung by using the electron microscope, nor can a significant fall in the NADPH content of the lung be measured (Keeling & Smith 1982). Thus it can be argued that when paraquat is accumulated into the lung, compensatory biochemical reactions take place that attempt to regenerate NADPH. The NADPH is utilized by the cycling of paraquat from its reduced and oxidized form and also in an attempt to detoxify H_2O_2 or lipid hydroperoxides via the glutathione peroxidase and reductase enzyme systems. Biosynthetic pathways such as fatty-acid synthesis that are highly dependent on NADPH (Wakil 1962) are inhibited owing to the rapid oxidation of NADPH. Only when these compensatory processes are no longer able to maintain NADPH levels in the lung can decreases in the amount of NADPH present be detected.

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That there are early significant biochemical changes in the redox state of paraquat-treated lungs is shown by the studies of Keeling *et al.* (1982). These studies demonstrated that following the administration of paraquat the amount of protein SH groups in the lung is reduced and there is an increase in protein SS forms. These protein SS forms are mostly found in the form of mixed disulphides where low molecular mass SH compounds (such as glutathione and cysteamine) react with protein. Keeling *et al.* (1982) found a direct correlation between the amount of mixed disulphides generated and the stimulation of the pentose phosphate pathway or indeed inhibition of fatty-acid synthesis. The formation of mixed disulphides between protein and glutathione may be part of a significant regulatory mechanism in rat tissues that maintains the integrity of cell membranes during oxidative stress (Isaacs & Brinkley 1977*a*, *b*). Increases in lung-mixed disulphides may be a factor that is responsible for regulating the pentose phosphate pathway and fatty-acid synthesis. It seems likely that the redox stress caused by paraquat in target cells results in an increase in glutathione oxidation (in an attempt to detoxify lipid hydroperoxides and H_2O_2). Any excess GSSG formed will react with protein sulphydryl to form mixed disulphides, perhaps mediated by a thiol transferase activity. As sulphydryl groups of enzymes react with oxidized glutathione to form mixed disulphides the enzyme activity of these proteins is altered (Isaacs & Brinkley 1977*a*, *b*; Moron *et al.* 1979), presumably by conformational changes in their structure. In this way, as GSSG reacts with the protein sulphydryl, the coincident activation of the pentose phosphate pathway would generate NADPH that in turn would lead to the reduction of GSSG to GSH. Thus the activation of the pentose phosphate pathway by GSSG in the cell would contribute to the regulation of the normal redox state in the lung cells. It is of interest that a greater understanding of the regulation of intermediary metabolism in the lung has come about from an attempt to understand the mechanism of paraquat toxicity.

It seems clear that the primary reaction involved in the mechanism of paraquat toxicity in the lung is the cycling from its cationic form to reduced form with the production of superoxide anion and consumption of electrons. Those authors who favour lipid peroxidation as the mechanism of paraquat damage suggest that the production of superoxide anion directly or indirectly, through the formation of more reactive species of oxygen such as hydroxyl radical, leads to the peroxidation of vital lipid membranes and hence cell death. On balance the evidence *in vitro* favours the formation of lipid peroxides by lung microsomes whereas the *in vivo* data is more tenuous. Those authors who have emphasized the importance of NADPH depletion in paraquat toxicity suggest that the depletion of this vital cofactor renders the lung unable to perform essential biosynthetic processes, and in turn is more susceptible to lipid peroxidation. There is no reason why these hypotheses need to be mutually exclusive.

In conclusion, therefore, if the glutathione peroxidase or reductase systems together with the endogenous levels of antioxidant are inadequate to defend against peroxidation, then it is possible that cell damage may occur. The depletion of NADPH by the redox cycling of paraquat will lead to additional utilization of the remaining NADPH in an attempt to provide reduced glutathione for the glutathione peroxidase enzyme activity. Thus NADPH will be depleted both in the production of superoxide anion and in a defence to it. Consequently, the pentose phosphate pathway is stimulated in an attempt to regenerate NADPH. The pathways dependent on NADPH appear to be inhibited either as a compensatory mechanism or because NADPH is depleted within the cell. With further generation of NADPH this cofactor is available either to regenerate reduced glutathione or it may be used for the further reduction

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of paraquat and cycling with oxygen. On balance, the evidence suggests that the concentration of paraquat in lung cells causes a severe redox stress that leads eventually to a sustained depletion in NADPH levels. This by itself, or in combination with lipid peroxidation, initiates the cascade of biochemical events that lead to cell death.

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