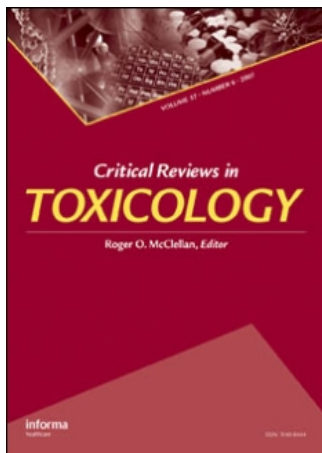


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### Paraquat Poisonings: Mechanisms of Lung Toxicity, Clinical Features, and Treatment

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# Paraquat Poisonings: Mechanisms of Lung Toxicity, Clinical Features, and Treatment

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Paraquat dichloride (methyl viologen; PQ) is an effective and widely used herbicide that has a proven safety record when appropriately applied to eliminate weeds. However, over the last decades, there have been numerous fatalities, mainly caused by accidental or voluntary ingestion. PQ poisoning is an extremely frustrating condition to manage clinically, due to the elevated morbidity and mortality observed so far and due to the lack of effective treatments to be used in humans. PQ mainly accumulates in the lung (pulmonary concentrations can be 6 to 10 times higher than those in the plasma), where it is retained even when blood levels start to decrease. The pulmonary effects can be explained by the participation of the polyamine transport system abundantly expressed in the membrane of alveolar cells type I, II, and Clara cells. Further downstream at the toxicodynamic level, the main molecular mechanism of PQ toxicity is based on redox cycling and intracellular oxidative stress generation. With this review we aimed to collect and describe the most pertinent and significant findings published in established scientific publications since the discovery of PQ, focusing on the most recent developments related to PQ lung toxicity and their relevance to the treatment of human poisonings. Considerable space is also dedicated to techniques for prognosis prediction, since these could allow development of rigorous clinical protocols that may produce comparable data for the evaluation of proposed therapies.

**Keywords** Chemistry, Clinical Features, History, Human Intoxication, Paraquat, Toxicokinetics, Treatment

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## ABBREVIATIONS

Ab, antibody.	GIT, gastrointestinal tract.
ACE, angiotensin-converting enzyme.	GPx, glutathione peroxidase.
AP-1, activator protein-1.	Gred, glutathione reductase.
ARDS, acute respiratory distress syndrome.	GSH, reduced glutathione.
ATP, adenosine triphosphate.	GSSG, oxidized glutathione.
BALF, bronchoalveolar lavage fluid.	H <sub>2</sub> O <sub>2</sub> , hydrogen peroxide.
BHs, bipyridylum herbicides.	HMP, hexose monophosphate pathway.
b.w., body weight.	HO·, hydroxyl radical.
CHP, charcoal hemoperfusion.	HRCT, high-resolution computed tomography.
CL <sub>Cr</sub> , creatinine clearance.	HWR, Haber–Weiss reaction.
CL <sub>PQ</sub> , paraquat clearance.	i.p., intraperitoneal.
C <sub>max</sub> , maximum plasma concentration.	i.v., intravenous.
CNS, central nervous system.	ICI, Imperial Chemical Industries (now Syngenta).
CP, cyclophosphamide.	K <sub>m</sub> , Michaelis–Menten constant.
CP51, 1-(2'-methoxyethyl)-2-methyl-3-hydroxypyridin-4-one.	LPO, lipid peroxidation.
Cyt c, cytochrome c.	MDA, malondialdehyde.
DEX, dexamethasone.	MINA, 4-methylisonicotinic acid.
DFO, desferoxamine.	MGBG, methylglyoxal bis-(guanyldiazide).
DL <sub>CO</sub> , lung carbon monoxide diffusing capacity.	MP, methylprednisolone.
DNA, deoxyribonucleic acid.	Na <sup>+</sup> , sodium.
ERG, electroretinogram.	NAC, <i>N</i> -acetylcysteine.
exEth, ethane in the expired breath.	NADP <sup>+</sup> , oxidized nicotinamide adenine dinucleotide phosphate.
Fe <sup>2+</sup> , ferrous ion.	NADPH, reduced nicotinamide adenine dinucleotide phosphate.
Fe <sup>3+</sup> , ferric ion.	NaSAL, sodium salicylate.
FiO <sub>2</sub> , fraction of inspired oxygen.	NF-κB, nuclear factor kappa-B.
FR, Fenton reaction.	NMN, <i>N</i> -methylnicotinamide.
FRD, ferredoxin.	NO, nitric oxide.
G6PD, glucose-6-phosphate dehydrogenase.	NOS, nitric oxide synthase.
GFR, glomerular filtration rate.	O <sub>2</sub> , oxygen.
GGO, ground-glass opacification.	O <sub>2</sub> <sup>-</sup> , superoxide radical.

PaCO<sub>2</sub>, partial pressure of carbon dioxide in arterial blood.

PAH, *p*-aminohippurate.

PaO<sub>2</sub>, partial pressure of oxygen in arterial blood.

PAO<sub>2</sub>, partial pressure of oxygen in the alveolus.

PEEP, positive end-expiratory pressure.

PFTs, pulmonary function tests.

P-gp, P-glycoprotein.

PQ or PQ<sup>2+</sup>, paraquat.

PQ<sup>•+</sup>, paraquat monocation free radical.

PUFAs, polyunsaturated fatty acids.

PUS, polyamine uptake system.

RNA, ribonucleic acid.

ROS, reactive oxygen species.

s.c., subcutaneous.

SH, thiol.

SOD, superoxide dismutase.

*t*<sub>1/2</sub>, half-life.

*T*<sub>max</sub>, time to maximum plasma concentration.

TPC, TUNEL-positive cells.

TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling.

VER, verapamil.

*V*<sub>max</sub>, maximal rate.

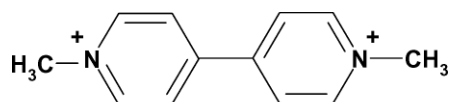
WBC, white blood cell.

XD, xanthine dehydrogenase.

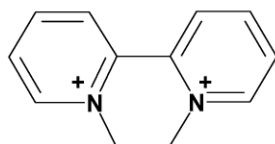
XO, xanthine oxidase.

## 1. HISTORY, USE, AND USEFULNESS OF PARAQUAT

Paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride; PQ) [CAS number 1910-42-5] is an herbicide belonging to the chemical family of bipyridylium (also called bipyridyl) quaternary ammonium herbicides. PQ and diquat (1,1'-ethylene-2,2'-dipyridylium dibromide; DQ) [CAS number 85-00-7] are the most commonly used herbicides of this group (Figure 1). They have similar chemical and physical properties and have a similar mode of action on plants (Calderbank, 1968). Of the two bipyridylium herbicides (BHs) in use, PQ is by far the most clinically significant in terms of number of intoxication cases, and it is the main subject of this review.



PQ (1,1'-dimethyl-4,4'-bipyridylium ion)



DQ (1,1'-ethylene-2,2'-dipyridylium ion)

FIG. 1. Chemical structures of paraquat (PQ) and diquat (DQ).

TABLE 1  
Some paraquat trade names

Paraquat	Paraquat–diquat mixtures
Crisquat	Preeglone
Dextrone X	Priglone
Esgram	Weedol
Gramoxone	

PQ was first described in 1882 (Weidel and Rosso, 1882). Its redox properties were discovered in 1933 by Michaelis and Hill (1933). By that time it was used as an oxidation–reduction indicator because an electron donation to the PQ ion (PQ<sup>2+</sup>) forms a stable [in the absence of molecular oxygen (O<sub>2</sub>)] free radical monocation (PQ<sup>•+</sup>) having a violet or blue color (Michaelis and Hill, 1933); hence, PQ is commonly called methyl viologen (Figure 1).

The PQ herbicidal properties were discovered at the Jealott's Hill International Research Centre, Bracknell, UK, in 1955, and in August 1962, PQ was introduced into the market as an herbicide by the Plant Protection Division Ltd of Imperial Chemical Industries (ICI; now Syngenta) (Homer et al., 1960; Calderbank, 1968; Smith and Heath, 1976). Gramoxone, manufactured by Syngenta, is the most common trade name for PQ, but the herbicide is also sold under many different trade names by several different companies (Table 1).

In spite of numerous intoxications, PQ is now registered and used in over 120 developed and developing countries throughout the world (Table 2). The main reasons for such widespread use are the following:

- PQ is an excellent herbicide for destroying weeds that may decrease crop yields. It is also used in pasture renovation and on noncrop areas such as public airports, electronic transformer stations, and around commercial buildings. Its success as a weed killer lies in the fact that small quantities of a PQ solution rapidly kill plants on contact with the leaves, and is also due to its low cost. In addition, PQ allows the roots to remain intact, thus holding the soil together and preventing soil erosion.
- PQ is highly hydrophilic and thus not absorbed through intact skin.
- Aerosolized PQ particles are large in diameter and thus do not reach the humans alveoli when exposed by inhalation route. Indeed, typical spray equipment generates droplet sizes with a median volume diameter over 100 μm.
- PQ is rapidly inactivated and metabolized once in the soil, preventing its accumulation in the ecosphere.

Since its introduction in the market, numerous successful practical uses of the herbicide have been implemented. PQ is an extremely effective, fast-acting, and nonselective foliage-applied contact herbicide, killing a wide range of grass and

TABLE 2

Countries in which paraquat is registered as of June 2005 (adapted from paraquat information center, [www.paraquat.com](http://www.paraquat.com))

Albania	El Salvador	Malaysia	Sierra Leone
Algeria	Ethiopia	Mali	Singapore
Angola	Fiji	Malta	Slovakia
Antigua & Barbuda	France	Mauritania	Somalia
Argentina	Gabon	Mauritius	South Africa
Australia	Gambia	Mexico	South Korea
Bahamas	Germany	Morocco	Spain
Bahrain	Ghana	Mozambique	Sri Lanka
Bangladesh	Greece	Myanmar	Sudan
Barbados	Grenada	Namibia	Suriname
Belgium	Guatemala	Netherlands	Swaziland
Belize	Guinea	New Zealand	Tahiti
Bolivia	Guinea-Bissau	Nicaragua	Taiwan
Botswana	Guyana	Niger	Tanzania
Brazil	Haiti	Nigeria	Thailand
Burkina Faso	Honduras	Oman	Trinidad & Tobago
Burundi	India	Pakistan	Turkey
Cameroon	Indonesia	Panama	Uganda
Canada	Iran	Papua New Guinea	United Kingdom
Cape Verde	Iraq	Paraguay	USA
Chad	Ireland	Peru	Uruguay
Chile	Israel	Philippines	Venezuela
China	Italy	Poland	Vietnam
Colombia	Jamaica	Portugal	Yemen
Costa Rica	Japan	Romania	Yugoslavia
Cote d'Ivoire	Jordan	Rwanda	Zambia
Croatia	Kenya	Sao Tome & Principe	Zimbabwe
Cuba	Lebanon	St Kitts & Nevis	
Czech Republic	Liberia	St Lucia	
Dominica	Macedonia	St Vincent & Grenadines	
Dominican Republic	Madagascar	Samoa	
Ecuador	Malawi	Senegal	

dicot weeds. PQ is rapidly inactivated by the majority of surrounding soils in the event of overspray (Amondham et al., 2006). Inactivation on contact with soil means that no biologically active residues remain in the soil, thus allowing planting or sowing to be carried out almost immediately after spraying. The  $PQ^{2+}$  is strongly attracted to the negative charge of soil clay particles, and once the equilibrium is established (Figure 2), PQ, at typical environmentally expected concentrations, becomes a strongly adsorbed residue that is biologically unavailable due to having an extremely low concentration in the soil solution.

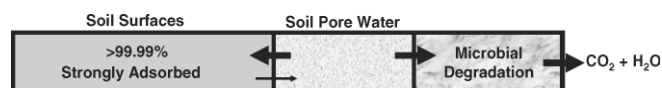


FIG. 2. Equilibrium dynamics for paraquat between the soil and soil solution. Adapted from Roberts et al. (2002).

The soil's natural deactivation capacity for this herbicide is several times the normally recommended application rate (Smith and Oehme, 1991), with existing evidence demonstrating that adsorption is capable of deactivating the equivalent of hundreds or even thousands of PQ applications over a wide range of soils. This also means that PQ is effectively immobilized in soils with no leaching to groundwater (Roberts et al., 2002).

Although the nonsystemic (contact) property of PQ makes it less than ideal for the long-term control of perennial weeds, the same property is of real advantage when parts of crop plants are sprayed accidentally and thus only the part receiving the spray is affected (Sagar, 1987). PQ is rainfast within only minutes of application. This property reduces the operator's dependence on weather and allows great precision in the timing of applications. In relation to the crop, PQ may be applied preharvest, preemergence, or preplant. The herbicidal activity becomes obvious as a rapid decolorization and desiccation of green plant tissue when

illuminated. It is the rupturing of the cell membranes, allowing water to escape from the plant material, that leads to the rapid desiccation of the foliage. PQ has also been used for control of aquatic weeds in irrigation ditches, from where residues disappear rapidly due to its strong adsorption to bottom mud and onto aquatic weeds (Grover et al., 1980). The best herbicidal results are achieved by spraying in late afternoon or evening. However, the herbicidal activity is slower in the dark, owing to the decrease of naturally occurring reducing agents characteristics of photosynthesis.

Product formulations differ among countries. Typically, PQ is available as a 10% to 30% concentrated solution (according to the manufacturer's instructions, correctly diluted spray solutions should contain no more than 0.05 to 0.2% of PQ ion) for agricultural use. PQ formulations are broadly neutral but can be irritant and corrosive, and the concentrate may also contain an aliphatic detergent to enhance entry of PQ into the cells and thus its toxicity. PQ can be applied safely when used according to the manufacturer's guidelines (Hart, 1987). It is generally with the liquid formulations (especially the concentrated ones) for agricultural use that the vast majority of fatal cases of intoxication have occurred. Proudfoot et al. (1987) reported a mortality of 65% in patients who ingested the concentrated formulation and only 4% in those who ingested the diluted solutions (2.5% w/v). Originally, marketed aqueous PQ formulations were brown in color. Due to mistakes with other common beverages such as coffee and cola drinks, among others, the color is now dark blue-green. The formulations also contain a powerful stenching and emetic agent. Recently, Syngenta scientists have developed a

formulation, Gramoxone Inteon, which contains a gelling agent (alginate) that is activated at the pH of stomach acid, and increased levels of an emetic and a purgative. Once formed, the gel minimizes and slows dispersion and passage of PQ to its site of absorption in the small intestine, allowing more time for productive emesis caused by the emetic, reducing the absorption of PQ into the blood (Heylings et al., 2007). The new formulation improved overall survival following PQ ingestion from 25.6% to 35.3% (Wilks et al., 2006).

### 1.1. Mode of Action as Herbicide

Herbicidal activity, as well as PQ-induced toxicity to mammals, was found to be linked to PQ redox potential (Bird and Kuhn, 1981). Initial work on the mode of action of BHs by Mees (1960) indicated that their ability to cause rapid kill is dependent on the photosynthetic activity of plants (sunlight) and on  $O_2$ . Zweig et al. (1965) further found that BHs cause a deviation of electron flow from Photosystem I (which normally transfers its electron to ferredoxin), leading to an inhibition of oxidized nicotinamide adenine dinucleotide phosphate ( $NADP^+$ ) reduction during photosynthesis (Figure 3). Resulting from this process,  $PQ^{+•}$  is produced in the cell at the expense of NADPH. Thus, PQ is only toxic to the green parts of the plant, where the photosynthesis occurs (Slade, 1966).  $PQ^{2+}$  is then rapidly reoxidized by the  $O_2$  produced in chloroplasts (Slade, 1966). During the reoxidation, a superoxide radical ( $O_2^{\cdot-}$ ) is generated, with the subsequent oxidation deleterious effects and consequent cell death. This redox cycle occurs until the supply

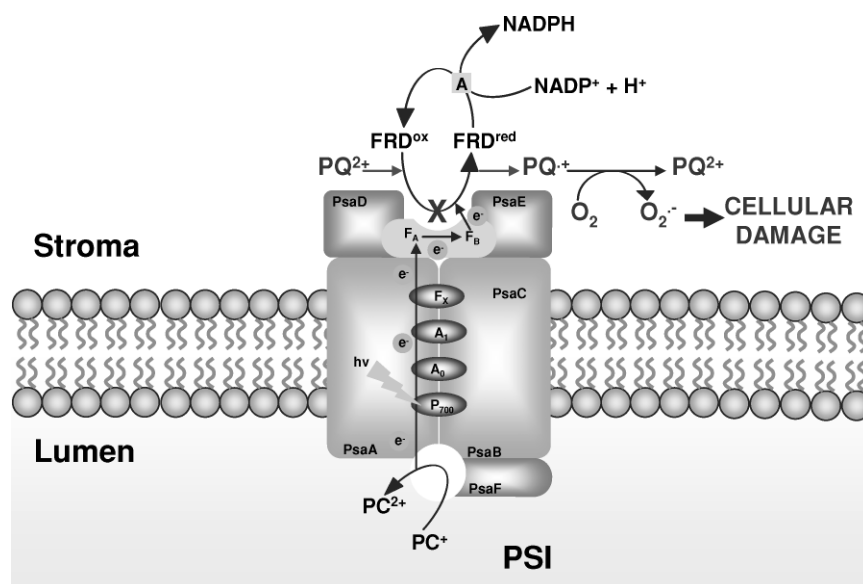


FIG. 3. Herbicidal mechanism of paraquat. In photosystem I (PSI), plastocyanin (PC) transfers its electron ( $e^-$ ) through a series of steps ( $P_{700}$ ,  $A_0$ ,  $A_1$ ,  $F_x$ ,  $F_A$ ) to ferredoxin (FRD) and finally to  $NADP^+$ . Paraquat ion ( $PQ^{2+}$ ) binds near the FRD binding site in PSI and accepts an  $e^-$ , becoming paraquat monocation free radical ( $PQ^{+•}$ ), which initiates a series of reactions leading to cell membrane disruption and plant death. The formation of such free radicals stops electron transport to  $NADP^+$  and effectively inhibits normal functioning of PSI. A, Ferredoxin- $NADP^+$  reductase.

of free electrons ceases. Therefore the mechanism of toxic action of PQ involves cyclic reduction–oxidation reactions, which produce reactive oxygen species (ROS) and depletion of reduced nicotinamide adenine dinucleotide phosphate (NADPH). After application, penetration through the leaf surface occurs almost immediately. This absorption is increased by high light intensity and humidity and by added nonionic adjuvant in the formulation, which ensure good spray retention and wetting of target foliage. An excellent review of this subject is given by Dodge (1971).

## 1.2. Biodegradation Pathways

When bound to soil, PQ is virtually biologically inactive, unavailable for either herbicidal or ecotoxicological action and unavailable for microbial degradation or photodecomposition (Burns and Audus, 1970; Smith and Oehme, 1991). The strong binding of PQ to clay minerals (e.g., bentonite) forms the basis of a suggested method for preventing systemic absorption in human poisoning cases (Smith et al., 1974a). Such soil-bound residues may persist essentially indefinitely, with only a 10% annual loss and a field half-life ( $t_{1/2}$ ) of 6.6 years (Hance et al., 1980). PQ is only significantly available for degradation during the immediate period after soil application (especially during the first 96 h), when the herbicide is only weakly adsorbed to the soil particles (Burns and Audus, 1970).

### 1.2.1. Photochemical Degradation

The photochemical degradation of PQ has been observed in laboratory conditions, as well as on the surface of plant material and soils. It is the predominant mechanism of PQ degradation in soils (Smith and Mayfield, 1978), and it is related to the availability of ultraviolet (UV) between the wavelengths of 290 and 310 nm during daylight hours (Slade, 1965, 1966). The main intermediates of photochemical PQ degradation on plants or soil surfaces are of low toxicity. They decompose easily and are not expected to produce adverse environmental effects.

**1.2.1.1. On Plant Surfaces.** In agricultural practice, much of the sprayed PQ is initially deposited on plant surfaces. Slade (1965, 1966) applied PQ dichloride droplets to maize, tomato, and broad-bean plants and studied the degradation pathways. Determinations carried out at intervals of 100 days showed that degradation was caused by photochemical decomposition on the leaf surfaces and not by metabolism. Degradation products isolated from plants sprayed with [ $^{14}\text{C}$ ]PQ dichloride included 4-carboxyl-1-methyl- $^{14}\text{C}$ -pyridylum chloride or 4-methylisonicotinic acid (MINA) and methylamine  $^{14}\text{C}$ -hydrochloride. The photochemical degradation of PQ dichloride continued after the plants were dead (Figure 4). The photochemical degradation of PQ is rapid. A 0.1% PQ solution was completely degraded in 3 days under a UV lamp (Slade, 1965). PQ

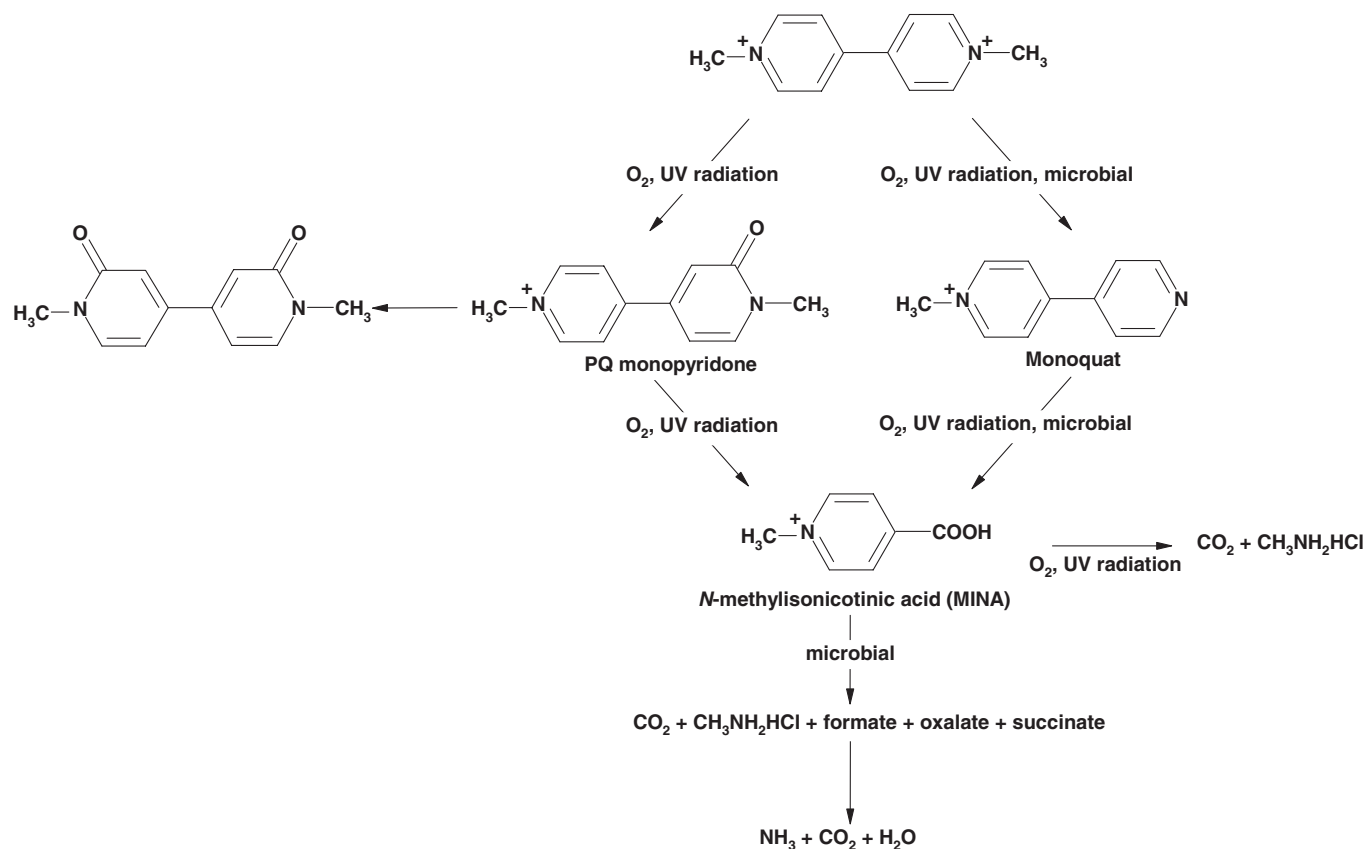


FIG. 4. Photochemical and microbial degradation of paraquat. Adapted from Slade (1965).



photodegradation products were not translocated from the desiccated leaves of the plants; nor were they found in the crops (cereals and fruits), when weeds were treated with PQ during 3–4 successive seasons (Slade, 1965).

**1.2.1.2. On Soil and Other Mineral Surfaces.** Slade (1966) showed that there was a breakdown, similar to that observed on plant surfaces, if spots of PQ on silica gel were directly exposed to sunlight. When [ $^{14}\text{C}$ ]PQ dichloride was sprayed on the bare soil surface of a field during a hot sunny period, traces of MINA were detected in the top inch of soil for the first few weeks afterward (Calderbank and Slade, 1976). Radioassay showed that the total soil residue did not markedly decrease during a 6- to 18-month period, so that, in agricultural practice, UV degradation of herbicide reaching the soil should be regarded as insignificant.

### 1.2.2. Microbial Degradation

Microbial PQ degradation was thoroughly reviewed by Haley (1979). In soils, it does not occur at appreciable rates due to the sequestering of the herbicide at mineral or organic anionic sites. Nevertheless, the biodegradation of PQ has been observed by a wide variety of soil microorganisms in aqueous solution. Baldwin et al. (1966) identified many soil microorganisms capable of degrading PQ. The herbicide was decomposed by *Corynebacterium fascians*, *Clostridium pasteurianum*, and *Lipomyces starkeyi*. Several other microorganisms were found to degrade PQ (Smith and Heath, 1976), but *Lipomyces starkeyi* proved to be the most active (Burns and Audus, 1970). In pure culture, degradation of PQ has been reported to occur under both aerobic and anaerobic conditions by *Clostridium pasteurianum* (Baldwin et al., 1966). In the case of the yeast *Lipomyces starkeyi*, the ability to degrade PQ was only seen under aerobic conditions (Funderburk, 1969).

Several biodegradation products of PQ have been characterized. The demethylated product, 1-methyl-,4,4'-bipyridylum or monoquat (Figure 4) was recovered from an unidentified bacterial culture, as well as the *N*-methyl betaine of isonicotinic acid or MINA (Summers, 1980). Whether these compounds occur sequentially in one degradation pathway or in separate pathways is unknown. The *N*-methyl betaine has been shown to be readily degraded in soils into methylamine and  $\text{CO}_2$  by microbial activity (Wright and Cain, 1970). The pyridylum ring carbons are known to be lost as  $\text{CO}_2$  by  $^{14}\text{C}$ -labeling studies. The methylamine can be utilized as a source of nitrogen and carbon. However, the enzymology of the degradation of PQ has not been reported and the other intermediates have not been identified.

## 2. CHEMISTRY OF PARAQUAT

The chemistry of PQ is dominated by its ability to act as a one-electron carrier. The electron can be transferred to PQ either *partially* (from a nucleophile or other electron-rich compound) or *completely* (from a reducing agent). In the first case, colored charge-transfer complexes are formed; in the second case, the blue-colored  $\text{PQ}^{\cdot+}$  is formed.

### 2.1. Physical and Chemical Properties

The physical and chemical properties of  $\text{PQ}^{2+}$  are summarized in Table 3. PQ is highly water soluble, slightly soluble in alcohol, and practically insoluble in organic solvents (Haley, 1979). PQ is nonexplosive and nonflammable in aqueous formulations. It is corrosive to metals and incompatible with alkylaryl-sulfonate wetting agents and strong oxidizing substances. Although nonionic surfactants may be used in combination, PQ is inactivated by anionic ones. It is stable in acid or neutral solutions but is readily hydrolysed by alkaline solutions (at  $\text{pH} > 12$ ). In the original container and under normal conditions, the shelf life of PQ is indefinitely long, and it is also stable at temperatures above the general environmental range.

The basic chemical nucleus of PQ (Figure 1) is a bipyridylum consisting of two quaternized pyridine rings bonded together such that their nitrogen atoms face diametrically away from one another. The quaternization is the result of the methyl radical addition (*para* position) to each of two nitrogen nuclei in the pyridine rings. The compound is, therefore, a *para*-substituted quaternary bipyridylum, hence its common designation PQ. In its usual oxidized form, it is ionized (bearing two positive charges) and it is most commonly manufactured as a dichloride salt. Chemically, PQ is thus 1,1'-dimethyl-4,4'-dipyridylum dichloride. The positive charges are largely resident on the nitrogen atoms, as shown by nuclear magnetic resonance (NMR) (Smith and Schneider, 1961). The herbicidal efficacy of PQ is related to the concentration of free  $\text{PQ}^{2+}$  in solution inside the chloroplast, but this will depend (in some cases, markedly) on the nature of the anion and any complexing agent with which it is applied (Homer and Tomlinson, 1959). For instance, many phenols form soluble crystalline complexes with PQ dichloride (White, 1969; Ledwith and Woods, 1970). This ready formation of complexes is possibly one of the factors that change the herbicidal activity of PQ among floral species and through the plant life span. Many plants constituents, such as lignin and tannin, are phenolic in nature and could cause immobilization of PQ (White, 1969; Ledwith and Woods, 1970).

### 2.2. Synthesis

PQ does not occur naturally. It was originally synthesized by Weidel and Rosso as reported in 1882 (Weidel and Rosso, 1882). There are several methods available for the synthesis of PQ. In the most common method, PQ is produced by coupling pyridine in the presence of sodium in anhydrous ammonia and quaternizing the 4,4'-bipyridyl with an excess of methyl chloride to obtain PQ dichloride (Figure 5). When bipyridyl is refluxed with methyl iodide or methyl bromide, the iodide and the bromide salt is obtained, respectively. The methyl sulfate salt can be obtained by heating 4,4'-bipyridyl with sodium acetate at  $70^\circ\text{C}$  for 2 h, then adding methyl sulfate and stirring for 15 min. Haley and Summers (Haley, 1979; Summers, 1980) thoroughly reviewed the published methods for PQ synthesis, and for the separation and

TABLE 3  
Physical and chemical properties of paraquat ion ( $PQ^{2+}$ )

Class	bipyridylium herbicide
Molecular formula	$C_{12}H_{14}N_2$
Molecular weight	186.3 (ion), 257.2 (dichloride) <sup>a</sup>
Common name	paraquat
IUPAC name	1,1-dimethyl-4,4-bipyridilium
CAS name	1,1-dimethyl-4,4-bipyridilium
Synonyms	methyl viologen
CAS number	4685-14-7 (ion), 1910-42-5 (dichloride), and 2074-50-2 (sulfate)
Specific gravity (20°C)	1.240–1.260 g/cm <sup>3</sup>
Physical state	white (pure salts), yellow (technical products) crystalline, odorless, hygroscopic powders
Melting point	PQ dichloride melts with decomposition at ~340°C to form poisonous vapors
Boiling point	PQ dichloride decomposes at ~340°C to form poisonous vapors
Solubility in water at 20°C	700 g/L
pH of liquid formulation	6.5–7.5
Vapor pressure	not measurable
$E'_0$ (relative to the normal hydrogen electrode)	–0.446 V
Octanol/water partition coefficient as log $P_{ow}$ (20°C)	–4.2
Dissociation constant	PQ ion does not dissociate
Relative gas density	8.88
UV spectrum (in water)	Single band centered at 257 nm (Kosower and Cotter, 1964)
X-ray analysis of the crystalline dichloride	Show two coplanar pyridine rings with two methyl groups (Russell and Wallwork, 1972)

<sup>a</sup> 1 g paraquat dichloride = 0.724 g of paraquat ion.

purification of bipyridylium salts. The yields obtainable vary from 20% to 96% of pure product. The only impurity permitted in the final product is the 4,4'-bipyridyl at a maximum level of 0.25% of the PQ content (Summers, 1980).

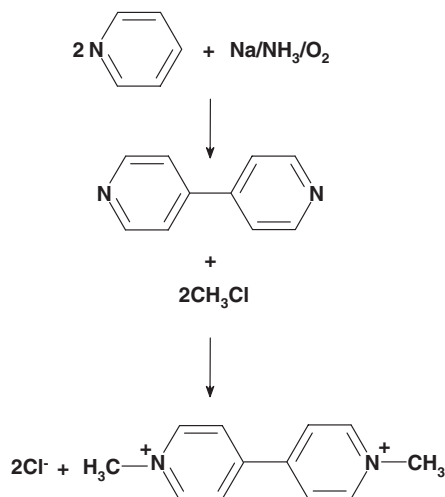


FIG. 5. Synthesis of paraquat.

### 2.3. Electrochemistry of Viologens and Paraquat Reduction

The viologens exist in three main oxidation states, namely,  $V^{2+} \leftrightarrow V^{\cdot+} \rightarrow V$ . The first reduction step is highly reversible and can be cycled many times without significant side reaction. The further reduction to the fully reduced state is less reversible, not only because the latter is frequently insoluble but also because it is an uncharged one. The compounds are also very stable chemically, although in more alkaline solutions they will dealkylate (Figure 6) as reported by Farrington et al. (1969). Because the methanol resulting from the dealkylation can be a reducing agent, solutions of methyl viologen in alkali can spontaneously be reduced and will then turn blue as the  $PQ^{\cdot+}$  is formed. Methanol is then itself oxidized to formaldehyde.

The PQ divalent cation is colorless, whereas the partially reduced  $PQ^{\cdot+}$  is blue colored and contains an odd electron. The odd electron is shared by all the nuclear carbon positions in the rings (Calderbank, 1968). This step is completely reversible, such that one equivalent of a reducing agent will reduce more than 50% of  $PQ^{2+}$  to  $PQ^{\cdot+}$  only if its reduction potential is more negative than that of PQ. Ito and Kuwana (1971) quoted the potential of the first reduction for  $PQ^{2+}$  as –0.446 V and the second is given as –0.88 V (relative to normal hydrogen

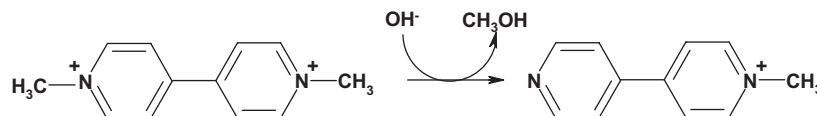


FIG. 6. Paraquat dealkylation in alkaline solutions.

electrode). A suitable reducing agent for generating the radical is sodium dithionite in alkaline solution ( $-1.13$  V). Considering that  $PQ^{+\cdot}$  carries an unpaired electron in a  $\pi$  antibonding orbital,  $PQ^{+\cdot}$  is remarkably unreactive. In addition, unpaired electron in  $PQ^{+\cdot}$  is not fixed. Delocalization of the unpaired electron (Figure 7) gives considerable resonance stabilization to the radical and thus it may diffuse outside the cell before reacting with  $O_2$ . It is a known fact that the greater the number of positive centers available to an odd electron, the greater the number of resonance structures and the higher the stability of the free radical. For review see Akhavein and Linscott (1968). The second step of PQ reduction is the addition of a second electron to the molecule

to originate the 1,1'-dimethyl-4,4'-dihydrobipyridyl. If it is required to stop the reduction at the radical stage, either a limited amount of the reducing agent must be used or the solution must be "poised" to the desired reducing potential ( $-0.56$  V for 99% conversion to radical) by adjusting the concentration of the reductant or the pH if relevant. The resulting fully reduced species is colorless (Michaelis and Hill, 1933).

### 3. TOXICOKINETICS OF PARAQUAT

The toxicokinetics of PQ has been studied in a variety of animal species, especially dogs, rats, and rabbits (Murray and Gibson, 1972; Hawksworth et al., 1981; Yonemitsu, 1986). The dog seems to be the most similar model of PQ to human toxicokinetics (Hawksworth et al., 1981).

#### 3.1. Absorption

Nearly all PQ poisonings result from ingestion. PQ is known to be very rapidly absorbed, apparently associated with the carrier-mediated transport system for choline on the brush-border membrane, though this absorption from the gastrointestinal tract (GIT) is low (Nagao et al., 1993a). Absorption occurs primarily in the small intestine (poorly from the stomach) and is estimated to be 1–5% in humans over 1–6 h period (Baselt and Cravey, 1989; Houze et al., 1990, 1995). Any recent food ingestion may decrease the amount of systemic absorption (Meredith and Vale, 1987; Bismuth et al., 1988). Although the plasma peak time ( $T_{max}$ ) is not known with certainty in humans, PQ may be detected in the urine as early as 1 h after ingestion, and according to data published by Proudfoot et al. (1979), Proudfoot (1995) and Smith (1988b), peak concentrations ( $C_{max}$ ) in humans are attained within 4 h and possibly within 2 h after intoxication. Smith et al. (1974a) reported that after oral administration of PQ to rats, plasma concentrations remained relatively constant for 30 h. During this period of time, concentrations in the lung rose progressively to several times the plasma concentration. If, during the first 30 h, plasma PQ concentrations were severely reduced by decreasing absorption of the herbicide from the GIT or increasing its elimination by extracorporeal techniques from the plasma, lethal concentrations wouldn't reach the lungs (Smith et al., 1974a). These authors also concluded that not only the  $C_{max}$  is responsible for determining the lung levels but also the maintenance of plasma levels from which the lung can take large amounts of PQ. The maintenance of such plasma concentrations in the rat has been shown to be the result of continued PQ absorption from the GIT over the first 30 h after oral administration. Absorption of PQ from the GIT into the human bloodstream is quite different from that seen in rats; concentrations decline

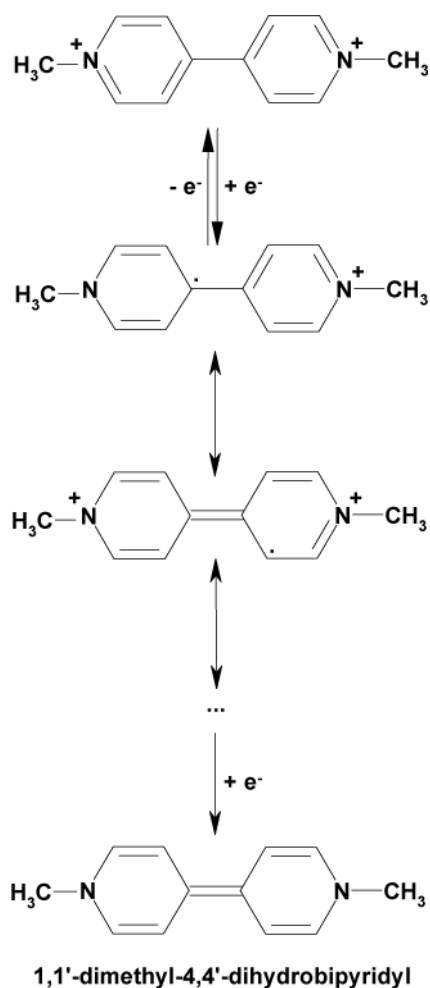


FIG. 7. Intermediate resonance structures of paraquat and full reduction.

rapidly over the first 15 h after  $T_{\max}$  to much lower levels than those described in rats due to tissue distribution, and more slowly thereafter (Smith, 1987). Thus, in humans, if adsorbents are to be effective in preventing PQ from entering the blood and consequently the lung, they must be administered within a few hours, or according to Bismuth et al. (1988), within the first few minutes after ingestion.

Daniel and Gage (1966) studied the absorption of [ $^{14}\text{C}$ ] PQ following oral and subcutaneous (s.c.) single-dose administration to rats. About 76–90% of the oral doses was found in the feces, and 11–20% in the urine; most of the s.c. dose (73–88%) was found in the urine and only 2–14.2% in the feces. These values bear no relation to their respective  $\text{LD}_{50}$  values (Conning et al., 1969). These studies evidenced that PQ was poorly absorbed from the gut. Rats, guinea pigs, and monkeys orally administered  $\text{LD}_{50}$  doses of [ $^{14}\text{C}$ ] PQ had low peak plasma concentrations (2.1–4.8 mg/L) (Murray and Gibson, 1972). Extensive corrosive injury to the GIT may increase the amount absorbed. The highest concentrations were found 1 to 6 h after an oral dose, depending upon the species used (Murray and Gibson, 1972).

Although almost all fatal exposures have resulted from the ingestion of PQ, a few case reports have involved rather extensive skin contamination (Samman and Johnston, 1969; Hearn and Keir, 1971; Vale et al., 1987; Smith, 1988a; Hoffer and Taitelman, 1989). PQ absorption through animal and human skin was studied in vitro (Walker et al., 1983). Human skin was shown to be highly impermeable to PQ, having a very low permeability coefficient of 0.73. Furthermore, human skin was found to be at least 40 times less permeable than the animal skins tested (including rat, rabbit, and guinea pig) (Walker et al., 1983). A study of the percutaneous absorption of PQ was undertaken in six human volunteers by Wester et al. (1984). It was observed that only minute quantities of PQ were absorbed through intact human skin over 24 h and that there was little difference among skin tested at different body sites in its ability to absorb PQ.

Fatal cases of s.c., intravenous (i.v.), intramuscular, or intraperitoneal (i.p) injection of PQ were also reported, with the doses being considerably lower than the lethal dose by ingestion route (Almog and Tal, 1967; Vale et al., 1987; Hsu et al., 2003).

Ocular exposure may cause local corrosive injury with ulceration and scarring likely resulting in a delayed slough of corneal epithelium 12–24 h after exposure, but not resulting in systemic toxicity (Cant and Lewis, 1968; McKeag et al., 2002).

Inhalation of PQ used in an agricultural/occupational setting does not allow sufficient absorption to cause acute systemic disease, because of droplet size (greater than 5  $\mu\text{m}$ ) that prevents deep lung exposure and absorption, low product vapor pressure, and low application concentration (Howard, 1983; Chester and Ward, 1984). Although no fatal cases have been reported from inhalation of PQ vapor or aerosols, toxicity has occurred from this route of exposure, since inhalation of PQ droplets may produce nasal and tracheobronchial irritation. An interesting episode in the history of the war against illicit marijuana use in which large

quantities of PQ were sprayed over culture fields in the early 1970s is described. By then, PQ was the herbicide of choice during aerial spraying of marijuana by the United States and Mexican governments. However, after spraying, growers simply harvested the crops before the plants were exposed to enough sunlight to damage the plants, resulting in an apparently healthy harvest although contaminated with PQ. Concerns regarding the smoking of PQ-sprayed marijuana in the early 1970s has proved unfounded, because PQ is destroyed by pyrolysis into a relatively nontoxic compound (4,4'-bipyridyl) during the smoking process (Groce and Kimbrough, 1982; Landrigan et al., 1983).

A fatal case of PQ absorbed per vagina of a 28-year-old woman (who inserted a tampon inadvertently soaked in PQ) as a consequence of respiratory, renal, and hepatic dysfunction was reported (Ong and Glew, 1989).

### 3.2. Distribution

Despite numerous studies, the distribution of PQ through the different tissues is still unclear. Dey et al. (1990) studied the toxicokinetics of [ $^{14}\text{C}$ ] PQ in rats exposed to a single s.c. injection. PQ was rapidly absorbed with a  $T_{\max}$  of 20 min. The toxicokinetic was best characterized by a two-compartment open model, the mean  $t_{1/2}$  being approximately 40 h. Peak concentrations in the kidney and lung tissues were at around 40 min. Nevertheless, the majority of the authors agree that the kinetics of PQ in the plasma is better described by a three-compartment open model. Murray and Gibson described a triexponential disappearance of [ $^{14}\text{C}$ ] PQ from the plasma after oral administration (126 mg/kg) in rats, guinea pigs, and monkeys (Murray and Gibson, 1972). The toxicokinetics of PQ appears to be similar in human and dog (Hawksworth et al., 1981; Vandenbogaerde et al., 1984). Hawksworth et al. (1981) described a plasma-concentration-time curve with a triexponential decline in dogs, suggesting a three-compartment model:

- Blood is assumed to be the central compartment. The concentrations found in plasma and erythrocytes are approximately the same at least in the rat (Sharp et al., 1972).
- The shallow compartment is thought to be composed of highly perfused tissues such as the kidney, liver, heart, etc. Rapid exchanges occur between this compartment and blood. The anatomy and physiology of the lung (a highly vascularized tissue) suggests therefore early exposure to any PQ circulating in the blood (Bismuth et al., 1987).
- The third compartment lies within the lungs, especially the pneumocytes type I and II and Clara cells, where exchanges with the central compartment are slow (Bismuth et al., 1987). The initial  $t_{1/2}$  of PQ in the lung was much greater than the  $t_{1/2}$  in other tissues and organs (e.g., kidney, liver, muscle, adrenal, spleen, heart, testis), explaining the highest PQ lung accumulation (Sharp et al., 1972). The kinetics of PQ in the rat

lungs shows a rapid decline with an elimination  $t_{1/2}$  of 20 min, followed by a slow decline with a  $t_{1/2}$  of about 50 h (Sharp et al., 1972). Peak concentration in lungs is reached 4–5 h after i.v. administration, and 5–7 h after ingestion, provided that renal function is normal (Sharp et al., 1972). Lethal concentrations may be achieved in the lung within 6 h of ingestion of 35 mg/kg (Houze et al., 1995). Patients are only rarely admitted to an experienced hospital in the treatment of PQ poisonings before the pulmonary peak. In the presence of renal failure (which normally occurs when more than 20 mg/kg PQ is ingested), peak pulmonary concentration is not achieved for 15–20 h, and may reach very high values (120 h or longer) (Hawksworth et al., 1981; Bismuth et al., 1987). Renal failure precludes elimination of PQ by its normal route. Furthermore, Hawksworth et al. (1981) suggested that an impairment of renal function by as little as 5% produces a fivefold higher concentration of the herbicide in the plasma. A critical plasma threshold is needed for active pulmonary uptake to occur (Manabe and Ogata, 1987). With time, however, it was shown that the concentration in the lung did fall to below that in muscle (due to the secondary  $t_{1/2}$  in the muscle). Considering that muscle represents a large percentage of the body mass, it may be considered an important reservoir of PQ (Murray and Gibson, 1972; Sharp et al., 1972).

Houze et al. (1990) studied the toxicokinetics of PQ in 18 cases of acute human poisoning. The concentration–time course was described by using a biexponential curve suggesting a two-compartment model with absorption, distribution, and elimination phases. Plasma PQ concentration exhibited a mean distribution half-life ( $t_{1/2\alpha}$ ) of 5 h and a mean elimination half-life ( $t_{1/2\beta}$ ) of 84 h. Tissue PQ distribution was ubiquitous with an apparent volume of distribution ranging from 1.2 to 1.6 L/kg. Muscle represented an important reservoir explaining the long persistence of PQ in plasma and urine for several weeks or months after poisoning (Smith, 1988b). The volume of distribution of PQ estimated from a kinetic study in one patient was 2.75 L/kg (Davies, 1987). Immunohistochemical studies were used to demonstrate the distribution and localization of PQ in several organs using the rat model. In the skin, PQ was localized in the ducts of sweat glands and sebaceous glands between 3 and 10 days after PQ i.v. injection (Nagao et al., 1993b). In the eyes, weak positive findings were observed in nerve fibers of retina between 3 and 10 days after the injection. In the cornea, PQ was localized in epithelial cells at the first 3 h and between 3 and 10 days after PQ administration. Since skin occupies a vast area of the body in animals, as an organ, it seems to be an important storage pool for the redistribution of PQ (Nagao et al., 1993b). PQ was also found in immune and haematopoietic systems (Nagao et al., 1994). In the bone marrow, PQ was localized in several types of blood cells (granulocyte, erythrocyte

and megakaryocyte) and their precursors between 24 h and 7 days after the i.v. administration. In the thymus, PQ was mainly localized in the medulla between 12 h and 7 days after administration, whereas in the spleen it was mainly localized in the red pulp between 12 h and 10 days after administration of PQ (Nagao et al., 1994). In the stomach, PQ was localized in the epithelial cells between 24 h and 10 days after i.v. administration, whereas in the esophagus, PQ was localized in epithelial cells and the lamina propria mucosa between 12 h and 10 days after administration. Three hours after the i.v. administration, PQ was localized in hepatocytes, and in the kidney, in the epithelial cells of the distal tubule. In the intestine, 3 h after injection, PQ was localized in the epithelial cells (Nagao et al., 1990).

Concerning the PQ binding to plasma proteins, controversial data exist. For many years, PQ was thought not to bind to plasma proteins (Lock and Ishmael, 1979). Jaiswal et al. (2002) then showed the binding of PQ to plasma albumin by using a fluorescence technique. These results were recently corroborated by Wang and coworkers (2007).

### 3.2.1. *Preferential Accumulation in the Lung*

Irrespective of the route of administration, the lung and the kidney are the organs showing the highest concentrations of PQ (Murray and Gibson, 1972; Sharp et al., 1972; Ilett et al., 1974). The distribution of [ $^{14}\text{C}$ ]PQ into various tissues after oral administration of 680 mol/kg to rats was followed as a function of time by Rose et al. (1976a). They showed that although the plasma concentration of PQ remained constant between 2 and 30 h after administration, PQ concentrations in the lung exhibited a time-dependent increase over the same period. None of the other studied organs showed this time-dependent accumulation. Rose et al. (1974) also demonstrated that slices of lung incubated with [ $^{14}\text{C}$ ]PQ exhibited a time-dependent accumulation of radioactivity. In addition, lung slices were the only tissue slices in which PQ accumulated at a concentration significantly higher than that in the medium. These studies demonstrated that lung, and no other major tissue, is able to accumulate PQ against a concentration gradient. After in vivo administration, PQ levels in the kidney did not show a time-dependent increase, but were nevertheless higher than those in the lung throughout the first 30 h (Rose et al., 1976a). These high concentrations of PQ in the kidney probably result from the fact that this organ represents the predominant route of elimination of PQ from the circulation and are likely to constitute extracellular rather than intracellular PQ. They may also underlie the observation that renal failure often occurs in PQ poisoning, especially during early stages. Taken together, these studies strongly suggest that the lungs are a specific target for the pathological effects of PQ because of its selective accumulation by this organ. PQ pulmonary concentrations can be 6 to 10 times higher than those in the plasma, and the compound is retained in the lung even when blood levels start to decrease.

TABLE 4  
Kinetic constants for the accumulation of paraquat into lung  
tissue slices from various species

Species	$K_m$ ( $\mu M$ )	$V_{max}$ (nmol of PQ/g tissue/h)
Rat	70	300
Mouse	68	556
Syrian hamster	77	452
Guinea pig	96	49
Rabbit	0.05	20
Humans	40	300
Monkey	70	50
Dog	60	10

Adapted from Rose et al. (1974).

### 3.2.2. Lung Accumulation Through the Polyamine Uptake System

Early work by Rose et al. (1974) demonstrated that the accumulation of PQ into rat lung slices occurred against a concentration gradient and could be abolished by metabolic inhibitors such as cyanide or rotenone, suggesting that the uptake is an adenosine triphosphate (ATP)-driven process. The accumulation also exhibited saturation kinetics with an apparent Michaelis-Menten constant ( $K_m$ ) of 70  $\mu M$  and a maximal rate ( $V_{max}$ ) of 300 nmol PQ  $\times$  g wet weight<sup>-1</sup>  $\times$  h<sup>-1</sup>. These observations, coupled with findings that PQ is neither metabolized by the lung (Conning et al., 1969; Ilett et al., 1974) nor becomes covalently bound to any degree (Ilett et al., 1974; Sullivan and Montgomery, 1983), which suggests that its accumulation is mediated through binding to, and subsequent translocation into, cells by a carrier system. Active accumulation of PQ via transport systems exhibiting similar kinetic parameters (Table 4) was also demonstrated in lung slices taken from other species [beagle dogs, New Zealand white rabbits, and cynomolgus monkeys (*Macaca fascicularis*)], including humans (Rose et al., 1976a). The kinetic constants for human and rat lung are statistically similar, suggesting that the rat may be a good experimental model for the study of PQ accumulation in the human lung. Thus, it seems likely that the human lung does possess a similar transport to that characterized in the rat lung, and this process accounts for the selective accumulation and hence the selective toxicity of PQ to the human lung. Carrier-mediated PQ uptake also occurs in the isolated perfused lung, into which accumulation of PQ to a concentration in excess of that in the perfusate has been observed (Rannels et al., 1985). However, the kinetics of this process appears somewhat different in comparison to the lung slices. The onset of active transport is preceded by an initial lag phase during which the intracellular PQ concentration approaches that in the perfusate, possibly because the endothelium functions as a barrier between the intravascular and the interstitial compartments. Only when concentrations proximal to the epithelium have risen sufficiently (relative to the  $K_m$  value for its uptake)

would active accumulation occur at a significant rate. Since in the isolated perfused lung the delivery of PQ occurs through the vasculature, the sequence or pattern of exposure of lung cells to PQ in this system may more closely resemble that occurring in vivo compared to the lung slice model, in which the epithelium becomes directly exposed. The observation that in vivo the rate of accumulation ( $V_{max}$ ) of PQ in the lung was only one-seventh of that found in vitro in lung slices led to a search for compounds present in plasma and capable of blocking the uptake of PQ in the lung (Lock et al., 1976). Subsequent to the identification of this transport system, a number of naturally occurring amines have been identified, which competitively inhibit the uptake of PQ into lung tissue and which themselves act as substrates and accumulated in rat lung slices in a saturable manner, obeying Michaelis-Menten kinetics. These amines include the diamines putrescine and cadaverine, the oligoamines spermidine and spermine (Smith, 1982; Wyatt et al., 1988), and the disulfide cystamine (Lewis et al., 1989; Figure 8). An important property of these specific polyamines is that they are positively charged at a physiological pH, and consequently they have a high affinity toward negatively charged cellular molecules. Thus polyamines are very soluble in water, and they exert strong cation-anion interactions with macromolecules, mainly with DNA and RNA (Marczynski, 1985), a feature that represents their best known direct physiological role in cellular functions such as cell growth, division, and differentiation (Janne et al., 1978; Heby, 1981). A possible gene coding for a polyamine transporter (TPO1) was isolated from eukaryotic cells and introduced into yeast cells (McNemar et al., 2001). Yeast cells heterologously expressing

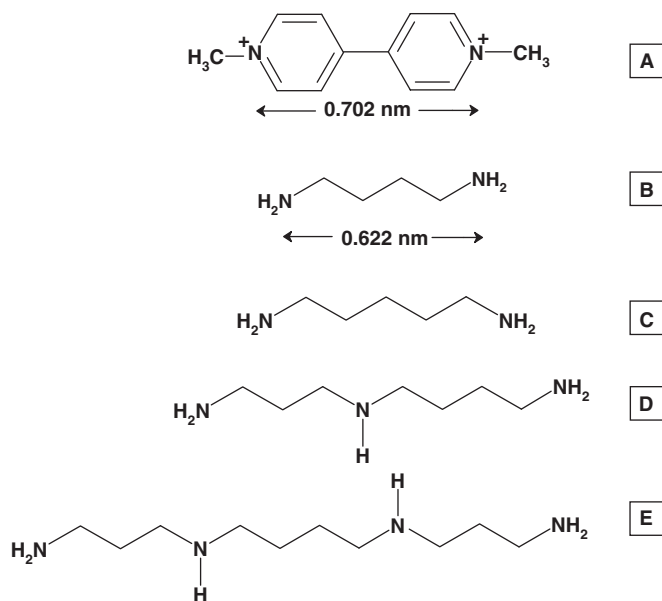


FIG. 8. Structures of paraquat (A) and putrescine (B), showing geometric standards of the distance between N atoms. The structures of cadaverin (C), spermidine (D), and spermine (E) are also shown.

TPO1 become sensitive to polyamines. For mammals, though, it is not known whether the transporter(s) is (are) located at the apical or basal side of the cells, and how the expression of the gene is regulated. A suggestion has been put forward that the polyamines, which, as already mentioned, are known to regulate cell growth, may play a role in the differentiation of alveolar epithelial type II cells to type I cells (Smith, 1982). It has also been proposed that cystamine, by representing a source of taurine, may have an antioxidant role in the lung (Lewis et al., 1989).

Subjects in whom acute fulminant poisoning occurs generally ingested more than 40 mg of PQ ion/kg of body weight (b.w.) (Vale et al., 1987). In these cases, the role of the pulmonary transport system clearly has a negligible role on the evolution of the intoxication, which progresses to multiorgan system failure. However, in cases of moderate poisoning, where, PQ plasma concentrations appear to be at the order of 10 to 20  $\mu\text{M}$ , kinetic considerations suggest that only cells actively accumulating PQ would achieve the intracellular concentrations necessary to cause significant cellular damage.

### 3.2.3. Structural Requirements for the Pulmonary Polyamine Uptake System

An important aim of earlier studies concerning pulmonary polyamine uptake system (PUS) was to discover the structural requirements for substrates of the transport system in order to find possible antagonists capable of preventing PQ from entering its target cells. Ross and Krieger (1981) established that to act as a substrate for the pulmonary PUS, a molecule must possess the following characteristics: (1) two or more positively charged nitrogen atoms, (2) maximum positivity of charge surrounding these nitrogens, (3) a nonpolar group between these charges, and (4) a minimum of steric hindrance. Gordonsmith et al. (1983) have demonstrated that the optimum distance (essential for binding and consequently, for transport) between the nitrogen centers is four methylene groups ( $\sim 0.622$  nm as it occurs in putrescine), although a spacing between four and seven methylene groups is tolerated. These assumptions explain how polyamines and PQ (with  $\sim 0.702$  nm between two positively charged nitrogens) can share a common uptake system, but also why PQ (with its steric hindrance of the nitrogens by the pyridine rings) is a less successful substrate (Smith, 1987). The affinity of the uptake system for the polyamines appeared to be sevenfold higher (i.e., exhibiting a lower apparent  $K_m$ ) than that of PQ (Smith, 1982). Although PQ proved to be a rather "poor" substrate (higher  $K_m$  than polyamines) for the PUS, it is undoubtedly "recognized" as a substrate, probably as a consequence of its structural similarity to these endogenous substrates (Figure 8), and is therefore mistakenly accumulated into the lung, especially in the alveolar type I and II cells and in the Clara cells, through this transport pathway (Smith, 1982). Later, O'Sullivan et al. (1991) showed that many putrescine analogues competitively inhibit putrescine and PQ uptake. The authors established that the inhibition

of putrescine uptake by analogues decreases with increasing *N*-alkylation and those analogues with a bulky substituent of the butyl chain do not inhibit the uptake at all. The strongest inhibition was found with *N*-(4-aminobutyl)aziridine. This cytotoxic compound does not seem to alter the polyamine  $V_{\max}$  but might fit into the substrate binding site of the transporter. The selective accumulation/retention of PQ in lung tissue provides a plausible explanation for this organ selectivity to damage in comparison with other tissues. Although the disposition of PQ in human tissues has not been as extensively studied as in experimental animals, the major organs affected in man are also the lung and kidney. Therefore, it seems likely that PQ is selectively accumulated in the human lung and excreted by the kidney.

DQ exposure produce signs and symptoms similar to those of PQ except for one important system—the pulmonary system (Jones and Vale, 2000). In contrast to PQ, DQ is not a substrate for the pulmonary PUS and therefore is not selectively pneumotoxic. In fact, DQ exhibits a much smaller intramolecular distance between the two charged nitrogen atoms, explaining its much greater safety margin (Rose and Smith, 1977).

### 3.2.4. Characterization of the Pulmonary Polyamine Uptake System

It is clear that there will be a range of endogenous and exogenous compounds that are capable of using this uptake system. Smith and Wyatt (1981) and Lewis et al. (1989) showed that the uptake of putrescine and cystamine in rat lung slices was not dependent on the sodium ( $\text{Na}^+$ ) concentration in the medium. In contrast to these observations, Rannels et al. (1989) found that, in type II pneumocytes the uptake of putrescine and spermidine was dependent on  $\text{Na}^+$ , whereas spermine uptake was not, indicating that polyamine uptake may take place via different uptake systems. However, in these experiments, the nature and concentration of the ions used to replace  $\text{Na}^+$  were probably critical factors, because it has been shown that a supplement of NaCl, LiCl, or choline significantly reduced the uptake of polyamines due to the increase of osmotic pressure (Rannels et al., 1989). On the other hand, Kumagai and Johnson (1988) showed that replacement of  $\text{Na}^+$  by mannitol or sucrose did not modulate putrescine uptake in rat enterocytes, whereas replacement by choline, lithium ( $\text{Li}^+$ ), *N*-methyl-D-glucamine, or tetramethylammonium did. It was hypothesized that cations can interact with the carrier but that no cotransport of  $\text{Na}^+$  is involved in putrescine uptake. Although these latter types of *in vitro* studies provide information about the intrinsic affinity of a compound for the incubated structure, they fail to give information on the actual behaviour occurring *in vivo*, since the anatomy and physiology of the studied tissue are disrupted. Using the isolated and artificial perfusion techniques, we demonstrated that the toxicokinetic behavior of PQ in the lung tissue appears to be modified by the iso-osmotic replacement of  $\text{Na}^+$  by lithium ( $\text{Li}^+$ ) in the perfusion medium (Dinis-Oliveira et al., 2006e). Although it seems that this condition does not significantly contribute to



improve the elimination of PQ from the extravascular structures of the lung, our results suggested that an impaired access to the lung tissue might be operating under  $\text{Na}^+$ -depleted conditions. Another issue is whether there is one or more pulmonary PUSs. In bovine arterial smooth muscle cells, Aziz et al. (1994) and Janne et al. (1978) found that putrescine was accumulated through an uptake system that is also used by spermidine, spermine, PQ, and methylglyoxal bis-(guanyldiazide) (MGBG), but spermidine and spermine were also accumulated through a different uptake system insensitive to putrescine and PQ and only partially sensitive to the presence of MGBG. Similarly, one study using suspensions of freshly isolated type II pneumocytes (Chen et al., 1992) showed that putrescine uptake was inhibited by PQ (and vice versa) in a partially competitive manner. These authors postulated that the PUS in type II cells for PQ and putrescine possessed two separate sites, one for each substrate, and that binding at one site leads to a conformational change in the other. However, such partially competitive inhibition was not found in other studies using hamster (Hoet et al., 1995) or human (Hoet et al., 1994) type II pneumocytes. In another study performed in rat lung slices the inhibition of PQ accumulation in presence of putrescine resulted from a process that appears to be competitive (Karl and Friedman, 1983).

### 3.2.5. Cellular Localization of the Polyamine Uptake System in the Lung

The problem of the localization of the PUS in the lung was addressed first by identifying the cellular targets for the toxicity of PQ and later by identifying the site of accumulation of radiolabeled PQ and/or polyamines. Smith and Wyatt (1981) performed morphological and functional studies to localize the site of cytotoxicity of PQ in lung slices taken from PQ (20 mg/kg)-exposed rats. Lung slices taken from rats 24 h after treatment evidenced morphological damage to type I and type II cells and their ability to take up putrescine (10  $\mu\text{M}$ ) or PQ (10  $\mu\text{M}$ ) was impaired, thus suggesting that type I or type II pneumocytes are the site of uptake of putrescine and PQ. Another experimental approach to determine the site of polyamine uptake resulted from autoradiography. Waddell and Marlowe (1980) showed that after the i.v. administration of [ $^{14}\text{C}$ ]PQ (10  $\mu\text{M}$ ) to mice, distribution of the label corresponded to that in alveolar type II cells. Studies with rat lung slices by Nemery et al. (1987) clearly demonstrated the presence of [ $^3\text{H}$ ]putrescine in alveolar type II cells and also in bronchiolar Clara cells (Figure 9).

Wyatt et al. (1988), who carried out both in vivo and in vitro studies, also showed uptake of [ $^3\text{H}$ ]PQ, [ $^3\text{H}$ ]putrescine, [ $^3\text{H}$ ]spermidine, and [ $^3\text{H}$ ]spermine by alveolar type II cells and, at least in vitro, also by Clara cells. Hoet and coworkers also visualized, by ultrastructural autoradiography, [ $^{14}\text{C}$ ]putrescine in both type I and type II cells of the alveolar epithelium, but not over the endothelium or any cells of the interstitium, in hamster (Hoet et al., 1995) and human (Hoet et al., 1993) lung slices (Figure 10). Dinsdale et al. (1991) also clearly demonstrated

labeling in the alveolar type I cell in rat by autoradiography at the electron-microscopic level. Saunders et al. (1989) suggested that alveolar macrophages were the site of putrescine and spermidine accumulation in rabbits, a species that shows a different response to PQ (Smith et al., 1978). Masek and Richards (1990) demonstrated that the toxicity of PQ to isolated mouse Clara cells could be decreased by addition of putrescine to the incubation medium. However, although this could be due to the inhibition of PQ accumulation into the cells, intracellular PQ levels were not determined.

The specific distribution of the PUS in a number of individual cell types is of considerable importance in attempting to understand the mechanism of PQ toxicity. Usually, data describing the amount of PQ present in the lung are expressed on a per gram wet weight basis. Since there are more than 40 different cell types in the lung (Sorokin, 1970), each with unique and functional activities, the concentration expressed on this basis will underestimate by perhaps as much as two orders of magnitude the concentration of PQ within specific cell types.

### 3.3. Metabolism

Only a small fraction of orally administered PQ is metabolized, with the greater part being excreted unchanged in the urine. Daniel and Cage (1966) undertook a study in rats using  $^{14}\text{C}$ -labeled PQ dichloride, and some evidence of metabolism by microorganisms in the gut, following oral dosing of rats, was found. Of the total oral dose of PQ, 30% of the label was present in the gut as metabolic products. Furthermore, metabolites were present in the urine after oral but not s.c. administration, suggesting the absorption of metabolites from the gut. Studies in vitro, using fecal homogenates, suggested that microbiological biotransformation was responsible for this effect. However, in another study, reported by Murray and Gibson (1972), with gavage administration of  $^{14}\text{C}$ -labeled PQ to rats, guinea pigs, and monkeys, formation of metabolites was not observed.

### 3.4. Elimination

According to former comments in this review, PQ is rapidly excreted by the kidneys. Daniel and Cage (1966) recovered virtually all of a PQ oral dose in the excreta of rats by two days. Absorbed PQ is almost completely eliminated unchanged by the renal system (Baselt and Cravey, 1989), which is accomplished by both glomerular filtration and active tubular secretion. Hawksworth et al. (1981) studied the elimination of PQ in dogs. After an i.v. administration of low doses of  $^{14}\text{C}$ -labeled PQ (30 to 50  $\mu\text{g/kg}$ ), it was rapidly excreted in the urine, with 80–90% being excreted in the first 6 h and urinary recovery being almost 100% complete by 24 h. The PQ clearance [ $\text{CL}_{\text{PQ}}$ , (28 ml/min)] was greater than the glomerular filtration rate (GFR), suggesting a process of active secretion, which may exceed 200 ml/min when renal function is normal (Bismuth et al., 1987). Tubular secretion was inhibited by *N*-methylnicotinamide (NMN) infusion, suggesting that PQ is



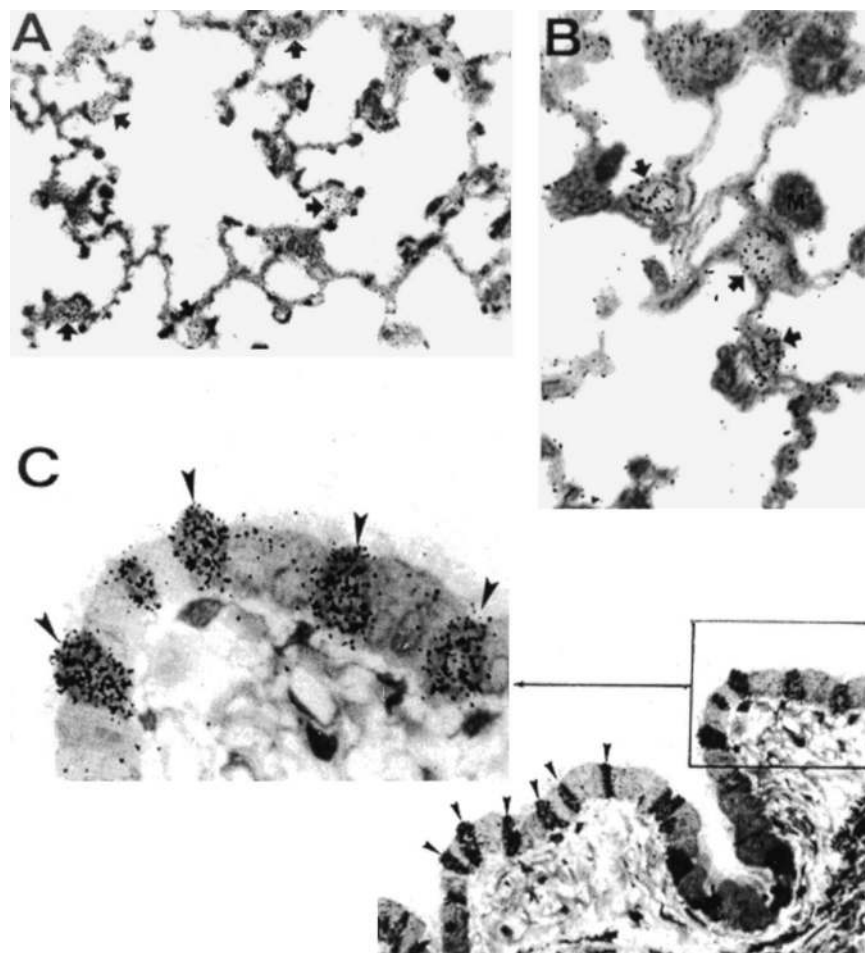


FIG. 9. Autoradiographs of rat lung tissue incubated with [ $^3\text{H}$ ]putrescine. Resin sections 1  $\mu\text{m}$  thick were stained with toluidine blue and examined by light microscopy. Labeling occurs in alveolar walls and in alveolar type II pneumocytes (A and B, arrows). There is no labeling in macrophages (B) or in walls of vessels, but Clara cells (arrowheads) in bronchiolar epithelium (C) show intense labeling. Original magnifications:  $\times 600$  in A;  $\times 1500$  in B and C. Reproduced with permission from Nemery et al. (1987).

secreted through an active transport process with high affinity for alkaline compounds (Hawksworth et al., 1981). After NMN administration,  $\text{CL}_{\text{PQ}}$  approximates to creatinine clearance ( $\text{CL}_{\text{Cr}}$ ). Following administration of large doses of PQ (20 mg/kg), the  $\text{CL}_{\text{PQ}}$  and  $\text{CL}_{\text{Cr}}$  decreased, due to renal tubular necrosis, reducing urinary output and  $\text{CL}_{\text{PQ}}$  by 10 to 20 times after the first few hours. Consequently, the urinary  $t_{1/2}$  increases (exceeding 120 h). Chan et al. (1997) studied the renal clearance of PQ in male Wistar rats using inulin as the marker of GFR. The obtained results demonstrated that the excretion of PQ was greater than the GFR, concentration dependent, and saturable, indicating that it was secreted by an active transport system. The excretion of PQ was predominantly dependent on the GFR with a small secretory component ( $K_m = 8.5 \pm 3.1 \mu\text{M}$ ,  $V_{\text{max}} = 114 \pm 19 \text{ nmol/kg/min}$ ). The  $\text{CL}_{\text{PQ}}$  was not inhibited by high doses of cimetidine, or *p*-aminohippurate (PAH). However, quinine and NMN reduced the fractional excretion of PQ, suggesting that they share the same cation transport system with PQ. Sharp et al.

(1972) reported a biphasic elimination of PQ from the plasma of rats after i.v. administration. The initial rapid phase had a 20–30 min  $t_{1/2}$ , and the slower phase a  $t_{1/2}$  of 56 h. Murray and Gibson (1972) also showed prolonged PQ elimination after oral administration to rats, guinea pigs, and monkeys. The urinary and fecal routes were equally important in all species studied. The fecal content was mainly due to elimination of unabsorbed PQ. Prolonged elimination of PQ in all tested animals indicated retention of the herbicide in the body. Despite the rapid PQ excretion, the kidneys are not very efficient at removing it from blood, since there is considerable reabsorption of PQ through the proximal convoluted tubules (Ferguson, 1971). This reabsorption appears to be a process of simple passive diffusion and is therefore reduced by rapid diuresis. This fact has considerable clinical significance. Biliary elimination (Daniel and Gage, 1966; Hughes et al., 1973; Nagao et al., 1990) could also represent an important excretory pathway due to the strong expression of P-glycoprotein (P-gp) at the canalicular membrane

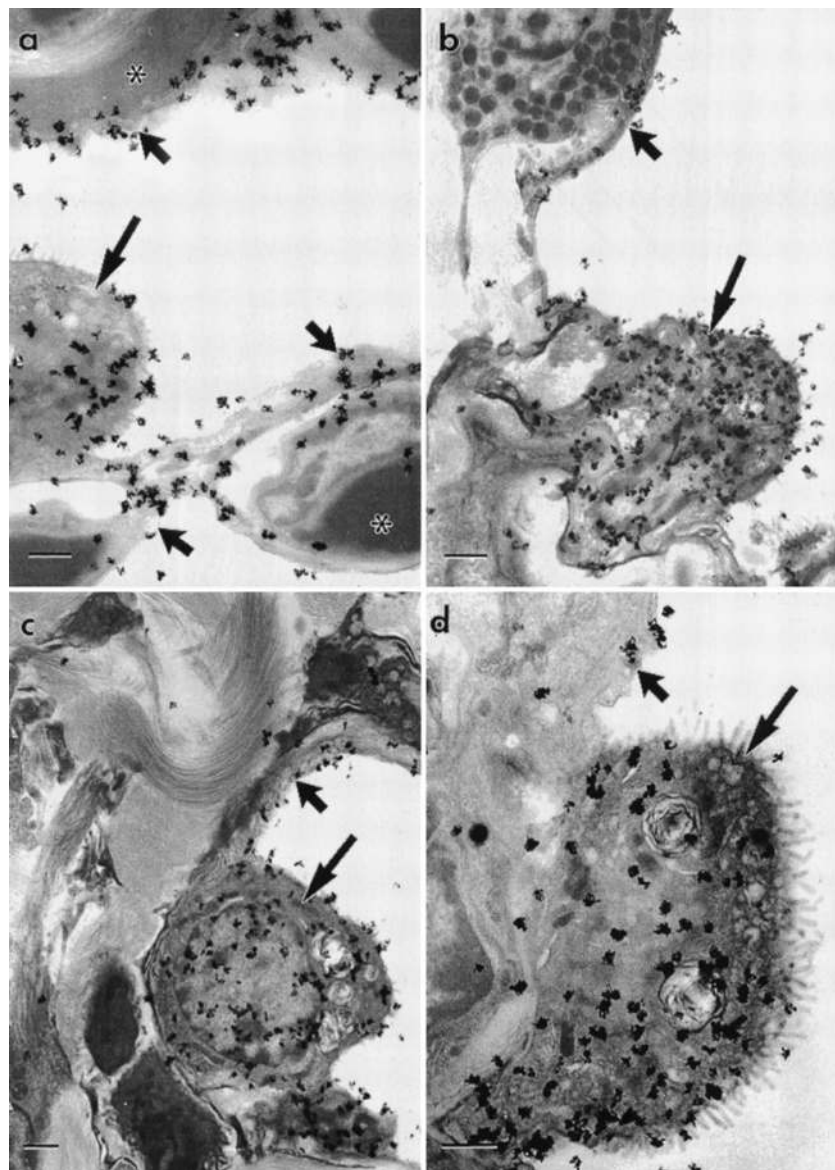


FIG. 10. Autoradiographs of human lung tissue incubated with  $2.5 \mu M$  [ $^3H$ ]putrescine. Unstained resin sections  $1 \mu m$  thick were examined by electron spectroscopic imaging. (a–d) Four different alveolar spaces lined with type II and type I pneumocytes. Silver grains are evident over type II pneumocytes (long arrows) and lining of alveoli (short arrows) but not over erythrocytes (\*) or paranuclear regions of endothelium. Cellular and noncellular components of alveolar interstitium were largely devoid of silver grains. Silver grains were uniformly distributed over both nucleus and cytoplasm of type II cells. Bars,  $1 \mu m$ . Reproduced with permission from Hoet et al. (1993).

of hepatocytes (Fardel et al., 2001, 2002) as recently described (see later discussion; Dinis-Oliveira et al., 2006b). From their studies, Nagao and coworkers (1990) also showed that PQ is secreted into the gut lumen from epithelial cells and that PQ secreted from liver into the duodenum is reabsorbed into the epithelial cells of the intestine.

Data from the limited human studies point to an elimination pattern similar to the excretion observed in experimental animals, with unchanged PQ elimination being essentially renal

through two pathways: glomerular filtration and tubular secretion (Bismuth et al., 1988). Tubular reabsorption is minimal (Beebejaun et al., 1971). With normal renal function,  $CL_{PQ}$  is much greater than  $CL_{Cr}$ , which enables excretion of high concentrations and large amounts of the herbicide within the first few hours of ingestion. Ingestion of large doses of PQ causes tubular necrosis with a rapid decrease in the GFR and tubular secretion, and a consequent increase of the elimination  $t_{1/2}$  (Bismuth et al., 1987; Bismuth et al., 1988). However, even without renal failure,

in humans, PQ excretion showed to be slower than in animals, since it was detected in the urine 7 days after ingestion (Carson, 1972) or as long as 26 days (Beebejaun et al., 1971). During this prolonged excretion time the concentration of PQ in blood was shown to be below the limit of detection; tissues act as depots from which PQ is released at a low rate (Carson, 1972). Nevertheless, in humans, over 90% is excreted unchanged within 12 to 24 h of ingestion, if renal function remains normal (Houze et al., 1990). Small amounts of PQ have been recovered in the bile postmortem. Thus enterohepatic recirculation may also exist in humans (Douze et al., 1975).

#### 4. BIOCHEMICAL MECHANISMS OF PARAQUAT TOXICITY

##### 4.1. Mechanism of Toxicity

A considerable amount of work has been done on the toxicodynamic mechanisms that underlie the toxicity of PQ. Most authors agree that upon entry into the cell, PQ undergoes a process of alternate reduction and reoxidation steps known as *redox cycling* (Figure 11): PQ is reduced enzymatically, mainly by NADPH-cytochrome P-450 reductase (Clejan and Cederbaum, 1989), NADH:ubiquinone oxidoreductase (complex I) (Fukushima et al., 1993; Yamada and Fukushima, 1993),

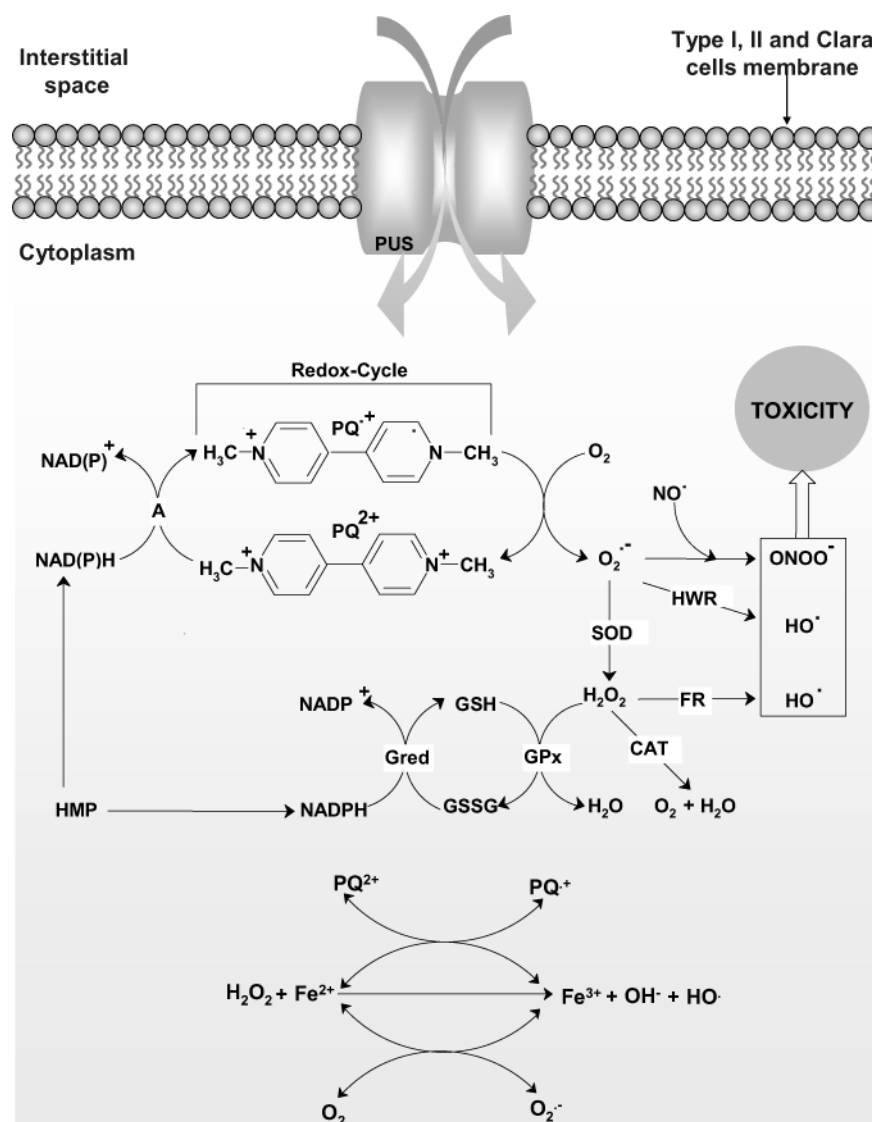


FIG. 11. Schematic representation of the mechanism of paraquat toxicity. A, Cellular diaphorases; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; Gred, glutathione reductase; PQ<sup>2+</sup>, paraquat; PQ<sup>•+</sup>, Paraquat monocation free radical; HMP, hexose monophosphate pathway; FR, Fenton reaction; HWR, Haber-Weiss reaction, PUS; polyamine uptake system. Adapted from Dinis-Oliveira et al. (2006c, 2006d, 2006e, 2007b).

xanthine oxidase (XO) (Winterbourn, 1981; Kelner et al., 1988; Waintrub et al., 1990; Kitazawa et al., 1991), and nitric oxide synthase (NOS) (Day et al., 1999) to form the  $PQ^{\cdot+}$  plus  $NADP^+$  or  $NAD^+$ . It is generally accepted that PQ uses cellular diaphorases, which are a class of enzymes that transfer electrons from  $NAD(P)H$  to small molecules, such as PQ (Dicker and Cederbaum, 1991; Aziz et al., 1994; Liochev and Fridovich, 1994; Day et al., 1999). The  $PQ^{\cdot+}$  is then rapidly reoxidized (returning to its original form) in the presence of  $O_2$  [lungs exhibit high alveolar  $O_2$  tension ( $PAO_2$ )] with subsequent generation of  $O_2^{\cdot-}$  (Bus et al., 1974; Dicker and Cederbaum, 1991). The reaction between  $PQ^{\cdot+}$  and  $O_2$  is very fast, with a rate constant of  $7.7 \times 10^8 M^{-1} s^{-1}$  (Farrington et al., 1973). The redox potential of PQ ( $PQ^{2+}/PQ^{\cdot+}$ ) is indeed very high ( $E'_0 = -0.45$  V), while that of molecular  $O_2$  ( $O_2/O_2^{\cdot-}$ ) is lower ( $E'_0 = -0.16$  V), thus facilitating electron flow from the reduced PQ to  $O_2$ . Provided that there is sufficient NADPH as an electron donor, and  $O_2$  as an electron acceptor, PQ will play a catalytic role in this redox cycling process, generating  $O_2^{\cdot-}$  at the expense of NADPH. This then sets in the well-known cascade leading to the production of other ROS, mainly hydrogen peroxide ( $H_2O_2$ ), by dismutation of  $O_2^{\cdot-}$ , and HO with consequent cellular deleterious effects (Smith, 1987). This mechanism of action is also responsible for the phytotoxic property of PQ (Dodge, 1971). Hydroxyl radicals may be generated by the Haber-Weiss reaction (Figure 11). This reaction is very slow but may be catalyzed by traces of transition metal ions or metal chelates (Fenton reaction) (Winterbourn, 1981; Richmond and Halliwell, 1982; Kohen and Chevion, 1985a, 1985b, 1985c).

#### 4.2. Biochemical Consequences of the Redox Cycling Process

Most authors agree that redox cycling of PQ is a prerequisite for its toxicity. However, the critical biochemical events in the toxic process are far from clear. It should be stressed that the several processes need not necessarily be mutually exclusive. It is quite possible that development of irreversible cell damage is the consequence of various events occurring independently of each other.

##### 4.2.1. Oxidation of NADPH

A decrease in the ratio  $NADPH/NADP^+$  on PQ-exposed lung tissue has been observed both in vitro (Sullivan and Montgomery, 1986) and in vivo (Witschi et al., 1977; Keeling et al., 1982). Although this is likely to be due partly to the oxidation of NADPH (an essential cofactor required for the maintenance of normal biochemical and physiological processes) in the reduction of PQ, NADPH is also used as a cofactor of glutathione reductase (Gred) in the regeneration of oxidized glutathione (GSSG) back to reduced glutathione (GSH). GSSG is formed during the reduction of peroxides to alcohol, or during the detoxification of  $H_2O_2$  into  $H_2O$  by glutathione peroxidase (GPx). Several authors have observed a marked stimulation of

the hexose monophosphate pathway (HMP) upon PQ treatment (Rose et al., 1976b; Bassett and Fisher, 1978; Keeling et al., 1982). Since this pathway represents the major cellular source of NADPH, this probably reflects an effort of the lung to maintain levels of reducing equivalents under conditions of oxidative stress, by stimulation of glucose-6-phosphate dehydrogenase (G6PD, the rate-limiting enzyme in the pathway). The studies of Keeling and coworkers (Keeling and Smith, 1982) demonstrated a loss of NADPH in PQ-treated lungs within a few hours after PQ exposure and before changes to the alveolar epithelium of the lung could be observed by electron microscopy. It has also been suggested that the activity of the enzyme G6PD is stimulated by GSSG, possibly through the formation of a mixed disulfide (Eggleston and Krebs, 1974). Since the lowering of the  $NADPH/NADP^+$  ratio is maintained despite the stimulation of the HMP, it is clear that this response is insufficient to overcome the prooxidant stress. As suggested by Smith and Nemery (1986), it is perhaps ironic that stimulation of the HMP may, in fact, merely make available more NADPH for the continued redox cycling of PQ and consequent ROS production. Assuming availability of NADPH and  $O_2$ , the redox cycling of PQ continues on and on, with the continued depletion of NADPH, and generation of  $O_2^{\cdot-}$ .

##### 4.2.2. Oxidation of Cellular Thiol (SH) Groups

Several reports suggest that the onset of PQ toxicity is accompanied by a decrease in the levels of intracellular SH groups, predominantly through the oxidation of reduced GSH to GSSG and to the formation of protein mixed disulfides (Keeling and Smith, 1982; Keeling et al., 1982; Dinis-Oliveira et al., 2006b). The oxidation of GSH to GSSG may occur through a direct effect of oxidizing species on the SH group. However, findings in GPx-deficient rat lungs (Glass et al., 1985) suggest that GSH is oxidized primarily through its role as a substrate in the GPx-mediated reduction of cellular  $H_2O_2$ . Both theories suggest that the inhibition of the reduction of GSSG, formed as a consequence of redox cycling of PQ, results in enhanced toxicity. The mechanism underlying this phenomenon is unclear. One possibility is that the effect is due to depletion of GSH, thus preventing its participation in direct scavenging of free radicals and/or preventing removal of peroxides by GPx. A second possibility is that it is not the decreased availability of GSH but the increase in GSSG levels that contributes to the toxic effect. Studies by Brigelius et al. (1982) have shown that increases in cellular levels of GSSG lead to the formation of protein-glutathione mixed disulfides, possibly through the mediation of SH transferase enzymes. The structure and consequent activities of many cellular enzymes appear to be sensitive to mixed disulfide formation, with some being inhibited while others are stimulated as a consequence. Increased levels of protein mixed disulfides have been observed in perfused liver (Brigelius et al., 1982) and in the lung (Keeling et al., 1982) of rats after exposure to PQ. Indeed, in the latter case, by administering various PQ doses, the authors

were able to demonstrate a direct linear relationship between the increase in levels of mixed disulfides and stimulation of the HMP activity. A similar relationship was demonstrated between mixed disulfide formation and inhibition of fatty acid synthesis. This provides good evidence that oxidative changes occurring subsequently to PQ exposure result in cellular metabolism alterations.

#### 4.2.3. Oxidative Damage to Lipids, Proteins, and DNA

Free radical-mediated membrane damage has been pointed to by many authors as a critical event in the mechanism of PQ toxicity. According to this hypothesis, electrophilic free radicals derived from the redox cycling of PQ are capable of abstracting allylic hydrogen atoms from membrane-associated polyunsaturated fatty acids (PUFAs). In this manner, when the generation of radicals spreads, it results in alterations of membrane structure and, ultimately, lipid peroxidation (LPO) (Yasaka et al., 1986). Indeed,  $\text{HO}^\bullet$  has been implicated in the initiation of membrane damage by LPO during the exposure to PQ in vitro (Bus et al., 1974, 1975; Shu et al., 1979) and in vivo (Bus et al., 1976; Burk et al., 1980; Dicker and Cederbaum, 1991). Curiously, clinical data concerning the LPO process in human PQ poisonings have been reported only rarely. Yasaka et al. (1981, 1986) noted an increase in serum concentrations of malondialdehyde (MDA), a marker for LPO, in one case. Kurisaki (1985) reported an increase of MDA in the lung and liver in seven patients who died from acute PQ poisoning. Recently, Ranjbar et al. (2002) investigated the oxidative stress in blood samples of workers in a pesticide factory, formulating PQ products for use in agriculture. Controls were age-matched workers with no history of pesticide exposure. It was concluded that PQ-formulating factory workers have elevated LPO and decreased antioxidant capacity and total thiol (SH) groups in blood, revealing their liability to oxidative stress upon sustained exposure to PQ.

The detection of hydrocarbons such as ethane or pentane in exhaled breath has attracted particular interest because these volatile hydrocarbons are known to appear within seconds after the release of free radicals from tissues and reflect the extent of peroxidized unsaturated fatty acids (Phillips, 1992; Kneepkens et al., 1994). Kazui et al. (1992) showed that the ethane in the expired breath (exEth) of rats reflects in vivo LPO. However, in another study, and in spite of gross pulmonary damage revealed by the autopsy, following intratracheal exposure of rats to PQ, exEth levels were not different from those of control animals (Schweich et al., 1994). The authors concluded that markers other than ethane must also be considered to detect this process in the lungs. Hong et al. (2005) reported the first clinical trial attempt to evaluate the exEth as a clinical marker of the degree of lung damage following acute PQ poisoning in 21 patients. The results indicated that even though the level of exEth was higher in the nonsurvivor group than in the survivor group, it is neither an independent predictor of survival nor a specific marker of lung injury in patients with acute PQ poisoning when

it is measured 24 h after acute PQ poisoning. Ishii et al. (2002) collected lung, kidney, and liver at autopsy, from seven victims poisoned with PQ. The authors identified and reported an increase of oxysterols [detected as 7-ketocholesterol (7-keto) and 7-hydroxycholesterol ( $7\alpha\text{-OH}$  and  $7\beta\text{-OH}$ )] in the lung and kidney in response to PQ ingestion. These authors suggested that oxysterols are suitable lipid markers of oxidative stress in man. Diene-conjugated 18:2 $\Delta$ 9,11-linoleic acid of plasma phospholipid of four patients (Situnayake et al., 1987) was also used as marker of LPO during the first few hours after PQ poisoning.

Besides lipids, ROS are also known to oxidatively modify DNA, carbohydrates, and proteins. One such modification is the addition of carbonyl groups to amino acid residues in proteins. Free radical damage to proteins has been implicated in the oxidative inactivation of several key metabolic enzymes. Fragmentation of polypeptide chains, increased sensitivity to denaturation, formation of protein-protein cross-linkages, and modification of amino acids side chains to hydroxyl or carbonyl derivatives are possible outcomes of oxidation reactions (Dean et al., 1997). In vivo studies have shown that PQ can cause lung protein oxidation assessed by carbonyl groups formation (Dinis-Oliveira et al., 2006a, 2006b).

Concerning DNA damage, PQ gave consistently positive results in assays for chromosomal damage (sister chromatid exchange, unscheduled DNA synthesis, and the comet assay) in mammalian cells (Sofuni et al., 1988; Ali et al., 1996; Dusinska et al., 1998). Using a human lung epithelial-like cell line (L132), Takeyama et al. (2004) showed that PQ induced DNA damage by G1 arrest. The same study also demonstrated that PQ could induce single-stranded DNA breaks after 2 h of treatment. Tokunaga et al. (1997) studied the effect of PQ on base modifications and showed an increase of 8-hydroxydeoxyguanosine (8-OH-dG) formation in various rat organs, particularly in brain, lung, and heart. In contrast, the formation of 8-hydroxyguanosine (8-OH-G), a marker for the oxidative damage to RNA, was not significantly affected by PQ. These results indicate that PQ causes base modifications as well as strand breaks as a consequence of the oxidative damage to DNA. When PQ was incubated with lung homogenates prepared from mice in the presence of calf thymus DNA, it caused damage to DNA in a concentration-dependent manner (Yamamoto and Mohanan, 2001). These results also suggest that the formation of  $\text{HO}^\bullet$  induced by PQ probably accounts for the DNA damage, since damage was attenuated by the co-treatment with melatonin, a potent scavenger  $\text{HO}^\bullet$ . More recently, Dinis-Oliveira et al. (2007a), using an in vivo rat model, showed a marked lung apoptosis assessed by the characteristic "ladder-like" pattern of DNA and by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay in the lung of rats as consequence of PQ exposure.

## 5. LUNG PATHOPHYSIOLOGY

The mechanism of PQ toxicity is very similar to that of DQ at the molecular level. However, the critical target organ differs

between the two compounds, so that the mammalian toxicology is quite different. While both herbicides affect the kidneys, PQ is selectively accumulated in the lungs through a saturable uptake process (Rose et al., 1974, 1976a; Smith, 1982; Smith and Nemery, 1992), and the systemic toxicity of PQ is dominated by lung toxicity. The pathological changes in the lung provoked by PQ have been investigated in various species of experimental animals. The rat, mouse, dog, and monkey develop lung damage similarly to that observed in humans (Conning et al., 1969; Murray and Gibson, 1972). The pathogenesis of PQ toxicity has been most extensively studied in the rat. There are two distinct phases in the development of pulmonary lesions (Smith et al., 1974b; Smith and Heath, 1976). These coincide with the early and late clinical stages. The initial stage involves acute damage to several organs, including liver, heart, kidneys, and lungs. Depending on the amount of PQ ingested, death may occur during this period and is associated with pulmonary, renal, and circulatory failure (Smith and Heath, 1976) (Table 5). Patients surviving this stage generally show a period of improvement. However, in most cases this is merely the prelude to the onset of the second stage, which involves damage almost exclusively to the lungs. Extensive pulmonary fibrosis ensues, resulting in dyspnea, cyanosis, and eventually death from respiratory failure. Nevertheless, it has been found that some species do not develop lung lesions. For example, the rabbit lung (Butler and Kleinerman, 1971) was not damaged by a single dose of PQ, although chronic administration to rabbits can induce lung damage (Seidenfeld et al., 1978).

### 5.1. Destructive Phase

The first toxicological effects to the lung correspond to a destructive phase in which the alveolar type I and type II epithelial cells are destroyed. This occurs within 1–3 days of dosing, although the speed at which it occurs depends on the given dose and the route of administration. Irrespective of these factors, the earliest observed pulmonary changes caused by PQ occur in the type I alveolar epithelial cells, which exhibit swelling (Kimbrough and Gaines, 1970; Sykes et al., 1977) accompanied by increases in their content of mitochondria and ribosomes, changes suggestive of increased metabolic activity (Smith et al., 1974b). Cell damage initially appears as mitochondrial swelling, followed by overt cell degeneration and cytoplasmic edema. The latter results in bulging of the cytoplasm into the alveolar space, and progresses to the rupture of the type I cell to expose the basement membrane (Smith and Heath, 1976). Early damage to type I alveolar cells by PQ may be explained by the fact that they cover a large surface area (approximately 93% of the alveolar epithelial surface area), representing 33% of alveolar epithelial cells. The main function of the type I alveolar cells, which are flat and actually form the alveolar vesicle, is the gas exchange between the air space and the capillaries. PQ deeply compromises lung function from the beginning of its toxic effects. The alveolar type II cell represents the only other lung cell type to show overt damage during this early phase of PQ toxicity. Dam-

age to the type II cell appears to lag slightly behind the type I cell injury, and first involves mitochondrial swelling and loss of the contents of the characteristic lamellar bodies (which are believed to contain surfactant) before frank cell destruction (Smith and Heath, 1976). The type II cells are more round shaped and are located at the distal border of the alveolar vesicles. They account for the remaining 7% by surface area and 67% by epithelial cell number. Their main functions are surfactant secretion, active transport of water and ions, and epithelial regeneration. The role of the surfactant (phospholipids, mainly phosphatidylcholine) is to form a thin film on top of a thin aqueous layer that covers the epithelial cells. This decreases the surface tension and thus prevents the lung collapse during expiration. They also act as a defense against toxic agents in consequence of their particular richness in NADPH-cytochrome P-450 reductase, and may undergo mitotic division and replace type I damaged cells. Notwithstanding the fact that some authors have observed morphological changes, including swelling (Brooks, 1971; Fukuda et al., 1985) and even vacuolization (Modee et al., 1972) of the capillary endothelium, the weight of the evidence suggests that these cells initially remain essentially undamaged, even at an ultrastructural level (Vijayaratnam and Corrin, 1971; Sykes et al., 1977). Certainly, the overt damage and destruction seen early in the epithelium do not manifest themselves in the endothelium. Dearden et al. (1982) observed endothelial damage in rats only 48 h after i.p. administration of PQ. In endothelial cells, on the septal side of the capillaries, the number of pinocytotic vesicles significantly increased from 48 to 96 h post-PQ. In endothelium adjacent to damaged epithelium, abnormalities included hydration, fragmentation, discontinuity, and widened intercellular junctions; these were maximal at 72–96 h post-PQ. These and other authors concluded that although other mechanisms are probably important, damaged pulmonary capillary endothelium seems to be a factor favoring the onset of an alveolitis, which is characterized by the production of a pulmonary hemorrhage proteinaceous edema and by the infiltration of the interstitial tissue and air spaces of the lung with inflammatory cells (Vijayaratnam and Corrin, 1971; Sykes et al., 1977). However, it should be noted that endothelial cell damage is notoriously difficult to demonstrate morphologically, even in instances in which there is functional evidence of microvascular impairment (Pietra, 1984). It has also been suggested that the destruction of the surfactant-producing type II cells results in increased surface tension within the alveoli, and that this draws fluid from the capillaries to produce edema (Gardiner, 1972). Alternatively, edema may also result from permeability changes in the alveolar wall subsequent to type I cell damage (Sykes et al., 1977). The inflammatory response that arises during this destructive phase, which is maintained throughout the proliferative phase, involves a rapid and extensive influx of inflammatory cells, mainly of polymorphonuclear leukocytes, macrophages (Clark et al., 1966; Brooks, 1971; Smith and Heath, 1974; Smith et al., 1974b; Sykes et al., 1977; Wasserman and Block, 1978; Fukuda et al., 1985), and eosinophils (Clark et al., 1966;

TABLE 5  
Phases of paraquat toxicity and associated clinical effects

Phases of toxicity	Ingested PQ ion dose (mg/kg b.w.)	Commercial formulation volume (PQ dichloride) for a 70-kg person	Toxicological effects		Prognosis
			Oral ingestion	Local exposure	
I. Asymptomatic or mild	<20 <sup>a</sup>	< 7.5 ml of 20% (m/v) concentrate	Nausea, emesis, diarrhea, intestinal hemorrhage, hemoptysis, oliguria. Renal and hepatic lesions are minimal or absent.	GIT, dermal and ocular irritation.	Full recovery is likely.
II. Moderate to severe	>20–30 but <40–50	7.5–15 ml of 20% (m/v) concentrate	Emesis and diarrhea followed by generalized symptomatology of systemic toxicity. Renal and hepatic failure may be present. Hypotension and tachycardia. Death usually due to pulmonary fibrosis.	Severe GIT, dermal, and ocular irritation, inflammation and ulceration of skin and mucous membranes.	Death occurs in the majority of cases, but may be delayed for 2–4 weeks.
III. Severe: acute fulminant toxicity	>40–55	> 15 ml of 20% (m/v) concentrate	Nausea, emesis, and diarrhea are followed by multiorgan failure (hepatic, renal, adrenal, pancreatic, CNS, cardiac, and respiratory failure). Patients do not survive long enough to demonstrate pulmonary fibrosis.	Marked ulcerations as in phase II. Esophageal perforation and mediastinitis can occur within 2–3 days of the ingestion.	Death usually occurs within 24 h (generally not delayed for more than a few days).

*Note.* GIT, gastrointestinal tract; CNS, central nervous system.

<sup>a</sup>Doses as low as 4 mg/Kg can cause death (Driesbach, 1983).

Vijeyaratnam and Corrin, 1971; Gardiner, 1972; Modee et al., 1972; Pietra, 1984; Fukuda et al., 1985; Candan and Alagozlu, 2001), into the interstitium and alveolar spaces. Most rats die within a few days after PQ exposure as a consequence of this extensive alveolitis and pulmonary edema. In human intoxication cases, the edema is generally not as extensive as seen in the rodent lung, and when it develops it is usually subject to clinical management.

## 5.2. Proliferative Phase

The second phase of PQ-induced lung toxicity involves the development of an extensive fibrosis in the lung, which is probably a compensatory repair mechanism to the damaged alveolar epithelial cells during alveolitis (Smith and Heath, 1976). If the degree of lung exposure to PQ is high, the alveolitis will be more widespread and severe, thereby resulting in a more extensive fibrosis and severe anoxia. Thus, the fibrosis may be

part of the normal reparative response of the lung to severe and extensive damage. The fibrosis associated with PQ toxicity is not exceptional or peculiar to the effects of PQ but is a response to an acute alveolitis that can also be induced by many other pulmonary toxins. The onset of the proliferative phase occurs several days after PQ ingestion. The earliest morphological indication of fibrotic development is the appearance of many profibroblasts in the alveolar spaces (Smith and Heath, 1976). These cells undergo rapid proliferation and differentiation to mature fibroblasts, which lay down collagen and ground substance to produce fibrosis. This fibrotic proliferation is very rapid, resulting in the loss of the normal alveolar architecture, interfering with gaseous exchange, and subsequently causing death from anoxia. Smith and Heath (1976) claimed that the localization of the fibroblasts (both immature and mature) and of the subsequent fibrotic lesion is entirely intra-alveolar. Other researchers have described interstitial in addition to intra-alveolar fibrosis (Fukuda et al., 1985), although they suggest that the intra-alveolar component is nevertheless more deleterious, because it is the latter that results in obliteration of the alveoli. The mechanisms for the development of this obliterating fibrosis are still poorly understood. Predominantly, in the event of interstitial or intra-alveolar fibrosis, whatever the cause, the normal architecture of the lung is destroyed due to the proliferation of fibroblasts and deposition of collagen, thereby reducing the effectiveness of gaseous exchange, leading to death as a consequence of severe anoxia. Using other experimental systems, not involving PQ, Witschi and coworkers have proposed that pulmonary fibrosis occurs when reepithelialization subsequent to epithelial damage is compromised in some manner (Witschi et al., 1980). Such a process would certainly appear to hold true also in the case of PQ, since the replacement of damaged type I cells (which constitute the majority of the epithelial surface area) is prevented by destruction of their progenitor type II cells. Moreover, Fukuda et al. (1985) suggested that secretion of proteolytic enzymes by stimulated inflammatory cells may result in degradation of alveolar basement membranes denuded by loss of the epithelium, and that this may also inhibit epithelial regeneration. This is consistent with the idea that within a given area of damage, reepithelialization and fibrotic proliferation represent mutually exclusive endpoints, and that the balance between the two is governed by the degree of epithelial damage. Thus, if reepithelialization is delayed (due to type II cell damage or to destruction of the basement membrane), fibrosis may occur. However, it appears that at least in the case of PQ, other factors may also play a role. In their review, Smith and Heath (1976) concluded that development of PQ-induced pulmonary fibrosis is independent of alveolar damage. They suggested that PQ may itself initiate the influx of pro-fibroblasts (Smith and Heath, 1976). This was evidenced to some degree by Conning et al. (1969), who demonstrated that macrophages treated with PQ caused a more rapid proliferation of cultured fibroblasts than did untreated macrophages, as well as by Schoenberger et al. (1984), who showed the release of a fibroblast growth factor by

PQ-exposed macrophages. Despite some advances toward understanding the nature of the PQ-induced fibrotic lung lesion, the ultimate mechanisms underlying this process, as with pulmonary fibrosis in general, remain elusive (Gharaee-Kermani and Phan, 2005).

## 6. CLINICAL AND EXPERIMENTAL OBSERVATIONS

Several studies have been performed to evaluate the acute toxicity of PQ administered by a variety of routes. An overall picture of the obtained results is summarized in Table 6. PQ sulfate and dichloride salts are equally toxic when expressed on the basis of PQ ion (Clark et al., 1966). There is a great variation in LD<sub>50</sub> values, depending upon the investigator, the laboratory where the work was done, and as result of the inherent differences in sensitivity between species, route of administration, and reproductive state. Evidence also exists of young animals being more susceptible (Clark et al., 1966). Also, individual animals of the same species show an unusually large variation in the time from dosing to death following identical dosage. When rats were weighed daily following a single oral or i.v. dose at a rate that would kill only a fraction of the tested animals, it was found that those minimally affected lost weight only briefly, and then began gradually to regain, whereas those that were severely affected continued to lose weight (Sharp et al., 1972). Rats lost at least as much body weight as would be expected during total deprivation of food and water (Peters, 1967). Weights of the two groups were statistically different after the first day following oral administration and on all days following i.v. administration. Weight loss appeared to be the result of lower food intake. Whether as the result of differences in absorption following oral administration, or of greater excretion, or sequestration regardless of the route of administration, minimally affected rats contained less PQ in their lungs, kidneys, and stomach during days 1–8 than did severely affected ones, including those that died (Sharp et al., 1972). In rats, this interval varies from 2 to 12 days, with some tendency for the deaths to be concentrated in an early and late peak (Clark et al., 1966). After rats had inhaled PQ, clinical signs and postmortem markers of toxicity were similar to those seen after oral, s.c., or i.p. administration. Aerosol LC<sub>50</sub> (mg/m<sup>3</sup>) values in PQ toxicity tests with mammals were directly related to the duration of exposure, PQ concentration in spray, and particle size [3  $\mu$ m (diameter) seemed most effective (Haley, 1979)].

Although histopathological alterations are generally similar among the rat, dog, monkey, and mice (Clark et al., 1966; Murray and Gibson, 1972), Butler (1975) found that the Syrian hamster is relatively resistant to interstitial fibrosis. Butler and Kleinerman (1971) also reported that rabbits did not develop the pulmonary changes typical of PQ poisoning in other species, despite doses of 2–100 mg/kg b.w. being administered i.p. and sacrifice of animals being delayed up to 1 month. The only findings in the lungs were occasional small interstitial infiltrates of lymphocytes and plasma cells, minimal alveolar hyperplasia, and some alveolar macrophages.



TABLE 6  
Paraquat LD<sub>50</sub> in various species

Species	Strain	Sex	Route	LD <sub>50</sub> (mg/kg b.w.) (95% confidence interval)	Reference
Mouse	NS	NS	per os	120	(Orme and Kegley)
			i.p.	30	
			i.v.	180	
Rat	Swiss-Webster	M	i.p.	39 (32.5–46.8)	(Sinow and Wei, 1973)
	Swiss-Webster	F	i.p.	30 (26.3–34.2)	(Bus et al., 1976)
	NS	F	i.p.	19 (16–21) <sup>a</sup>	(Clark et al., 1966)
	NS	F	i.p.	16 (10–26)	(Mehani, 1972)
	NS	NS	i.v.	21	(Orme and Kegley)
	NS	F	per os	112(104–122) <sup>a</sup>	(Clark et al., 1966)
	NS	F	per os	150 (139–162) <sup>a</sup>	(Clark et al., 1966)
	Sherman	M	per os	100 <sup>b</sup>	(Kimbrough and Gaines, 1970)
	Sherman	F	per os	110 <sup>b</sup>	(Kimbrough and Gaines, 1970)
	NS	F	per os	150 (110–173)	(Mehani, 1972)
	Sprague-Dawley	M	per os	126	(Murray and Gibson, 1972)
	NS	NS	per os	57	(Orme and Kegley)
	Sherman	M	dermal	80 <sup>b</sup>	(Kimbrough and Gaines, 1970)
	Sherman	F	dermal	90 <sup>b</sup>	(Kimbrough and Gaines, 1970)
Rabbit	NS	M	per os	50 (45–58)	(Mehani, 1972)
	NS	M	i.p.	25 (15–30)	(Mehani, 1972)
	NS		dermal	236	(Clark et al., 1966)
Cats	NS	F	per os	35 (27–46) <sup>a</sup>	(Clark et al., 1966)
Dog	Beagles	M	s.c.	1.8(1.0–6.1)	(Nagata et al., 1992)
		F	s.c.	3.5 (2.4–10.1)	(Nagata et al., 1992)
	NS	NS	oral	25	(Orme and Kegley)
Monkeys	Cynomolgus ( <i>Macaca fascicularis</i> )	M and F	per os	50	(Murray and Gibson, 1972)
		M	per os	70 <sup>a</sup>	(Purser and Rose, 1979)
Guinea pigs	NS	M	per os	30 (22–41) <sup>a</sup>	(Clark et al., 1966)
	Sprague-Dawley	M and F	per os	22	(Murray and Gibson, 1972)
	NS	F	i.p.	3 <sup>a</sup>	(Clark et al., 1966)

Note: NS, not stated; M, male; F, female.

<sup>a</sup>Dose quoted as paraquat ion.

<sup>b</sup>As dimethyl sulfate.

Human deaths from acute PQ poisoning started to be reported in medical literature in 1966, when Bullivant (1966) reported two fatalities in New Zealand due to accidental ingestion of PQ and mentioned a previous fatality that had occurred in Ireland in 1964. During 1967 and 1968, there were no less than 13 cases of PQ poisonings reported in the literature, 9 of which were fatal (Malone et al., 1971). Most of the initial reports on PQ involved accidental poisonings, usually related to storage of the herbicide in soft drink, wine, beer, or other common beverage bottles, or in inappropriately labeled containers, as well as due to poor worker-protection practices. PQ soon gained reputation, not only among the medical community but also among the general public, as being one of the most toxic substances available for which there was an apparent inability of therapeutic efforts to

alter the outcome. As little as a mouthful (approximately 20 ml) of a 20% solution of PQ produces a dose of about 55 mg/kg in an average 70-kg adult, which may be fatal. The lowest fatal dose recorded for adult humans is 17 mg/kg, but lower doses may be fatal for children (Wesseling et al., 2001).

### 6.1. Clinical Symptoms and Manifestations of Paraquat Intoxication

The effects of PQ are local and systemic, with the former being concentration dependent, while the latter are dose dependent (Proudfoot, 1999). Although the local effects can be severe, there are the systemic effects, largely referable to the respiratory system, which are potentially lethal. Findings suggest that PQ

may cause fatal poisonings by ingestion of small amounts and by dermal absorption of PQ (Wesseling et al., 1997).

#### 6.1.1. Poisoning by the Oral Route

Acute PQ poisonings are mostly due to ingestion of the concentrate liquid herbicide formulations.

The symptomatology of human PQ poisonings can be divided into three different presentations depending on the amount ingested (Table 5) (Vale et al., 1987; Pond, 1990; Bismuth et al., 1995).

**6.1.1.1. Asymptomatic or Mild Toxicity.** Ingestion of PQ in a dose of less than 20–30 mg/kg produces no symptoms or only mild GIT symptoms (nausea, irritation and diarrhea) (Vale et al., 1987; Pond, 1990). Renal and hepatic lesions are either minimal or absent. An initial decrease in the  $DL_{CO}$  is frequently noted, but development of clinical or radiological pulmonary fibrosis is rare. Full recovery is expected in all cases without sequelae (Vale et al., 1987).

**6.1.1.2. Moderate to Severe Toxicity.** Patients who ingest >20–30 but <40–50 mg/kg (Vale et al., 1987; Hudson et al., 1991; Bismuth et al., 1995) of PQ, equivalent to a single swallow (7.5–15 ml of a 20% solution for a 70-kg patient), are most likely to die from pulmonary fibrosis, which progresses after a few days to a few weeks. This ingested dose produces a more indolent illness. Patients develop upper GIT irritation/corrosion, acute tubular necrosis (12–48 h after ingestion), pulmonary hemorrhage (24–48 h after ingestion), and pulmonary fibrosis (1–2 wk after exposure). It typically evolves in three phases, described next.

**6.1.1.2.1. First Phase.** The initial phase is characterized by the emergence of lesions due to the herbicide's corrosivity. Corrosive effects are very similar to the effects induced by alkali (Stephens et al., 1981). Immediately after ingestion, patients frequently complain of lips, buccopharyngeal, esophageal, epigastric, and gastric pain (Bismuth et al., 1982; Vale et al., 1987). Most patients with PQ poisoning develop the characteristic lesions on the tongue (usually swollen, commonly known as the "PQ tongue"). Patients with such lesions can be completely aphonic and aphagic and may thus require total parenteral nutrition. Flexible fiberoptic esophagogastroscope frequently shows mucosal lesions (Zargar et al., 1991). This should be done between 4 and 8 h after ingestion. If the initial endoscopy is negative, it should be repeated at 36 h, because the corrosive lesions can sometimes manifest later. These are usually superficial, although several cases of esophageal and gastric ulceration preceding perforation and massive GIT hemorrhage have been reported (Malone et al., 1971; Ackrill et al., 1978). Other signs of GIT irritation such as nausea and vomiting may occur. Indeed vomiting almost always ensues, even in the absence of emetic agents in the commercial preparation. Secondarily, abdominal colic and diarrhea are noted occasionally.

**6.1.1.2.2. Second Phase.** Between the second and fifth days following ingestion, renal failure and hepatocellular necrosis develop. Functional renal insufficiency is often noted,

caused partly by hypovolemia secondary to GIT fluid losses and a decreased or total lack of oral fluid intake. PQ itself has direct renal toxicity. It generally causes a pure tubulopathy with proximal predominance (Vaziri et al., 1979; Bairaktari et al., 1998; Gil et al., 2005). Such renal tubulopathies usually evolve – as with all causes of tubular necrosis—to full recovery without sequelae (Bairaktari et al., 1998; Gil et al., 2005). Although the degree of renal failure may be mild by most standards, renal failure impairs the main route of excretion available and therefore may contribute significantly to the mortality produced by PQ. In a study reporting the nephrotoxicity of PQ in vitro and in vivo, proximal tubular function was monitored by measuring the accumulation of PAH and NMN using renal cortical slices from Swiss-Webster mice poisoned with PQ at the  $LD_{50}$  for i.p. administration (50 mg/kg b.w.) (Ecker et al., 1975). Tubular function in intact Swiss-Webster mice was estimated using disappearance of phenolsulfonphthalein and [ $^{14}C$ ]PQ from plasma in vivo. Glomerular function was estimated using disappearance of othalamate from the plasma of animals injected i.v. with PQ at a dose of 50 mg/kg b.w. Accumulation of PAH and NMN by renal cortical slices in vitro was not greatly altered. In vivo disappearance of phenolsulfonphthalein and [ $^{14}C$ ]PQ from plasma was greatly reduced, but iothalamate disappearance was little affected. These authors concluded that the nephrotoxicity attributable to PQ affects primarily the proximal tubule (Ecker et al., 1975). These findings are supported by in vitro experiments in which the proximal renal epithelial cell line (LLC-PK<sub>1</sub>) was found to be more susceptible to the toxic effects of PQ when compared to a distal epithelial cell line, MDCK (Chan et al., 1996a). It has been noted that the uptake of PQ by rat renal tubular cells in culture is saturable (Chan et al., 1996b). Besides being filtered in the glomerulus (Chan et al., 1996b), PQ is secreted in the proximal tubule, which is followed by its intracellular accumulation in proximal tubule cells through an active basolateral uptake mechanism (Chan et al., 1997). Renal failure proceeds gradually and may produce an unusually rapid rise in serum creatinine relatively to the rise in blood urea nitrogen (low BUN/creatinine ratio) (Chen et al., 1994a). The observation of an unusually high creatinine value in a case of upper GIT bleeding (where one might expect to observe an unusually large increase in BUN but not creatinine) led to the diagnosis of PQ toxicity even though the patient denied ingestion. Liver toxicity, as revealed by elevated liver enzymes in plasma, jaundice, and histopathological changes in the liver at examination postmortem, is sometimes seen in cases of poisoning with PQ in humans. The liver lesion caused by PQ displays a picture of centrilobular hepatocellular necrosis and cholestasis and usually moderate (Vale et al., 1987).

**6.1.1.2.3. Third Phase.** Delayed development of pulmonary fibrosis is responsible for the generally poor prognosis in acute PQ poisoning. Clinically and radiographically, this appears several days after ingestion. In the typical form, the interstitial lesion extends inexorably. The diagnosis of pulmonary

fibrosis can, in fact, be made by pulmonary function tests (PFTs) well before arterial  $O_2$  tension ( $PaO_2$ ) decreases (which is a signal of a rapid, often fatal clinical evolution). Early gas diffusion disturbances are responsible for alterations in gases concentrations, which precede radiological manifestations. Radiological lung changes do not always parallel the severity of clinical symptoms; they have been reported to be diffuse, coarse, reticulonodular infiltrates (Bier and Osborne, 1978). Chest X-ray may be normal, particularly in those patients who die soon after ingestion, due to multiorgan failure. More often, patchy infiltration develops, which may progress to ground-glass opacification (GGO) of one or both lung fields (Vale et al., 1987). Im et al. (1991) analyzed retrospectively 42 patients with a history of PQ ingestion and abnormal findings on chest radiographs. Radiographic changes during the first week after ingestion included diffuse consolidation (26/39), pneumomediastinum with or without pneumothorax (15/39), and cardiomegaly with widening of the superior mediastinum (8/39). Small cystic and linear shadows began to appear at the end of the first week and represented the preponderant parenchymal abnormality observed after 2–4 weeks. Focal honeycombing was the major parenchymal abnormality after 4 wk. High-resolution computed tomography (HRCT) of the lung 9 months after PQ exposure revealed localized fibrosis containing small cysts. Pulmonary fibrosis leads to a rapid development of refractory hypoxemia, resulting in death over a period of 5 days to several weeks. Neither spontaneous nor assisted artificial ventilation can delay the fatal outcome. In the final stage, if sepsis (which frequently occurs in these patients, even when they are treated with antibiotics) does not intervene, the PQ poisoning evolves toward decerebration during mechanical ventilation, with an inspired  $O_2$  fraction ( $F_{iO_2}$ ) of 100% and a  $PaO_2$  under 30 mm Hg. Lee et al. (1995) reviewed the findings of HRCT scans of the lungs in 16 patients with PQ poisoning. The most common pattern on initial HRCT scans was GGO (bilateral and diffuse in distribution), present alone or as part of a mixed pattern in 13 patients. Consolidation was present in six patients, irregular lines in three, and nodules in two patients. On follow-up HRCT scans, the GGO had changed to consolidation with bronchiectasis. Additional irregular lines and traction bronchiectasis also were observed. More recently, the specific radiologic and functional sequential changes of PQ-induced pulmonary damage were well characterized using HRCT and PFTs in long-term follow-up of PQ-poisoned survivors (Huh et al., 2006). Among the cohort of 27 patients who had ingested PQ, the HRCT findings showed a normal ( $n = 14$ ) and an abnormal group ( $n = 13$ ). Increased PQ ingestion in the abnormal group was associated with more rapid and severe pulmonary changes. All the patients with normal HRCT findings survived. When the serial changes of HRCT are observed, initial GGO indicates primary lung damage from PQ, because this pattern reflects alveolar edema and inflammatory cell infiltration. GGO on HRCT peaked on day 7 after ingestion. Between 2 weeks and 1 month, consolidation increased and pulmonary fibrosis progressed, and slow improvements were observed for up to

six months. Compared with the PFTs results obtained at 1 and 6.5 months, expiratory volume in first second ( $FEV_1$ ), forced vital capacity (FVC), and lung carbon monoxide diffusing capacity ( $DL_{CO}$ ) all improved slightly. Lung changes after PQ intoxication may be functionally and radiologically reversible following treatment. Although most patients who have radiological lung changes go on to develop progressive and ultimately fatal lung damage, there are case reports in which patients have developed radiological changes but have survived and improved progressively (see also section 9 for details).

**6.1.1.3. Severe: Acute Fulminant Toxicity.** Patients who ingest greater than 40 mg/kg ( $>15$  ml of a 20% solution for a 70-kg patient) usually die within hours to a few days, at most (Bismuth et al., 1982; Pond, 1990). These patients experience multiple organ failure, including acute respiratory distress syndrome (ARDS), cerebral edema, myocardial necrosis, with cardiac, neurologic, adrenal, pancreatic, hepatic (with jaundice), and renal failure (Nagi, 1970; Russell et al., 1981; Bismuth et al., 1982; Reif and Lewinsohn, 1983; Pond, 1990; Florkowski et al., 1992). Death may occur even before the development of significant chest radiographic abnormalities (Pond, 1990). Alveolitis is observed, with clinical signs of acute noncardiogenic pulmonary edema and rapidly progressive hypoxemia, even in patients treated with salt and fluid restriction. Acute pneumonitis, shock, metabolic acidosis, and convulsions have been reported. Nausea, vomiting, and abdominal pain are also present. Bloody diarrhea may be present.

#### 6.1.2. Exposure by Dermal Route

Although deliberate ingestion is responsible for most cases of serious PQ toxicity, morbidity and mortality can result from other routes of exposure. Indeed, the most important accidental exposure routes for people applying PQ are dermic and inhalation; in normal use, ingestion is unlikely. Local toxicity is produced by direct injury to tissues with which the herbicide comes into contact due to PQ corrosive effects. Local effects include skin damage (blistering), as well as nails, nose, and lips ulcers (Samman and Johnston, 1969; Hearn and Keir, 1971; Vale et al., 1987; Smith, 1988a; Hoffer and Taitelman, 1989). Contact with concentrated PQ solutions may cause localized discoloration or a transverse band of white discoloration affecting the nail plate, although the latter may not occur until several weeks after exposure. Transverse ridging and furrowing of the nail, progressing to gross irregular deformity of the nail plate or total loss of the nail, may also occur (Samman and Johnston, 1969). Normal nail growth follows. The extent and severity of such damage is mainly dependent on the concentration of PQ in the formulation rather than the dose (as for GIT lesions). In general, systemic toxicity in humans, after percutaneous exposure, seems unusual as reported by Hoffer and Taitelman (1989), who described 15 consecutive cases of single exposures of the skin or eyes during contact with PQ at working places. From these data it is

apparent that a single exposure of healthy skin to PQ solutions only causes local lesions. However, patients with dermal PQ repeated exposures may have significant skin irritation or can even die. Most of the fatal cases occurred in developing countries. In all of these cases, one or more of the following factors were present: previous skin damage, caused either by PQ itself or by mechanical or other chemical means, and prolonged skin contact to clothes soaked in concentrated PQ, or less concentrated solutions if the skin is not washed immediately after exposure (according to the manufacturer's instructions, correctly diluted spray solutions should contain no more than 0.05 to 0.2% of PQ ion) (Wohlfahrt, 1982). The lowest known concentration of PQ leading to fatal poisoning by dermal route is 5 g/L (Smith, 1988a). Athanaselis et al. (1983) reported the poisoning of a 64-year-old spray operator via the skin. Fluid had leaked down his back for several hours, causing irritation of the skin. Two days later the sprayman visited a doctor, who advised hospitalization. The patient rejected this advice but was admitted 3 days later into hospital. He died, 12 h after admission, due to toxic shock and renal and respiratory insufficiency. At autopsy, the findings were typical of PQ poisoning, with fibrosing interstitial pneumonitis and intra-alveolar hemorrhage, renal tubular cell degeneration, cholestasis, and necrosis of the back skin. Another peculiar case of a fatality from transdermal exposure to PQ was reported in Papua New Guinea (Binns, 1976). The patient, evidently thinking that PQ (20% PQ w/v) would kill lice, applied the formulation to his scalp and beard. This produced painful sores and he steadily deteriorated until dying 6 days after applying the PQ to his skin. At autopsy, there were skin lesions as well as solid and hemorrhagic lungs. Garnier et al. (1994) reported two cases of percutaneous exposure. In the first case a 36-year-old man applied a 20% concentrate to his whole body to cure scabies. He developed extensive erythema followed by blistering and 2 days later he was admitted to hospital. Transient renal failure ensued. Dyspnea appeared 1 week after admission and he deteriorated, dying 26 days after exposure. In the second case, death followed PQ application to beard and scalp to treat lice (Garnier et al., 1994). An agricultural worker developed persistent hepatic cholestasis after an episode of acute PQ poisoning through skin absorption (Bataller et al., 2000). Several other cases of percutaneous PQ intoxication with respiratory lesions were also reported (Newhouse et al., 1978; Okonek et al., 1983; Papiris et al., 1995; Soloukides et al., 2007). Senanayake et al. (1993), analyzing 85 Sri Lanka PQ applicators, found no clinically important differences in any of the measurements made between the study group and the two control groups. In particular, the results of the PFTs were similar to those of the control groups. The same was true for the haematological screen and blood tests for liver and kidney function. The incidence of skin damage, nose bleeds, and nail damage in the study group was slightly higher than in the control groups. The results of this study showed that long-term spraying of PQ, at the concentrations used, produced no adverse health effects, in particular no lung damage, attributable to the occupational

use of the herbicide. These results were consistent with a cross-sectional study undertaken by Castro-Gutierrez et al. (1997) in Nicaragua in order to evaluate any relationship between respiratory health and PQ exposure. One hundred and thirty-four exposed plantation workers who had been exposed to PQ (0.1–0.2%) over more than 2 years during spraying were questioned and their lung function was examined. More than half of the workers (53%) had experienced a skin rash or burn from PQ exposure, 25% had nosebleeds, 58% had nail damage, and 42% had splashed their eyes that in several workers, this led to a continued blurred vision, in one case to an opacified cornea. A statistically significant dose-response relationship was observed between intensity of exposure (as indicated by a history of skin rash or burn) and the prevalence of dyspnea (95% confidence interval (CI): 2.4–9.0), and the prevalence of episodic shortness of breath accompanied by wheezing (95% CI: 1.4–6.3). No statistical significance was observed between PQ exposure and PFTs alterations. Dalvie et al. (1999) analyzed 126 PQ applicators in the Western Cape region of South Africa and did not observe an association of PQ exposure and PFTs changes, although they did report a positive association of chronic PQ exposure and arterial oxygen desaturation during maximum exercise. Notwithstanding the interest of these studies, a major shortcoming is the lack of confirmation of poisoning by detection of PQ in urine and/or plasma.

#### 6.1.3. Ocular Irritation

Direct eye contact with concentrated solutions will produce corrosive ocular injury, dependent on contact time and concentration. Ocular exposure may produce severe corneal and conjunctival injury and anterior uveitis (Cant and Lewis, 1968). Local effects to the eye may heal only slowly and with scarring (Nirei et al., 1993; McKeag et al., 2002). McKeag et al. (2002) described the clinical appearance and progress of bilateral ocular injury caused by PQ on a 69-year-old fruit farmer, who splashed a 20% solution of PQ into both his eyes.

Cingolani et al. (2006) investigated the effects of PQ in mice retina. There was no significant decline in electroretinogram (ERG) a- or b-wave amplitudes after i.v. injection of 1  $\mu$ l of 0.5 mM PQ in C57Bl/6 mice, but loss of ERG function occurred after injection of the same volume of 0.75 or 1 mM PQ. Histology in PQ-injected eyes showed condensation of chromatin and thinning of the inner and outer nuclear layers, indicating cell death, and terminal deoxynucleotidyl transferase-mediated dUTP-biotinide end labeling (TUNEL) demonstrated that one mechanism of cell death was apoptosis. Fluorescence in the retina and retinal pigmented epithelium after intraocular injection of PQ, followed by perfusion with hydroethidine, indicated high levels of  $O_2^-$ , and oxidative damage was demonstrated by staining for acrolein, and enzyme-linked immunosorbent assay (ELISA) for carbonyl protein adducts. PQ-induced damage to the outer nuclear layer was greater in BALB/c mice

than in C57Bl/6 mice, suggesting strain differences in the oxidative defense system of photoreceptors and/or other modifier genes.

#### 6.1.4. *Exposure by Inhalation*

The large size of PQ droplets produced by most commercial agricultural spraying equipment (typically greater than 100  $\mu\text{m}$ ) generally precludes serious poisoning by the inhalational route (Howard, 1983; Wojeck et al., 1983; Senanayake et al., 1993; Garnier, 1995).

#### 6.1.5. *Muscle Toxicity*

Myopathy associated with PQ poisoning was reported for the first time by Saunders et al. in 1985. The examination of skeletal muscles obtained at both the biopsy and autopsy revealed findings of extensive degeneration and fibrosis. Koppel et al. reported, in 1994, that extensive myonecrosis was observed in a specimen of postmortem intercostal muscle of a 52-year-old woman who had ingested an unknown dose of PQ and died on day 11 after ingestion. Vyver et al. (1985) reported a case of a patient who died 5 days after ingestion of PQ, where levels were higher in the skeletal muscle and an increase of creatinine kinase levels appeared on the fourth day after admission. More recently (Tabata et al., 1999), degeneration of skeletal muscle, mainly of the rectus abdominis, psoas major, and diaphragm, was also reported. Laboratory data revealed that the plasma creatinine kinase values (1796 mU/ml) were highest on day 5, after which the levels decreased steadily; however, they were maintained at about 900 mU/ml even on day 8.

#### 6.1.6. *Appearance at Autopsy*

The appearance at autopsy depends on exposure dose and on the survival time. Following severe intoxications, corrosive injuries of the lips are likely. The mucous membranes of the mouth, pharynx, esophagus, larynx, and the upper trachea are intensely congested and may be covered with a yellowish-green epithelial slough. The gastric mucosa is likely to be congested and may contain small hemorrhages. The kidneys may be swollen and perhaps rather pale, and the liver may be also somewhat pale. Centrilobular necrosis and cholestasis of the liver with extensive bile-duct loss, renal tubular necrosis were reported by several authors (Situnayake et al., 1987; Takegoshi et al., 1988; Soontornniyomkij and Bunyaratvej, 1992). Ultrastructurally, dilatation of bile canaliculi with decrease of microvilli and thickening of pericanalicular ectoplasm was found in the hepatocytes (Takegoshi et al., 1988). Cortical necrosis of the adrenal glands and mild acute pancreatitis were also documented (Soontornniyomkij and Bunyaratvej, 1992). Lungs are heavy, filling and holding the shape of the thoracic cavity, congested, edematous, but microscopically there is not yet any fibrosis or epithelial pro-

liferation. There may be early pneumonia, and pulmonary hemorrhage is common, in addition to the severe edema. Aspiration pneumonitis (100% of cases) and pneumothorax with pneumomediastinum (18.75% of cases) were remarked autopsy findings in those dying from PQ poisoning (Daisley and Simmons, 1999). In cases of moderate intoxications and consequently with longer survivals, the appearances at autopsy are different. The changes in the mucous membranes of the oropharynx have resolved and the liver and kidneys are usually of normal appearance, at least on naked-eye examination, although there may be microscopic evidence of cellular damage. The dramatic changes are to be found in the lungs, where they form the classical picture of PQ poisoning. On gross examination the lungs are usually of reduced size, with a solid appearance and of dark gray color. Section reveals a firm, obviously fibrotic structure, which is apparently completely airless. Microscopic examination reveals a grossly abnormal tissue with abundant fibrosis, often virtually obliterating the alveoli. Many plump fibroblasts are to be seen in alveolar walls and alveolar spaces. Hyaline membranes are common, possibly a result of ventilation with high concentrations of  $\text{O}_2$ . In general, the longer the survival time, the more marked is the proliferation of fibroblasts in the alveoli, and the more airless the lung tissue becomes (Carson and Carson, 1976). On whole sections, there is obliteration of air spaces with multiple areas of fibrosis. There may be active proliferation of the bronchial epithelium, forming small adenomata within the parenchyma. At later stages, there is less inflammation.

## 6.2. *Intoxications During Pregnancy*

In a fatal case of PQ poisoning in a pregnant woman, who developed the typical symptoms and signs of PQ poisoning and, at postmortem, had the typical lung pathology of PQ poisoning, the fetal lungs were normal (Fennelly et al., 1968). However, Talbot and Fu (Talbot et al., 1988), who reported the clinical cases of nine pregnant women who ingested PQ, measured its levels in maternal, fetal, and cord blood in one case and showed that PQ crosses the placenta and is concentrated to levels 4–6 times greater than the maternal blood. Amnioscopy in another case showed PQ levels in amniotic fluid were nearly twice that of maternal blood. The fetus appears to tolerate maternal PQ poisoning while it is dependent on the maternal circulation. The condition of the fetus worsened (developed signs of PQ poisoning) at delivery (due to exposure to atmospheric  $\text{O}_2$ ), or in utero if the gestational age was greater than 30 weeks. Poor late-gestational survival may be due to the fact that type II pneumocytes appear between 28 and 32 weeks of gestation. All fetuses died, whether or not emergency cesarean operation was carried out. Jeng et al. (2005) presented a case of moderate PQ poisoning from suicidal ingestion in a woman in the third trimester of pregnancy. Despite initial deterioration of renal and liver function, she had a normal spontaneous delivery of a healthy baby girl 14 weeks after the exposure. The child reached developmental milestones normally and appeared healthy and well nourished at age 5.

During the earlier embryogenesis stages, PQ exposure may lead to spontaneous abortion or to teratogenic effects (Rutledge, 1997).

## 7. PREDICTING HUMAN OUTCOME IN PARAQUAT POISONING

Patients who have strong dermal PQ exposure and all who have ingested PQ require hospitalization and experimental therapy. PQ poisoning is one of those intoxications for which it is possible to predict the severity and prognosis for individual patients using specific laboratory tests and information from the medical history. Successful prediction of those who may survive PQ poisoning can prevent inappropriately aggressive treatments, which are normally elaborate, expensive, and have not clearly improved the survival rate (Bismuth et al., 1987; Hampson and Pond, 1988), in those who have no hope of survival and in those only minimally poisoned. Possible prognostic factors in PQ poisonings are the formulation involved, whether or not it was diluted, the amount ingested, the time since ingestion, the presence or absence of food in the gut (time since the last meal before PQ ingestion), whether spontaneous emesis has occurred (the color of the vomitus), treatment already administered, particularly decontamination measures, and plasma and urinary PQ concentrations (Proudfoot et al., 1979; Bismuth et al., 1982; Hart et al., 1984; Scherrmann et al., 1987; Ikebuchi et al., 1993; Proudfoot, 1995). Suicidal PQ poisonings are generally more severe than accidental poisonings due to higher ingestions. A recent meal, which delays and reduces absorption, can improve prognosis (Bismuth et al., 1982). A person with acute PQ ingestion is likely to come to the emergency department initially complaining only of an acute corrosive injury, so the differential diagnosis should encompass all corrosive agents. A careful physical examination should include search for oral, skin, or mucous membrane lesions. Endoscopy visualization of significant ulcerations in the esophagus or stomach within the first 24 h of exposure indicates a poor prognosis (Bismuth et al., 1982). The extent and depth of ulceration reflect the concentration and dose of PQ that has had contact with the mucosal surfaces and, indirectly, the amount of PQ absorbed systemically. The development of renal failure is indicative of more severe toxicity and a worse prognosis than would be predicted for a patient in whom renal function is preserved (Bismuth et al., 1982; Vale et al., 1987; Baselt and Cravey, 1989) (see also section 7.3). Almost all patients with renal failure from PQ have significant lung toxicity, but there are occasional reports of renal failure without significant lung toxicity (Dolan et al., 1984).

The measurement of plasma PQ concentration is the most reliable method for assessing the prognosis, since the severity and rate at which the toxic signs evolve depends on the amount of PQ absorbed systemically. Intoxicated patients should be watched and treated expectantly until PQ levels are reported to be nonexistent. PQ poisoning has been reported in children (McDonagh and Martin, 1970). The clinical ap-

proach for intoxicated children does not differ from that for adults.

### 7.1. Paraquat Quantification in Biological Samples

Positive semiquantitative urine tests should be followed by quantitative plasma and urine PQ levels. Several laboratory analytical methods are available for measuring PQ in biological samples. PQ may be detected or quantified in a great variety of biological fluids and tissues, and also in various materials suspected to be the source of PQ ingestion or exposure. In emergency situations, PQ can be measured in plasma, urine, gastric aspirate, and dialysates. In the field of forensic toxicology, PQ can be assayed in several tissues or in whole blood. The analytical methods for PQ quantification have been reviewed by Haley, Summers and Scherrmann (Haley, 1979; Summers, 1980; Scherrmann, 1995). In this review only the most applied qualitative and quantitative tests are described.

#### 7.1.1. Qualitative and Semiquantitative Test

The dithionite test is based on the reduction of PQ by freshly prepared 1% aqueous sodium dithionite in 0.1 N NaOH to form the stable blue radical ion ( $\lambda_{\max}$  603 nm) (Tompsett, 1970) as described earlier. A visual inspection gives immediate perception of the presence of blue free radical, in comparison with negative and positive controls. Evaluation of the color intensity can be related to a semiquantitative scale (see later description). The test is rapid, specific, and requires the availability of nonoxidized sodium dithionite. DQ undergoes a similar reduction to form a yellow-green cation ( $\lambda_{\max}$  760 nm).

#### 7.1.2. Quantitative Test: Spectrophotometry

Probably the easiest, rapidest, simplest, and the method with the lowest detection limit for PQ quantification is based on second- or fourth-derivative spectrophotometry (Fell et al., 1981; Jarvie et al., 1981; Fuke et al., 1992; Kuo et al., 2001). In this mode, the normal absorption spectrum is transformed to the second- or fourth-derivative spectra of the PQ cation radical. The sodium dithionite color reaction is used to detect PQ, and matrix interference is eliminated by the use of a chemical deproteinization technique with sulfosalicylic acid in order to give a clear supernatant, compatible with spectrophotometry. Derivative spectroscopy confers an advantage over classical spectrophotometric detection by enhancing the  $PQ^{\cdot+}$  peak and suppressing the broader absorption bands resulting from nonspecific matrix absorption such as with diquat, hemolysis, bilirubin, or lipemia.

##### 7.1.2.1. Reagents and Their Preparation.

- A 1.38-mg amount of PQ dichloride (Sigma, St. Louis, MO, and other manufacturers) is dissolved in 1 ml distilled water (1 mg/ml as  $PQ^{2+}$ ).
- Deproteinization reagent: 50 g sulfosalicylic acid is dissolved in 100 ml distilled water.

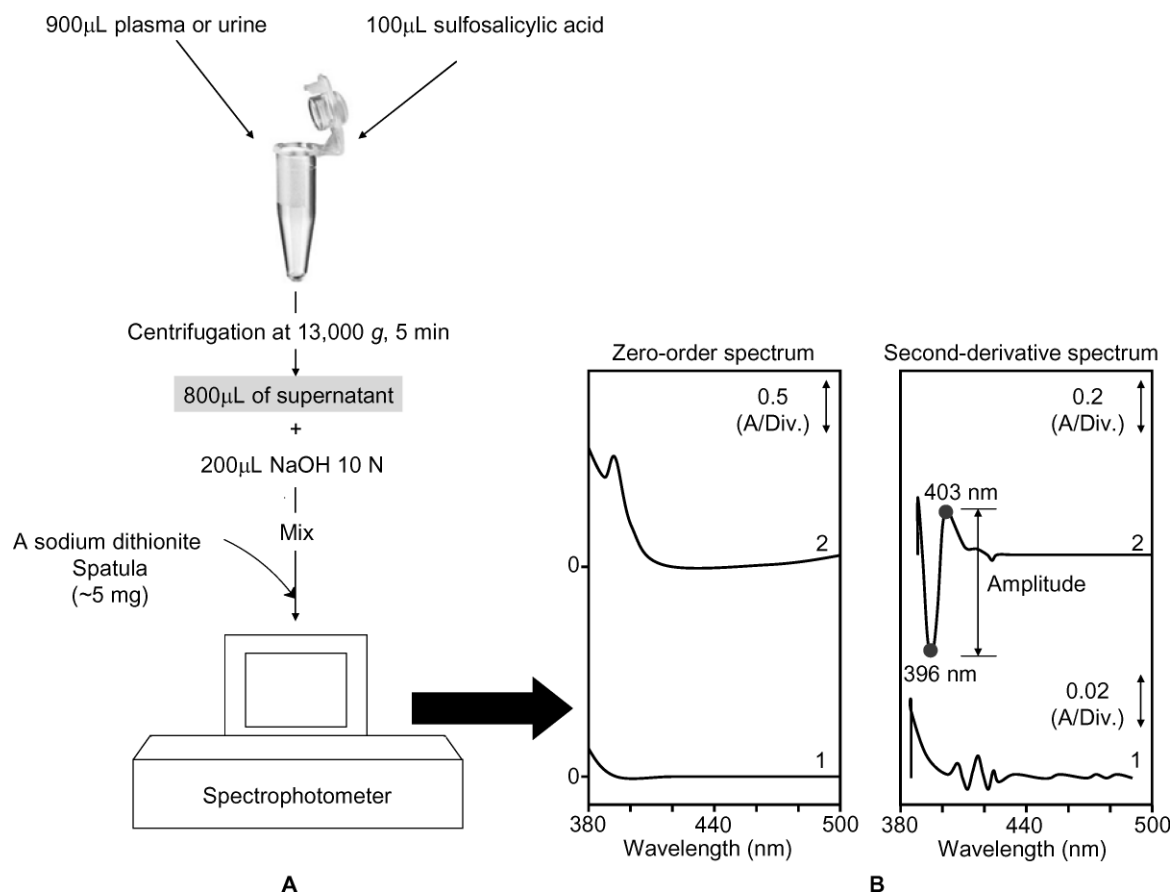


FIG. 12. (A) Pretreatment procedures for paraquat in urine and plasma before the second derivative spectrophotometric analysis. (B) Zero-order and second-derivative spectra. The qualitative analysis is made by observing the presence of inflection points at about 396 and 403 nm. The quantification is made with amplitudes measurable between 396 and 403 nm.

- Alkaline reagent: 40 g NaOH is dissolved in 100 ml distilled water.
- Chromogenic reagent: sodium dithionite ( $\text{Na}_2\text{S}_2\text{O}_4$ ).

#### 7.1.2.2. Analytical Conditions.

- Instrument: ultraviolet–visible (UV-Vis) spectrophotometer with a differential analyzing system.
- Cell: plastic-made semimicro cell with an optical path length of 1.0 cm.

7.1.2.3. Procedures. The procedures for urine and blood plasma specimens are detailed here and are shown in Figure 12:

1. 900  $\mu\text{L}$  of blood plasma and urine is mixed well with 100  $\mu\text{L}$  of the deproteinization reagent solution.
2. The mixture is centrifuged at 13,000 g for 5 min.

3. 800  $\mu\text{L}$  of the supernatant solution is mixed with 200  $\mu\text{L}$  of alkaline reagent.
4. A spatula (~5 mg) of the chromogenic reagent is added to give the a blue color characteristic of the  $\text{PQ}^{+}$ .
5. The data of a zero-order spectrum is obtained by scanning from 500 to 380 nm (wavelength space  $\Delta\lambda = 0.5$  nm) and then second-differentiated (derivative wavelength space  $\Delta\lambda = 4$  nm). A qualitative and quantitative analysis of PQ is performed at the amplitude peaks of 396–403 nm of the second-derivative spectrum.

The calibration curves are constructed by spiking various concentrations of PQ into blank specimens, and processing in the same way as above. The calibration curve in the 0.2–8.0  $\mu\text{g}/\text{ml}$  range obeys Beer's law. Using these experimental conditions, the intra- and interday coefficients of variation showed values lower than 5% and the detection limit of the method was 0.10  $\mu\text{g}/\text{ml}$  (Dinis-Oliveira et al., 2006d, 2006e).

## 7.2. Predicting the Outcome from Plasma Paraquat Concentrations

The prognosis for a patient with PQ ingestion can be fairly determined by measuring plasma PQ concentration and its relationship to time of ingestion. PQ concentrations data should be obtained before starting any treatment that could decrease the levels. A nomogram was initially presented by Proudfoot et al. (1979; Proudfoot, 1995) after quantification of PQ plasma concentrations at various times postingestion in 79 poisoned victims. Those whose concentrations were below 2.0, 0.6, 0.3, 0.16, and 0.1 mg/L at 4, 6, 10, 16, and 24 h after ingestion, respectively, survived. Subsequently, this nomogram was refined by Hart et al. (1984) by examining plasma PQ concentrations from a larger group of patients ( $n = 218$ ) (Figure 13). Hart et al. (1984) produced a contour graph of plasma PQ-to-time relationships for 10, 20, 30, 50, 70, and 90% probability of survival. The 50% probability curve reported by Hart et al. (1984) correlated well with the predictive line separating survival from death developed by Proudfoot et al. (1979). Hart et al. (1984) confirmed the difficulty of Proudfoot et al. (1979) in predicting outcome from plasma concentrations data within the first 3 h. Schermann et al. (1987) extended this predictive curve up to day 7 after intoxication, and showed that those patients who presented, within 8 h, plasma PQ concentrations of 10 mg/L or above, usually died from cardiogenic shock within 24 h, while those with lower concentrations (but above the predictive line), died of pulmonary fibrosis and respiratory failure latter than 24 h after ingestion. Bismuth et al. (1982) soon confirmed the value of the line with 100% accurate prediction of the outcome in 17 patients admitted 25 h or less after ingestion. Similarly, Schermann et al. (1987) accurately predicted the outcome in 45 cases. Suzuki et al. (1991) combined the data of Proudfoot

et al. (1979), Bismuth et al. (1982), Schermann et al. (1987), and Sawada et al. (1988) with those from a further group of 78 patients, and concluded that the predictive line correctly identified 101 of the 102 deaths and 61 of the 63 survivors evaluated within 24 h of PQ ingestion. Although the nomogram can provide a fairly accurate prognosis, helping in predicting illness severity and death probability if PQ levels can be obtained immediately, it is inevitable that any predictive line will fail occasionally. Estimation of the time interval since ingestion is prone to error, particularly during the first few hours when plasma PQ concentrations decline rapidly, and a time error of even 0.5 to 1.0 h may radically alter the relationship of a concentration to the predictive line. In addition, plasma PQ concentrations may not be entirely accurate since they may be assayed by one of several methods and publications seldom make clear whether the concentration reported is that of PQ ion or PQ salt, and there is the possibility of interindividual variation in susceptibility to the toxic agent, a matter on which there is little, if any, knowledge.

Sawada et al. (1988) reported an objective index for the prognosis of PQ poisoning based on a study of serum PQ concentrations in 30 patients, 20 of whom died and 10 of whom survived. The severity index of PQ poisoning (SIPP) is derived from the time (in hours) until the start of treatment from the time of PQ ingestion, multiplied by the serum PQ level ( $\mu\text{g/ml}$ ) on hospital admission.

$$\text{SIPP} = [\text{serum level of PQ } (\mu\text{g/ml})] \\ \times [\text{time from ingestion to treatment (h)}]$$

When the SIPP score is less than 10, patients may survive; a score of 50 separates late deaths due to respiratory failure ( $10 < \text{SIPP} < 50$ ) from early deaths secondary to circulatory failure ( $\text{SIPP} > 50$ ). Although this is an important study, the original publication by Sawada et al. (1988) states that the test was carried out in serum rather than plasma. This is important since PQ serum concentrations are around threefold lower than those in plasma obtained from the same blood sample, and this may have caused some difficulties in the interpretation of results of subsequent studies that based on using plasma PQ quantifications. Subsequently, Yamamoto et al. (2000), by analyzing 43 patients, showed that PQ poisoning is characterized by high  $\text{O}_2$  consumption with high oxygen extraction, with the degree of derangement based on the SIPP. The authors also suggested that the development of a marked imbalance between increased  $\text{O}_2$  demand and decreased  $\text{O}_2$  supply, because of myocardial depression, might be a possible cause of death related to circulatory failure. Suzuki et al. (1991) compared SIPP with the predictive line in 167 patients admitted within 24 h of PQ ingestion. The outcome predicted by the Proudfoot's line was wrong in 1 fatal case and in 2 who survived; in comparison, SIPP failed to predict accurately the outcome in 1 survivor and 10 fatal cases. These differences were statistically significant, and Suzuki et al. (1991) concluded that the predictive line was more accurate than

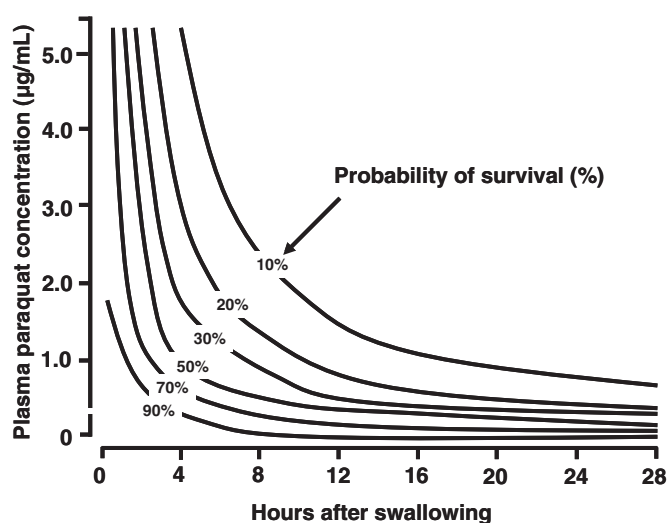


FIG. 13. Nomogram showing relation between plasma paraquat concentrations ( $\mu\text{g/ml}$ ), time after ingestion, and probability of survival. Adapted from Hart et al. (1984).



SIPP in predicting outcome from PQ concentrations in samples taken in the first 24 h after ingestion.

The severity assessment of PQ poisoning using an interesting formula was reported by Ikebuchi et al. in 1993, by performing multivariate analysis on data from 128 poisoned patients. As a result, the TIP or toxicological index of PQ was implemented. However, only 21 patients who survived were included in this study. A discriminant function ( $D$ ) score  $>0.1$  predicts survival and  $D < 0.1$  predicts death.  $D$  was calculated as follows:

$$D = 1.3114 - 0.1617 (\ln T) - 0.5408 (\ln [\ln(C \times 1000)])$$

where  $T$  is the time since ingestion (h) and  $C$  the plasma PQ concentration ( $\mu\text{g/ml}$ ).

The probability of survival was also estimated by Jones et al. (1999), who plotted the logarithm of the plasma PQ concentration (mg/L) versus the logarithm of the time since ingestion. The predicted probability of survival for any specified time and concentration was calculated according to the following ratio:

$$\exp(\text{logit})/[1 + \exp(\text{logit})],$$

where

$$\text{logit} = 0.58 - 2.33 \times \log(\text{plasma PQ}) - 1.15 \times \log(\text{h since ingestion})$$

The authors proposed that this equation may be helpful in predicting who will survive PQ up to at least 200 h after ingestion, and could be used as a research tool for studies on efficacy of PQ poisoning treatments.

More recently, Huang et al. (2003, 2006) successfully applied the Acute Physiology and Chronic Health Evaluation (APACHE) II system (Knaus et al., 1985) in predicting the in-hospital mortality of 64 patients with acute PQ poisoning over a period of 12 years. The study demonstrated that the APACHE II score is positively correlated with plasma PQ concentration and with ingested amount of PQ. Nonsurvivors ( $n = 46$ ) had a higher APACHE II score ( $23.3 \pm 12.7$ ) than survivors ( $n = 18$ ) ( $6.1 \pm 4.2$ ). All patients who had an APACHE II score greater than 20 died before discharge. APACHE II score greater than 13 predicted in-hospital mortality, with 67% sensitivity and 94% specificity. The authors concluded that the APACHE II score

is a simple, reproducible, and practical tool for evaluating the severity of acute PQ poisoning.

### 7.3. Predicting Outcome from Urine Paraquat Concentrations

Although plasma PQ concentrations have a greater predictive value, urine data may contribute to a more rapid evaluation of prognosis (Scherrmann et al., 1987). In addition, certain of the available urinary tests can be carried out in nearly all hospitals. A simple urine semiquantitative test can confirm the presence of PQ when the urine concentration is about  $1.0 \mu\text{g/ml}$  or greater, by adding 10 ml of urine to 2 ml of a freshly prepared 1% sodium dithionite in 1  $N$  sodium hydroxide (Berry and Grove, 1971; Widdop, 1976; Braithwaite, 1987). This qualitative urine test is very easy to perform, and there is a good correlation between the amount of PQ present and the intensity of the formed blue color (Figure 14). The darker the color, the worse is the patient's prognosis (Scherrmann et al., 1987). The patient's urine should be tested serially for 24 h after ingestion. However, a negative result should be interpreted cautiously, because early urinary semiquantitative testing may underestimate the amount of PQ systemically absorbed. Scherrmann et al. (1987) measured the urine PQ concentration in 53 patients, comparing results with the semiquantitative urine test. Almost all patients with urinary PQ concentrations less than  $1 \mu\text{g/ml}$ , within 24 h of ingestion, survived. Patients with semiquantitative urine test results showing more than ++ (navy blue;  $>10 \mu\text{g/ml}$ ) within 24 h following ingestion have a high probability of death (Figures 14 and 15). In contrast, patients showing less than  $\pm$  (pale blue;  $<1 \mu\text{g/ml}$ ) may survive (Scherrmann et al., 1987). Again, these results should be interpreted with caution since PQ-induced acute renal failure influences urine PQ excretion and may lead to false-negative results.

### 7.4. Additional Laboratory Tests

Despite the fact that plasma concentration is the most reliable prognosis factor in PQ poisoning, its measurement is not readily available in all hospitals. For this reason, intensive care treatment must often be undertaken without any information concerning plasma levels. Nevertheless, almost all hospital laboratories can

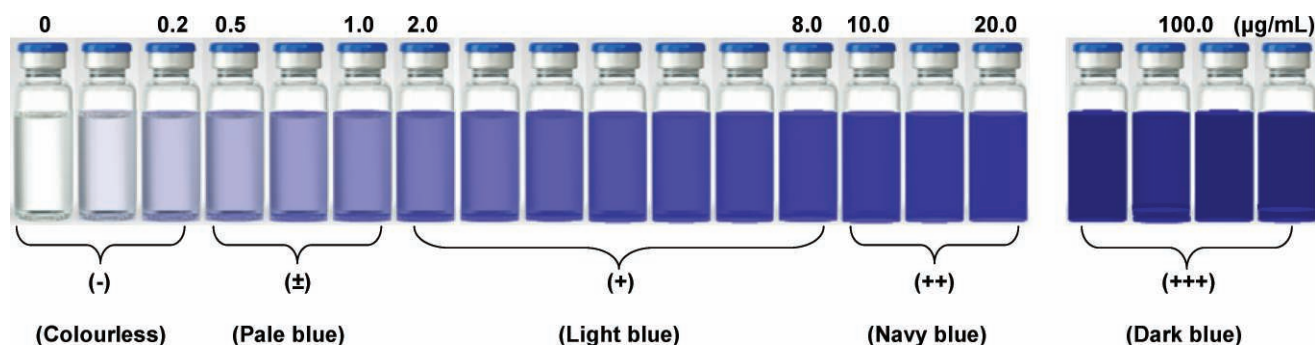


FIG. 14. Representative qualitative urinary test for paraquat. Correlation between the paraquat concentration ( $\mu\text{g/ml}$ ) and the intensity of the blue color change.

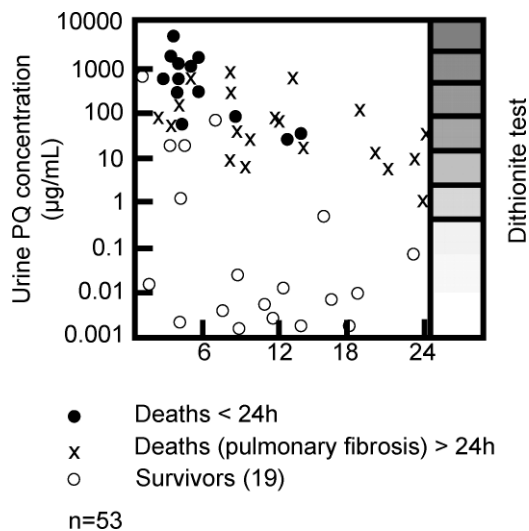


FIG. 15. Relationship between urine paraquat concentrations and survival. Adapted from Scherrmann et al. (1987). For dithionite test colors, see Figure 14.

perform usual tests, such as complete blood count, blood biochemistry, and arterial blood gases. These tests are generally available immediately after patient admission. Lee et al. (2002) reviewed 602 PQ-poisoned patients and reported a correlation between acute death from PQ poisoning and usual admission laboratory data. These authors concluded that besides dermal or inhalational route, and exposure to low PQ quantities, other factors are also good prognosis factors, namely, young age, lower degrees of leukocytosis and acidosis (the nonsurvivors presented lower levels of  $\text{HCO}_3^-$  in arterial blood and thus lower pH), and the absence of renal, hepatic, and pancreatic failures on admission after acute PQ poisoning in multiple logistic regression analysis. Heart rate, respiratory rate, hemoglobin, BUN, serum creatinine, aspartate aminotransferase, alanine aminotransferase, total bilirubin, amylase, and glucose were also significantly lower in survivors than in nonsurvivors. Therefore, the authors concluded that lower differences between the two groups over time may indicate a lower degree of PQ exposure or absorption, or a lower vulnerability to PQ. This supports the hypothesis that the prognosis in humans is influenced by individual sensitivity (Hart et al., 1984) and inaccuracies in assessment of the ingestion-to-presentation interval. Increased levels of serum aminotransferases, bilirubin, or amylase were also detected by Bismuth et al. (1982) in severely poisoned patients. Analysis of admission laboratory data indicated that the prognosis of patients with acute PQ poisoning depends on renal function and acid-base balance (Bismuth et al., 1982). White blood cell (WBC) count at admission is also emphasized as an index of predicting outcomes in PQ poisoning by Kaojarern and Ongphiphadhanakul (1991). These results suggest that certain admission laboratory data may provide as much information for predicting the prognosis as do plasma PQ concentrations.

A respiratory index (RI) has been devised to measure pulmonary function trends in PQ exposures (Suzuki et al., 1989).

This may be of more value in patients who present more than 36 h after PQ ingestion. In a series of 51 patients, all 43 patients with an RI greater than or equal to 1.5 died; all 8 with an RI less than 1.5 survived. This RI was calculated according to the following ratio:

$$\text{RI} = A - \text{aDO}_2/\text{PO}_2$$

where  $A - \text{aDO}_2$  is calculated:

$$713 \times \text{FiO}_2 - \text{PaCO}_2[\text{FiO}_2 + (1 - \text{FiO}_2)/R] - \text{PaO}_2$$

The respiratory quotient ( $R$ ) was assumed to be 0.8 (Bismuth and Hall, 1995). However, the RI is subject to some limitations, and is probably of less value than plasma PQ concentrations in early cases than in those who present 36 h or longer after ingestion. Further assessment of the RI is required.

Kao et al. (1999) investigated changes in lung ventilation (LV) and alveolar permeability (AP) in patients with PQ intoxication, using  $^{99\text{m}}\text{Tc}$  diethylenetriamine pentaacetate (DTPA) radioaerosol lung scintigraphy. Traditional  $^{99\text{m}}\text{Tc}$  macroaggregated albumin (MAA) perfusion lung imaging was also performed for comparison. Those patients (69%) with abnormal AP died. The authors concluded that AP may help predict outcome in patients with PQ intoxication.

Analyzing the serum of 21 PQ-poisoned patients, Nakamura et al. (2001) showed that serum concentrations of type IV collagen and tissue inhibitor of metalloproteinase-1 (TIMP-1) increased with time in nonsurvivors but did not change in survivors. The authors concluded that these parameters may be useful indicators of severity and/or prognosis for the development of respiratory failure in patients with PQ poisoning.

Finally, pneumoproteinemia, a recent concept in the assessment of lung diseases, seems to be promising in predicting the outcome of PQ poisonings; in ARDS several types of serum or bronchoalveolar lavage fluid (BALF) biomarkers, such as surfactant protein (SP)-A, -B, and -D, and Clara cell (CC) 16 have been evaluated with success (Doyle et al., 1995, 1998; Hermans and Bernard, 1998; Kuroki et al., 1998; Hermans and Bernard, 1999). Pan et al. (2002) observed an increase of serum SP-D in rats exposed (i.p.) to PQ plus  $\text{O}_2$ . These authors proposed that serum SP-D may be a useful biomarker of lung injury and type II cell hyperplasia, at least in rodents, as a consequence of PQ exposure. This innovative approach to evaluate PQ-induced lung injury was firstly investigated in humans by Hantson et al. (2006). These authors described a case report of a 20-year-old man who ingested 100 ml of a 20% PQ formulation. Serum CC16 increased gradually with the progression of renal impairment, and serum SP-A and SP-B levels increased before any significant changes in pulmonary gas exchanges. The SP-A, SP-B, and CC16 levels in BALF were within normal limits. The immunostaining studies using antibodies (Ab) directed against CC16, SP-A, and SP-B were performed on postmortem lung tissue specimens and showed that the labeling for SP-A and -B was reduced or absent following PQ toxicity, while Clara cells were relatively preserved (Hantson et al., 2006). More recently,

Gil et al. (2007) correlated the changes in plasma SP-D concentrations with disease severity in 12 patients with acute PQ intoxication. The results showed that SP-D did not increase during the course of PQ intoxication; nor did SP-D predict survival. However, SP-D was positively correlated with PaO<sub>2</sub>, and the authors concluded that a low concentration of plasma SP-D could reflect injury to pneumocytes. Further studies, including a higher number of cases, are clearly required to validate the use of these pneumoproteins as biomarkers of PQ-induced lung toxicity.

## 8. TREATMENT

The high toxicity of PQ, coupled with its widespread use and ready accessibility, results in many human exposures, by both unintentional and deliberate self-poisonings. Unless the exposure is negligible, all PQ poisonings require immediate treatment and close monitoring in a hospital setting. The "window of opportunity" for any effective treatment of PQ poisoning is very narrow, only a few hours at most. All attempts should be made to obtain an accurate history for any agrochemical exposure.

In view of the proposed mechanisms of PQ toxicity, it has been possible, at several points, to interrupt the toxic pathway (Dinis-Oliveira et al., 2006d). Management has been directed primarily at removing PQ from the GIT (preventing absorption), increasing its excretion from blood, and, measures aimed to prevent pulmonary damage with anti-inflammatory agents and some newer drugs. For many years, the leading PQ manufacturer, Syngenta, has been providing free diagnosis and treatment kits (obtained via local Syngenta office or by e-mail to [cltestkitsupply@syngenta.com](mailto:cltestkitsupply@syngenta.com)), and has been participating in initiatives with global and local suicide prevention agencies en-

couraging safe and secure storage. In Figure 16, a flow chart guide currently used in the management of PQ poisoned patients is presented.

### 8.1. Preventing Paraquat Absorption

The key to successful treatment of an acute PQ exposure relies almost entirely on aggressive early decontamination measures to limit absorption. If there has been dermal exposure, either primarily or secondarily from contact with contaminated vomitus, cloth should be removed immediately and the skin washed gently but thoroughly with soap and water to prevent transdermal absorption. Harsh scrubbing should not be conducted because the resultant skin abrasion could actually increase the transdermal absorption of PQ. PQ-exposed eyes should be irrigated with copious amounts of tepid water or normal saline for at least 15 to 20 min. Since PQ avidly binds to clay, oral administration of mineral adsorbents may be useful as a prehospital treatment for minimizing PQ absorption. Measures to limit absorption that have been employed include the addition of an emetic to all PQ formulations, induction of emesis with syrup of ipecac, whole gut lavage, and oral administration of mineral adsorbents. In 1975, the manufacturer of PQ (ICI) added a potent emetic, PP796 (a phosphodiesterase inhibitor), to liquid and solid PQ formulations. There are a few published laboratory experiments reporting the use of emetic-containing PQ formulations. A study in rats suggested that the emetic used in proprietary PQ preparations may itself possess cardiorespiratory toxicity when given i.v., although the relevance of this finding to human PQ ingestions is uncertain (Noguchi et al., 1985). Unfortunately, despite the occurrence of earlier vomiting, Bramley and Hart (1983) were unable to

FIG. 16. Flowchart guide usually be followed for the management of paraquat poisonings (data are based on human evidences with successful outcomes). PQ, paraquat; i.v., intravenous; PaO<sub>2</sub>, partial pressure of oxygen in arterial blood; O<sub>2</sub>, oxygen; NO, nitric oxide; CHP, charcoal hemoperfusion; CP, cyclophosphamide; MP, methylprednisolone; DFO, desferoxamine; NAC, *N*-acetylcysteine; DEX, dexamethasone; WBC, white blood cells. <sup>1</sup>If systemic toxicity is suspected, test urine for PQ. There is little data for time to peak plasma levels by skin absorption, but if the urine is negative for 24 h, systemic toxicity can probably be disregarded. If the urine test is positive or if there is any doubt about potential systemic toxicity, assess blood concentrations and treat for systemic toxicity as described for ingestion. <sup>2</sup>Risk of inducing bleeding, perforation or scarring due to additional trauma to fragilized tissues. Gastric lavage without administration of an adsorbent has not shown any clinical benefit and therefore it is not advisable. <sup>3</sup>Or in 250 ml of cathartics (it will increase gut motility and improve excretion of the adsorbent-PQ complex) via nasogastric tube. The blue color highlights the treatments that should be performed in order to prevent GIT absorption. <sup>4</sup>Maximum dose is 50 g. <sup>5</sup>Repeated doses of cathartics may result in fluid and electrolyte imbalances, particularly in children, and are therefore not recommended. <sup>6</sup>Particularly important as a mean to correct dehydration, accelerating excretion, reducing tubular concentrations, and correcting any metabolic acidosis. However, fluid balance must be monitored to avoid fluid overload if renal failure develops. In this case hemodialysis or hemofiltration may be required. <sup>7</sup>CHP may be beneficial if the procedure can be instituted within 4 h (when PQ exhibits high plasma concentrations) after ingestion, and its efficacy is maintained even when plasma concentration is less than 0.2 mg/L. Due to the slow redistribution back to circulation and consequent prolonged PQ elimination, seven CHP sessions may be useful. <sup>8</sup>Plasma should be analyzed rather than serum, because serum PQ concentrations are approximately threefold lower than those in plasma obtained from the same blood sample. If only serum is available, results should be interpreted with caution in relation to survival curves. Plasma should be stored in plastic and not in glass tubes because PQ adsorbs onto glass surfaces. The first quarter of this figure is adapted from "Paraquat Poisoning: Practical Guide to Diagnosis, First Aid and Hospital Treatment," published by the Health Assessment and Environmental Safety Department of Syngenta and the Medical Toxicology Unit, Guy's & St Thomas' Hospital NHS Trust, London ([http://www.paraquat.com/portals/5/paraquat\\_booklet.pdf](http://www.paraquat.com/portals/5/paraquat_booklet.pdf)).

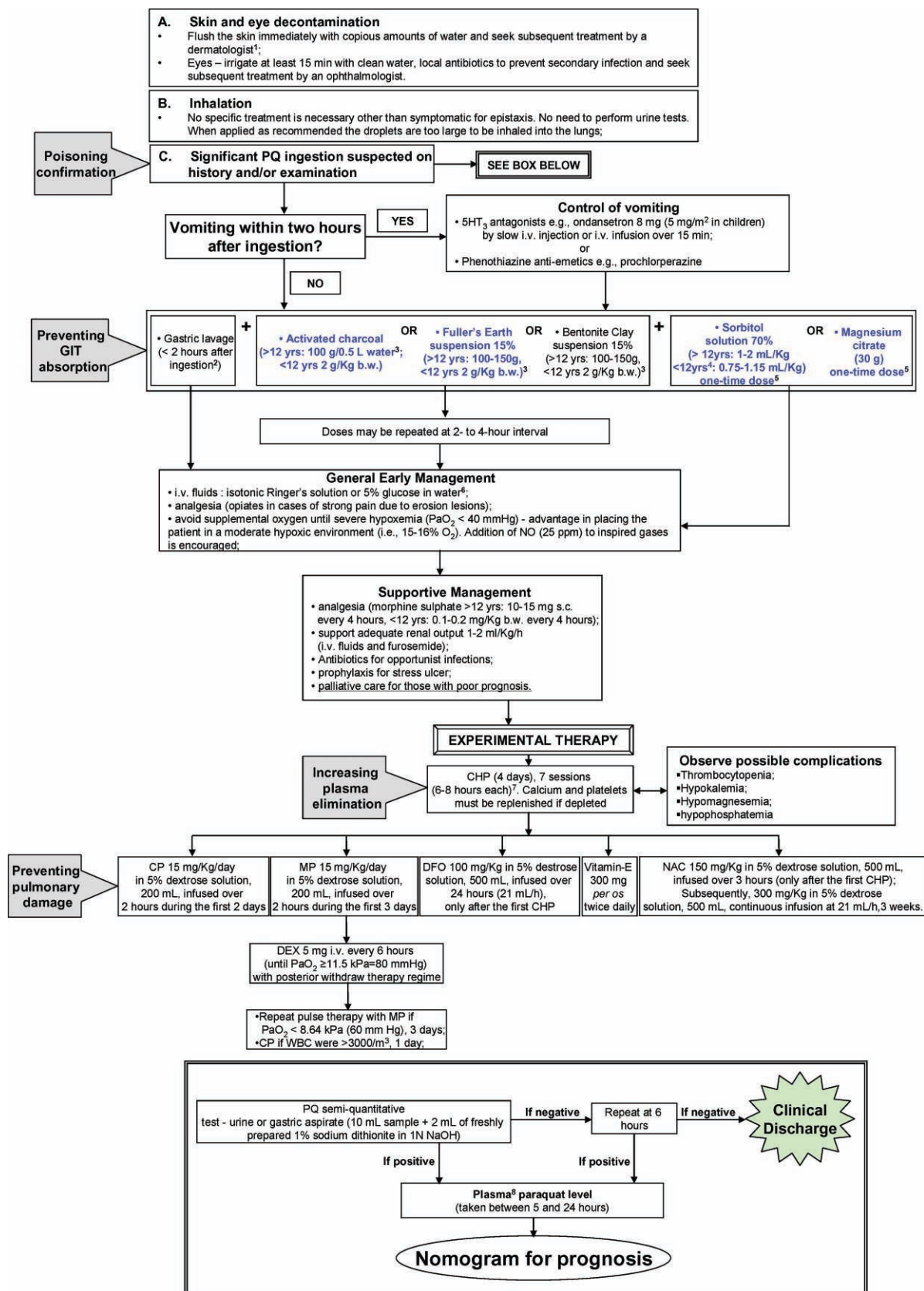


FIG. 16. (Figure legend on previous page)

demonstrate an improved prognosis in patients who had ingested emetic-containing, rather than non-emetic-containing, PQ formulations. Subsequent reports (Onyon and Volans, 1987) from the same study have also failed to record any significant reduction in mortality since the introduction of the emetic PP796. A decrease in the mortality due to PQ poisoning, as a result of the introduction of the emetic-containing formulation also failed to be noted by other investigators (Bismuth et al., 1982). No clinical or experimental studies involving the use of Ipecac syrup in PQ poisoning have been reported. Although syrup of Ipecac may be of value in a home setting if immediately available (administered within 1 h and only in an alert, conscious patient), its use should be discouraged since it may induce repeated vomiting and may reduce the benefit of subsequent hospital treatments, namely, mineral adsorbents. The risk of worsening the GIT corrosive injury must also be balanced against the lethality of the amount ingested.

There have been only two clinical studies published where the authors made specific mention to the efficacy of gastric lavage. Bismuth et al. (1982) were not able to establish the value of gastric lavage in a review involving 28 patients. Bramley and Hart (1983) were unable to demonstrate an improved prognosis resulting from the use of gastric lavage in a study of 262 cases of PQ poisoning referred to a poison information center in the United Kingdom. Without administration of an adsorbent, gastric lavage should never be used. The use of sterilized diatomaceous clays (bentonite and Fuller's earth) in the gastric lavage solution has been performed prior to their continued GIT administration (along with the cathartic magnesium sulfate) (Meredith and Vale, 1987; Vale et al., 1987). There are additional theoretical objections to gastric lavage following PQ ingestion. Ulceration of the oropharyngeal and esophagogastric mucosal surfaces by concentrated PQ formulations is likely to make the procedure hazardous, and risk of further injury exists (Meredith and Vale, 1987).

Although Fuller's earth and bentonite are recommended as adsorbents (administered orally or via nasogastric tube) in PQ ingestions, the ready availability and the equal if not greater efficacy of activated charcoal to bind PQ make it the agent of choice (Okonek et al., 1976, 1982a; Okonek et al., 1982b). Attention should be paid to the brand of activated charcoal, which can influence the adsorptive capacity, probably because of the large variation of surface area (Meredith and Vale, 1995). More recently, Nakamura et al. (2000), studying the in vitro adsorption characteristics of varying particle sizes (mesh) of activated charcoal for PQ, showed that particle size did not influence the amount adsorbed. However, the authors also showed that smaller the particle size of activated charcoal, the faster PQ is removed due to the larger contact surface area. Therefore, it may be assumed that the increase of contact surface area accelerated the removal of PQ onto activated charcoals. Activated charcoal in suspension with magnesium citrate effectively adsorbs PQ, an effect that is maximal at pH 7.8 as observed in vitro and in vivo by the improvement of rats survival rate to 94% in opposition to 63%

in the activated charcoal and Fuller's earth groups, and 69% in the magnesium citrate group (Gaudreault et al., 1985). Activated charcoal, 100 g for adults and 2 g/kg b.w. for children, should be given unless there is a contraindication, such as protracted vomiting or severe burns of the oral mucous membranes. Rapid control of repeated vomiting with antiemetics and promotility agents is essential when the patient cannot retain the adsorbent. A total of three doses of activated charcoal at 2-h intervals may be useful to avoid enterohepatic recirculation (Douze et al., 1975; Mullick et al., 1981; Nagao et al., 1990; Ameno et al., 1994; Dinis-Oliveira et al., 2006b). The airways should be protected appropriately to prevent aspiration of gastric contents as previously described (Daisley and Simmons, 1999; Ruiz-Bailén et al., 2001).

Yamashita et al. (1987) have reported the results of gastric and intestinal lavage with the cation-exchange resin Kayexalate in PQ-poisoned patients. Six of 11 patients treated in this manner survived, while 11 patients who did not receive Kayexalate died. Unfortunately, it is not possible to judge whether the severity of poisoning was comparable in the two groups of patients because blood PQ concentrations were not provided.

PQ has very low bioavailability but peak concentrations occur very early. Thus, these procedures to prevent absorption are only likely to work if given very soon after poisoning (within 1–2 h). In practice, however, they are used very frequently irrespective of the delay between poisoning and treatment.

## 8.2. Increasing Paraquat Elimination

In cases of PQ poisoning by ingestion, once GIT decontamination has been performed, there remain two additional treatment strategies. The first is to attempt to alter the herbicide's *toxicokinetics* (i.e., its distribution in the body after ingestion). The second is to attempt to modify its *toxicodynamics* (i.e., the herbicide's effects on the target organs).

### 8.2.1. Extracorporeal Elimination

The goal of extracorporeal elimination procedures is to remove PQ from the circulation and prevent its uptake by pneumocytes and Clara cells. The only method that has been shown to be efficient and to enhance the extracorporeal elimination of PQ is charcoal hemoperfusion (CHP), with  $CL_{PQ}$  values that may be as high as 170 ml/min. These high clearances, however, do not allow extrapolation of the efficacy of these procedures in removing clinically significant quantities of PQ, as the amount removed depends on the plasma level, which always decreases rapidly. Most toxicologists currently recommend rapid initiation of CHP to lower plasma PQ levels and to limit pulmonary and other organs' uptake of PQ. Patients with plasma levels of 3 mg/L or greater should probably not be considered for CHP treatment because of the uniformly poor prognosis and a lack of demonstrated efficacy for the procedure (Hampson and Pond, 1988). Tabei et al. (1982) studied, in vitro and in vivo, the efficiency

of CHP for the removal of PQ. At a flow rate of 200 ml/min, 93–99% of PQ in 4 L of solution (5, 10, 100 ppm) was removed in less than 160 min. The elimination  $t_{1/2}$  was 16 min and 10 s. At 100 ml/min, it was 49 min 30 s. Of 23 PQ poisoning cases, 15 patients underwent CHP, of which 10 died of respiratory failure within 28 days ( $7.6 \pm 2.9$ ) and 5 survived without pulmonary complications. Of 8 patients who did not receive CHP, 6 died of respiratory failure within 97 days ( $33.4 \pm 18.8$ ), even when their general condition was good upon admission. In one patient, whose PQ concentration in blood was followed, 99% of the PQ was removed from circulating blood by a single CHP. Authors concluded that CHP is effective for the removal of PQ from blood in vivo and from solution in vitro. CHP may thus improve survival after PQ ingestion. Analyzing 105 patients, who had swallowed 1 to 3 mouthfuls of PQ solution (24.5% w/v), Hong et al. (2003) also concluded that adequate CHP appears to be an indispensable treatment for patients with acute PQ poisoning. When CHP is effective (i.e., when PQ levels in venous outlet approximates to zero), the plasma PQ concentration drops dramatically within 1–3 h. Unless the procedure is begun at an early stage, when PQ is concentrated in the central compartment, a poor total body  $CL_{PQ}$  by extracorporeal techniques and a rise in plasma concentrations for several hours following completion of CHP may ensue, which can be explained by extensive PQ tissue distribution (a rebound in plasma concentrations is observed) and its slow redistribution back into the circulation following termination of the extracorporeal procedure (De Broe et al., 1986). Because of these factors, Okonek et al. (1979, 1982b) proposed that “continuous” (repeated) CHP should be performed. Nevertheless, treating PQ poisoning with CHP has been the subject of considerable controversy, with the weight of evidence in the published literature showing a lack of clinical efficacy in several cases (Bismuth et al., 1982; Castro et al., 2005). Furthermore, the renal  $CL_{PQ}$  under normal kidneys function is 3–10 times more efficient than  $CL_{PQ}$  by means of CHP (Proudfoot et al., 1987). CHP is also often complicated by thrombocytopenia, as platelets adhere to the cartridge (Winchester, 2002). Notwithstanding the fact that it is extremely difficult to make CHP the first-line therapy in PQ poisoned patients with this level of evidence, it is perceivable that a 6- to -8-h course of CHP may be beneficial if the procedure can be instituted within 4 h (when PQ exhibit high plasma concentrations) after ingestion, and its efficacy is maintained even when plasma concentration is less than 0.2 mg/L. Due to the slow redistribution back to circulation and consequent prolonged PQ elimination, seven CHP sessions may be useful (Figure 16).

Serial and combined CHP with hemodialysis (HD) have also been recommended, particularly during the first 24 h after exposure (Proudfoot et al., 1979; Tabei et al., 1982). The  $CL_{PQ}$  achieved with HD is good when PQ plasma levels are high (around 10 mg/L). However,  $CL_{PQ}$  drops remarkably when the plasma concentration is less than 1 mg/L. The  $CL_{PQ}$  achievable by hemodialysis (HD) can be as high as 150 mL/min (Okonek et al., 1982b). However, considering that the plasma PQ con-

centration is usually relatively low, the actual amount of PQ removed by this extracorporeal procedure may be clinically insignificant compared with the ingested dose (Proudfoot et al., 1987). In addition, the  $CL_{PQ}$  with HD decreases considerably when the plasma concentration falls to less than 0.5 mg/L. Nevertheless, HD should be used, when indicated, for the treatment of PQ-induced renal failure.

Plasmapheresis was also tentatively used in acute PQ poisonings, though with no success (Tsatsakis et al., 1996).

### 8.2.2. Forced Diuresis and Peritoneal Dialysis

Other therapies that have been investigated include removal of PQ from the blood by forced diuresis and peritoneal dialysis. Forced diuresis was initially popular in the management of patients with PQ poisoning (Kerr et al., 1968; Fennelly et al., 1971). However, forced diuresis is not very effective since PQ tubular reabsorption is small (Lock and Ishmael, 1979). Moreover, pulmonary edema, a complication of PQ poisoning as well as of forced diuresis, increases morbidity and makes patient management more difficult. Fluid replacement must be undertaken with careful monitoring of respiratory function and urine output. Nevertheless, furosemide and the early administration of i.v. fluids and electrolytes, to maintain adequate urine flow (achieving an urine output of 1 to 2 ml/kg/h), are important because the kidneys are the major physiological route for PQ excretion. A brisk urine flow supports both glomerular filtration and tubular secretion of PQ and delays the onset of an acute oliguric renal failure. PQ may cause peripheral vasodilatation with “third spacing” (Webb and Leopold, 1983) and intrarenal vasoconstriction (Lock and Ishmael, 1979). These mechanisms account for a functional component of the early stages of PQ-induced renal failure, which is mostly reversible. Unfortunately, this may occur in the first several hours following PQ poisoning, with decreases of  $CL_{PQ}$  together with increases of its  $t_{1/2}$  and consequent generation of lethal concentrations of PQ in the lung tissue (Bismuth et al., 1987). Bismuth et al. (1982) suggested that functional renal failure has no prognostic value, while organic renal failure (proximal acute renal tubular necrosis) has greater importance in predicting the outcome from PQ poisoning. This functional renal impairment should be promptly corrected with i.v. volume expansion to allow maximal renal excretion of systemically absorbed PQ before the onset of renal tubular necrosis from direct action of the herbicide on the kidney (Lock and Ishmael, 1979).

Peritoneal dialysis is a poor mean of removing PQ (Carson, 1972). Indeed, this technique is only able to eliminate small quantities of the herbicide when plasma levels are very high.

### 8.3. Supportive Therapies

Patients poisoned with PQ are always dehydrated to some extent due to GIT fluid losses (Webb and Leopold, 1983; Williams et al., 1984). Besides maintaining kidney perfusion, early i.v.



fluids and electrolytes administration are also important to correct dehydration.

Reduction of O<sub>2</sub> supply (hypooxygenation) has been tried because of evidence in animals of a relationship between the FiO<sub>2</sub> and the severity of the pulmonary damage (Fisher et al., 1973; Rhodes et al., 1976; Kehrer et al., 1979). Animals poisoned with PQ die more quickly in an O<sub>2</sub>-enriched atmosphere than do poisoned animals breathing room air (Fisher et al., 1973; Kehrer et al., 1979). Similarly, poisoned animals kept in a somewhat hypoxic atmosphere had lower mortality rates than animals kept in room air (Rhodes et al., 1976). Oxygen may increase lung injury by providing additional substrate for O<sub>2</sub><sup>-</sup> formation. In humans, O<sub>2</sub> also appears to accelerate lung damage, and thus artificial ventilation with low O<sub>2</sub> concentrations (<21%) in the breathing mixtures [with nitric oxide (NO) ventilation] were suggested to restrict the concentration of inhaled O<sub>2</sub> as long as possible, unless PaO<sub>2</sub> fell below 40 mm Hg as occurs in hypoxemic patients (Chollet et al., 1983). The use of low FiO<sub>2</sub> requires positive end-expiratory pressure (PEEP) and continuous positive pressure breathing. Supplemental O<sub>2</sub> is given when necessary for symptomatic relief, but mechanical ventilation would not be offered as a treatment option for a patient who is obviously in impending respiratory failure due to pulmonary fibrosis.

Relief of pain and anxiety is essential. Because medical therapy is so highly unsuccessful in reversing moderate to severe PQ ingestions, the health care providers, their patients, and the patients' families are often bewildered. The art of medicine is crucial here as a multidisciplinary support. Honesty about prognosis, without taking away hope, and emphasizing what can be done (i.e., pain relief and pastoral and social service care) are keystone approaches to a grim situation (Vale et al., 1987).

First attempted in 1968 (Matthew et al., 1968), lung transplantation has been used in highly selected patients but mostly with unsuccessful outcomes (Saunders et al., 1985). Since muscles are important body reservoirs for PQ, the herbicide release from muscles may occur when weaning from mechanical ventilation is started, resulting in a new lung injury.

#### 8.4. Measures to Prevent Lung Damage

Research into the mechanisms of PQ toxicity and the development of antidotes, although very productive in terms of the information gained about free radical-mediated toxicity and the polyamines and their uptake pathways (Smith, 1988b), has yielded little hope for PQ-poisoned patients. No antidote has been currently recommended on the basis of convincing evidence obtained in patients. Certain of these compounds appear to give promising results in experimental animals, partly because they are administered either prophylactically or early in the course of the poisoning. Confirmation of efficacy is not available from patient clinical data because of the heterogeneous nature of the patient populations, a lack of prospective, controlled trials without numerous confounding variables, and the fact that patients may be presented at emergency rooms after most of the PQ has been eliminated from the body.

As a *first approach*, most potential antidotes have been directed toward compounds that detoxify the O<sub>2</sub><sup>-</sup> or the other subsequently formed ROS. These have included:

**Superoxide dismutase or mimetic enzymes.** Under normal circumstances, O<sub>2</sub><sup>-</sup> produced by PQ and other chemicals is kept under control by the superoxide dismutase (SOD) enzymes. The use of SOD as a treatment to ameliorate PQ-induced injuries has produced variable results. Exogenously administered SOD conferred protection in young rats that had been challenged with PQ (Autor, 1977). Also, in adult rats, SOD reduced the mortality to PQ challenge from ~80 to 45% over a 28-day period (Wasserman and Block, 1978). In contrast, the results from most other studies, in which SOD had been employed as an antioxidant treatment for PQ toxicity, demonstrated that when SOD was administered by continuous i.v. infusion, it failed to ameliorate the toxic effects of the herbicide (Block, 1979). In addition, SOD administered by the parenteral route was not effective in human poisonings (Fairshter et al., 1979). Although these differences are not easily explained, it has been reported that the lack of SOD effectiveness in protecting against PQ toxicity can be attributed to its physicochemical properties; this enzyme cannot enter the target cell membrane because of its high molecular size (which prevents intracellular transport) or its charge (which prevents its adherence to targets) (Freeman et al., 1985). More recently, in order to circumvent these problems, investigators have used low-molecular-weight metalloporphyrin SOD mimetics or liposomal encapsulated SOD for the purpose of successfully treating oxidative stress-induced injuries. More precisely, Day and Crapo (1996) employed the low-molecular-weight metalloporphyrin SOD mimetic tetrakis-(4-benzoic acid) porphyrin (MnTBAP) to protect mice against PQ-induced lung injury. This SOD mimetic has been demonstrated to penetrate cell membranes, retain intracellular activity, and also protect endothelial cells against intracellular PQ-induced injury *in vitro* (Day and Crapo, 1996). However, no studies have been performed to examine the role of liposomal encapsulated SOD against PQ-induced human injuries yet.

**Vitamin E ( $\alpha$ -tocopherol).** Vitamin E is a lipid-soluble vitamin that exerts its antioxidant effects by scavenging free radicals and stabilizing membranes containing PUFAs (Burton, 1994). The role of vitamin E in PQ toxicity was demonstrated in several studies where deficiency of vitamin E potentiated the development of acute PQ toxicity in animals. It was shown that vitamin E deficiency shortened and decreased survival, worsened histologic lung damage in rats (Block, 1979), and significantly reduced the LD<sub>50</sub> in mice (Bus et al., 1975) exposed to PQ. Moreover, the potentiation of acute PQ toxicity by vitamin E deficiency was reversed by its administration (Block, 1979). Although the mechanism(s) by which vitamin E protects against PQ toxicity is not fully understood, it may be attributed to the vitamin's antioxidant properties in preventing LPO or by scavenging O<sub>2</sub><sup>-</sup> and thus preventing its toxicity. Although vitamin

E confers protection against PQ-induced injuries in vitamin E-deficient animals, normal animals receive little benefit from additional pharmacologic supplementation with vitamin E. In a study in male rats, the i.p. administration of vitamin E, either 30 min after i.p. PQ LD<sub>50</sub> challenge followed by a second injection 24 h later, or 2 h before PQ challenge, followed by a second injection 26 h later, did not alter the acute mortality nor reduced the characteristic pathological lung changes observed at death (Redetzki et al., 1980). Moreover, in investigating the extent of lipid peroxidation, expressed as serum malondialdehyde level, in patients with subacute toxic reactions from PQ poisoning, it was shown that the administration of vitamin E to humans (100–4000 mg/day) was ineffective in protecting against PQ poisoning and did not affect the levels of malondialdehyde (Yasaka et al., 1986).

The failure of vitamin E to protect against PQ and other oxidants is unclear at the present time. It has been suggested that this ineffectiveness might be related to the solubility of vitamin E, since lipid-soluble antioxidants take too long to diffuse through cellular membranes. To overcome this major limitation in patients requiring emergency treatment, water-soluble analogs of  $\alpha$ -tocopherol, which can be safely administered i.v. (Petty et al., 1990), or liposomal  $\alpha$ -tocopherol preparations (Suntres and Shek, 1995) might offer a better treatment effect. Shahar et al. (1980) reported recovery in a child who had ingested a potentially lethal dose of PQ and was treated with vitamin E. Similar results with vitamin E cotherapy were also obtained by our group (Dinis-Oliveira et al., 2006d). However, Harley et al. (1977) noted no effect in another case.

**Vitamin C (ascorbic acid).** Ascorbic acid, a water-soluble vitamin, is effective in scavenging free radicals, including HO $\cdot$ , aqueous peroxy radicals, and O<sub>2</sub> $\cdot^-$ . Ascorbic acid acts as a two-electron reducing agent and confers protection by releasing an electron to reduce free radicals, thus neutralizing these compounds in the extracellular aqueous environment, prior to their reaction with biological molecules (Evans and Halliwell, 2001). Moreover, the antioxidant potential of ascorbic acid is attributed not only to its ability to quench ROS, but also to its ability to regenerate other small molecule antioxidants, such as  $\alpha$ -tocopherol, GSH, and  $\beta$ -carotene (Evans and Halliwell, 2001). Intravenously administered vitamin C shortly prior to PQ challenge protected against tissue damage as evidenced by a reduction of the exEth, a reliable index of oxidative damage (Kang et al., 1998). Although prior administration of ascorbic acid confers protection against PQ toxicity, the use of ascorbic acid in treating PQ-induced tissue injuries has resulted in unfavorable consequences. Apparently, ascorbic acid can accelerate the generation of HO $\cdot$  by reducing oxidized free transition metal ions [e.g., ferric ion (Fe<sup>3+</sup>)] in the aqueous phase (Buettner and Jurkiewicz, 1996; Halliwell, 1996; Carr and Frei, 1999; Evans and Halliwell, 2001). Results show that, during extensive cellular damage transition metals are released into the aqueous phase

(Kohen and Chevion, 1985c; Halliwell, 1996). Ascorbic acid, given at a time when the extensive tissue damage induced by PQ is in progress, aggravates the oxidative damage (Kang et al., 1998). The exacerbation of the oxidative damage following the interaction of transition metals with ascorbic acid during the progressive stages of PQ toxicity was significantly reduced by pretreating these animals with desferrioxamine (DFO), a chelator that tightly binds the ferric iron just prior to PQ administration (Kang et al., 1998). In PQ-poisoned patients, ascorbic acid showed to be an important free radical scavenger (Hong et al., 2002).

**Desferrioxamine (desferrioxamine).** Iron and PQ do appear to behave synergistically in the generation of HO $\cdot$ , by an iron-driven Fenton reaction (Figure 11). Physiologically, free iron (i.e., iron bound to low-molecular-weight chelators) exists predominately in the ferric (Fe<sup>3+</sup>) state, and the foregoing reaction does not proceed at a toxicologically significant rate. However, based on evidence from *in vitro* studies (Vile and Winterbourn, 1988), it has been suggested that the presence of PQ facilitate the reduction of Fe<sup>3+</sup> to ferrous ion (Fe<sup>2+</sup>), thus significantly enhancing the rate of HO $\cdot$  generation as long as sufficient H<sub>2</sub>O<sub>2</sub> is available (van Asbeck et al., 1989). The reduction of Fe<sup>3+</sup> may be achieved directly by the PQ $^{+}$  (Vile and Winterbourn, 1988), or indirectly by the O<sub>2</sub> $\cdot^-$  generated through reduction of O<sub>2</sub> by PQ $^{+}$  (McCord and Day, 1978) (Figure 11), or by ascorbic acid (Buettner and Jurkiewicz, 1996; Halliwell, 1996; Carr and Frei, 1999; Evans and Halliwell, 2001). The importance of iron and other transition metals in the PQ-related damage has been demonstrated by both *in vitro* and *in vivo* studies, where iron chelation prevented PQ toxicity (Kohen and Chevion, 1985a, 1985b, 1985c; van Asbeck et al., 1989; Van der Wai et al., 1992), a treatment that also depends on the lipophilicity of the chelating agents. The administration of DFO by continuous i.v. infusion to vitamin E-deficient rats significantly reduced mortality produced by PQ (van Asbeck et al., 1989). It has been shown that DFO can exert its protective effects, not only by inhibiting the PQ-induced generation of HO $\cdot$ , but also by blocking the uptake of PQ by the alveolar type II cells (Van der Wai et al., 1992). Administration of more lipophilic chelating agents, such as 1-(2'-methoxyethyl)-2-methyl-3-hydroxypyridin-4-one (CP51), also increased the survival of PQ-challenged rats with a normal vitamin E status. Moreover, the protective effect of CP51 was also demonstrated during *in vitro* experiments where CP51 prevented the PQ-induced lysis of alveolar type II cells (Van der Wai et al., 1992). Although experimentation with iron chelators against PQ-induced toxicity seems promising, the potential efficacy and optimal doses of the iron chelation therapy in human poisoning have not been assessed. In addition to other therapeutic approaches, our group reported (Dinis-Oliveira et al., 2006d) the use of DFO (100 mg/kg in 500 ml of 5% dextrose solution in continuous i.v. perfusion at 21 ml/h during 24 h only after the first CHP session) in a successful clinical case of PQ poisoning. Comparable results, using a similar



DFO dose, were also previously reported by Lheureux et al. (1995).

**Clofibrate.** Clofibrate increases the expression of hepatic catalase, which is an antioxidant enzyme (Goldenberg et al., 1976). Clofibrate has a protective effect on PQ-induced pulmonary toxicity and on mortality, but only when rats are treated before PQ administration (Frank et al., 1982). However, clofibrate has no effect on the antioxidant enzymes in the lung, and therefore its protection against experimental PQ-induced lung toxicity seems not to be due to an antioxidant effect (Frank et al., 1982). No clinical studies for this drug have been reported.

**Low-molecular-weight thiol-containing antioxidants.** Since compounds containing SH groups are among the most important endogenous antioxidants, their therapeutic use has been proposed in oxidant lung injury (Deneke, 2000). GSH is the most abundant non-protein SH in living organisms and it plays a crucial role in intracellular protection against ROS and other free radicals (Anderson, 1997). GSH can function as a nucleophile to form conjugates with many xenobiotics and/or their metabolites and can also serve as a reductant in the metabolism of  $H_2O_2$  and other organic hydroperoxides, a reaction catalyzed by GPx found in cytosols and mitochondria (Anderson, 1997; Deneke, 2000). Although in vitro studies have shown that alveolar type II cells can be supplemented with exogenous GSH to protect against PQ-induced injury (Hagen et al., 1986), the antioxidant effectiveness of exogenously administered GSH for the treatment of pulmonary injuries against PQ or other oxidants has been hindered by its rapid hydrolysis in the circulation (Smith et al., 1992).

**N-Acetylcysteine (NAC),** the acetylated variant of the amino acid L-cysteine, is a cell-permeable precursor of GSH and an excellent source of SH groups (Patterson and Rhoades, 1988). NAC is converted in the body into metabolites capable of stimulating GSH synthesis, promoting detoxification and acting directly as free radical scavenger (Kelly, 1998; Deneke, 2000). It has been shown that the administration of 20 mg/kg of NAC prior to PQ intoxication protects against its toxicity in rats, leading to less edema and cellular infiltration in the lung than control animals without NAC pretreatment (Wegener et al., 1988). Also, the incubation of NAC with alveolar type II cells, which are known to be specific targets of PQ toxicity in vivo, enhanced the GSH content of these cells and consequently prevented the PQ-induced cytotoxicity (Hoffer et al., 1996; Çeçen et al., 2002). In another study, the administration of NAC to PQ-intoxicated animals did not affect the survival rate, although it delayed the PQ-induced release of chemoattractants for neutrophils in the BALF and significantly reduced the infiltration of inflammatory cells, suggesting that NAC can confer its protective effect by delaying inflammation (Hoffer et al., 1993, 1996). A more recent study, using also both in vivo and in vitro experiments, demonstrated that NAC posttreatment in PQ-intoxicated rats can

effectively increase the survival rate and abolish the PQ-induced oxidative stress and inflammatory response (Yeh et al., 2006). Different NAC dosage and time schedule administration may explain the discrepancies. In vitro exposure of human alveolar cells to PQ produced apoptotic cell death, probably via oxidative stress mechanisms, and this toxic effect was inhibited by NAC, an effect attributed to the direct scavenging activity mediated by the SH group of NAC (Cappelletti et al., 1998). Clinically, there are a few case reports describing the successful treatment of PQ poisoned patients, using NAC in the treatment cocktail (Lheureux et al., 1995; Drault et al., 1999; Lopez Lago et al., 2002; Dinis-Oliveira et al., 2006d).

The toxicity of PQ in mice was significantly decreased by the administration of thiosulfite or sulfite, which also abolished the PQ-induced depletion of the GSH in liver induced by PQ (Yamamoto, 1993). In culture, cystamine, the disulfide form of the naturally occurring SH compound cysteamine, prevented PQ-induced Clara cell damage at low PQ concentrations (Masek and Richards, 1990). In mice, L-cystine protected against the toxicity of PQ by maintaining GSH levels in the lung cells (Kojima et al., 1992). Diethylmaleate, a GSH-depleting agent, increases PQ toxicity in rats (Bus et al., 1975). No clinical studies using thiosulfite or sulfite treatment have been reported.

**Metallothionein (MT)** is a metal-binding protein of low molecular weight, containing cysteine as one-third of its total amino acids (Deneke, 2000). This protein has been shown to be an efficient scavenger of ROS, such as  $O_2^-$  and  $HO^{\cdot}$  (Miles et al., 2000). Synthesis of MT can be induced by essential metals, such as zinc and copper. Induction of metallothionein in the lungs of mice after zinc administration has been shown to protect against the lethality and pulmonary toxicity of PQ (Sato et al., 1996). Although intrapulmonary MT levels are low, they are readily induced by s.c. administration of PQ to mice (Bauman et al., 1991). Nakagawa et al. (Bauman et al., 1991; Nakagawa et al., 1995, 1998) have also found that PQ-induced MT synthesis in the liver as a consequence of ROS production. The protective role of MT in PQ toxicity has also been demonstrated in transgenic mice deficient in MT genes. In these experiments, it was shown that tissues in MT-null mice were more susceptible to PQ-induced oxidative stress than normal mice, as evidenced by increases in LPO (Sato et al., 1996). Similarly, Lazo et al. (1995) showed that embryonic cells derived from MT-null mice were more susceptible to ROS produced by PQ. A major reason for the increase in the susceptibility of these tissues to PQ has been attributed to the lower basal levels of nonprotein SH groups, including MT and GSH, which constitute the first line of defence against oxidative stress-induced injuries (Deneke, 2000).

**Xanthine oxidase inhibitors.** Xanthine dehydrogenase (XD) and xanthine oxidase (XO) are two forms of the same enzyme that differ in the electron acceptor used in the final step of catalysis. In the case of XD, the final electron acceptor is  $NAD^+$  (dehydrogenase activity), whereas in the case of XO the

final electron acceptor is  $O_2$  (oxidase activity). XD is converted to XO by oxidation of cysteine residues (Cys<sub>893</sub> and Cys<sub>1326</sub> of the human enzyme) and/or proteolytic cleavage. Under normal physiologic conditions, XD is the predominant form of the enzyme found in vivo. XD/XO catalyzes an important physiologic reaction, the sequential oxidation of hypoxanthine to xanthine and uric acid. Accordingly to some studies (Kitazawa et al., 1991; Matsubara et al., 1996), PQ mediates the electron-transfer reaction with XD/XO by reduction/reoxidation cycling. PQ takes electrons away from reduced XD/XO, reducing itself. Consequently, PQ accelerates the generation of  $O_2^-$  via XD/XO system. In rats fed with a tungsten-enriched diet, which inhibits the XD/XO activity by replacing the molybdenum ion within the enzyme, the mortality due to PQ decreased significantly compared with rats fed with a standard diet (Kitazawa et al., 1991). Pretreatment with oxypurinol (1000 mg/kg s.c.) partially prevented the PQ toxicity in rats. In addition, PQ exposure showed to increase XO lung activity (Waintrub et al., 1990). The role of XD/XO in PQ toxicity was also investigated using cultured bovine pulmonary artery endothelial cells (Sakai et al., 1995). Tungsten and allopurinol inhibited the increase of XO activity and decreased  $O_2^-$  release and the subsequent formation of other ROS (Sakai et al., 1993, 1995). The effects of these treatments have not been investigated in human poisonings by PQ.

**Selenium.** Selenium (Se) is an essential trace element, and a large portion of body Se is present in the form of cellular GPx (Behne and Wolters, 1983). The Se-containing enzyme GPx plays an important protective role against PQ toxicity. This protective effect of Se has been reported by several authors (Cagen and Gibson, 1977; Omaye et al., 1978; Burk et al., 1980). Se-dependent GPx is able to reduce, and thereby detoxify, both organic and inorganic hydroperoxides using GSH as a reducing agent. More recent studies indicated that the Se-containing enzyme GPx is the major, if not the only, structural form of body Se that protects mice against the lethal oxidative stress caused by high levels of PQ; it seems less important, however, in protecting mice against the moderate oxidative stress by a low level of PQ (Cheng et al., 1998). Se-containing enzyme GPx plays also a critical role in maintaining the redox status of mice under acute oxidative stress, and protects against PQ-induced oxidative destruction of lipids and protein in vivo (Cheng et al., 1999). Nevertheless, Se was not yet used in the treatment of human poisonings by PQ.

**Niacin and riboflavin.** Niacin (500 mg/kg b.w.) decreases the mortality rate in rats from 75 to 55% (Brown et al., 1981). At least in the isolated perfused rat lung, niacin was shown to protect against PQ-induced lung toxicity (Ghazi-Khansari et al., 2005).

By stimulating the activity of Gred, Schvartsman et al. (1984) observed an improvement of the survival rate after treatment with riboflavin plus vitamin C in PQ-intoxicated rats, while

riboflavin given alone did not afford any protection. No human studies using these vitamins have been reported for PQ poisoning.

**Oils and other fatty acids.** The role of nutrients in modulating PQ toxicity in experimental animals has also been investigated, though not as extensively as for antioxidants. It was noted that an intramuscular injection of commercial corn oil, which was used for the administration of lipophilic anti-inflammatory agents, reduced the lethality of a single oral dose of PQ in mice from 70 to 50%. Similarly, the injection of other fresh commercial vegetable oils bearing different ratios of unsaturated to saturated fat as well as fish oils (cod liver and menhaden oils) also reduced PQ lethality (Fritz et al., 1994). The mechanism underlying the protective effect conferred by these oils is not clear, but it does not appear to be due to their vitamin E content or due to alteration in the absorption or distribution of PQ (Fritz et al., 1994). On the other hand, loaded hepatocytes with  $\alpha$ -linolenic acid underwent LPO to a greater extent and at much lower PQ concentrations than normal unloaded hepatocytes (Sugihara et al., 1995). It has been demonstrated that an increase in monosaturated fatty acids or a reduction in PUFA in lipid membranes decreases the susceptibility of membranes to oxidant attack (Fritz et al., 1994; Sugihara et al., 1995). The effect of soy protein, soy isoflavones and saponins on PQ-induced oxidative stress was investigated in rats. Rats were fed on experimental diets containing casein, soy protein, and casein with soy isoflavones and saponins. The diets were supplemented with 0.025% PQ. The obtained results suggested that an intake of soy protein itself, but not soy isoflavones and saponins, reduces PQ-induced oxidative stress in rats (Aoki et al., 2002). These approaches were never tested in human poisonings by PQ.

**Angiotensin-converting enzyme inhibitors.** Recently, inhibitors of the angiotensin-converting enzyme (ACE), which catalyzes the conversion of angiotensin I to the vasoconstrictor peptide angiotensin II was reported to prevent PQ-toxicity in animal models (Candan and Alagozlu, 2001; Mohammadi-Karakani et al., 2006). Several physiological roles of angiotensin II have been clarified not only in relation to the pathogenesis of hypertension but also regarding the stimulation of fibroblast proliferation and collagen synthesis (Booz et al., 1993; Lasky and Ortiz, 2001). Lisinopril decreased the amount of hydroxyproline in the lung tissue of the PQ-exposed rats (Mohammadi-Karakani et al., 2006). The antifibrotic effect of lisinopril was shown to be due to inhibition of angiotensin II synthesis, which results in the stimulation of fibroblast proliferation and collagen synthesis. Also, when captopril is administered to PQ-poisoned rats it prevented PQ toxicity by improving the disrupted antioxidant capacity, lowering LPO, and preventing lung tissue fibrosis (Candan and Alagozlu, 2001). Lisinopril, unlike captopril, does not contain SH groups in its structural formula, which may be the reason for lisinopril not having any effect on LPO (Bagchi et al.,

1989). More recently, the beneficial effect of ACE inhibitors in preventing pulmonary fibrosis as consequence of PQ-exposure was also corroborated by Ghazi-Khansari et al. (2007). Nevertheless, human studies are not yet available assessing the benefit of this treatment in PQ poisoning.

A *second approach* has been followed to decrease the redox cycling of PQ. Methylene blue, for example, competes 100 to 600 times more effectively than PQ for reduction by three different flavo-containing enzymes—XO, NADH cytochrome *c* reductase, and NADPH cytochrome *c* reductase—resulting in decreased  $O_2^-$  production (Kelner et al., 1988). However, studies of this treatment modality for acute PQ poisoning are lacking.

A *third approach* has been followed to prevent the accumulation of PQ in the alveolar epithelial cells via the PUS. In tissue culture, spermidine uptake by epithelial type II cells is inhibited by PQ (Rannels et al., 1985, 1989). In Clara cells culture, putrescine and spermidine reduce PQ-induced damage, indicating that they compete for the same transporter (Masek and Richards, 1990). This has been shown to be possible in vitro. Studies in vivo, however, have not shown any antidotal effect (Dunbar et al., 1988). Indeed, putrescine infused to rats and achieving a plasma concentration fourfold that of PQ was unable to decrease either its accumulation in the lungs or its toxic effects (Dunbar et al., 1988). Other substances such as D-propranolol and imipramine may decrease the pulmonary accumulation of PQ in vitro (Drew et al., 1979). However, in vivo studies did not confirm these data and showed no protective effect of these agents (Drew et al., 1979; Bateman, 1987). Chlorpromazine inhibited PQ uptake and increased its efflux in vitro (Siddik et al., 1979). Unfortunately, in vivo, chlorpromazine potentiated the PQ toxicity by reducing urinary excretion and increasing pulmonary PQ concentrations simultaneously (Koyama et al., 1987). In humans, uncontrolled studies showed no positive effect of D-propranolol (Fairshter et al., 1979). The use of anti-PQ antigen-binding fragments (Fab) from cleaved Ab to treat poisoning or some other PQ-sequestering agents to remove PQ from lung cells was also tested. Antibodies from IgG- and IgM-secreting cell lines have been raised in murine hybridomas and show high selectivity and affinity for PQ (Bowles et al., 1988; Johnston et al., 1988). PQ-specific Ab inhibit the uptake of PQ in vitro by type II alveolar cells from the rat and reduce toxicity (Wright et al., 1987; Chen et al., 1994b). After i.v. injection of 0.1 mg/kg PQ, the plasma PQ concentration in rats pretreated with anti-PQ Ab was increased and the amount excreted in the urine was significantly decreased compared with controls (Nagao et al., 1989). However, although using anti-PQ Ab can successfully sequester PQ in the plasma compartment of rats and mice, it could not prevent PQ from accumulating in tissues, such as the lung; nor could it favor its release (Cadot et al., 1985; Nagao et al., 1989). In fact, such in vitro and in vivo studies suggest that as the concentrations of PQ in the lung are not changed, PQ Ab neither prevent PQ uptake by the lung nor favor its release. Moreover, it was predicted that a 100- to 200- g Fab Ab fragment dose would be required for an adult human, an amount beyond production ca-

pabilities (Wright et al., 1987). More recently a single-chain Fv (scFv) fragment specific for PQ was produced from hybridoma cells secreting a PQ-specific murine monoclonal Ab, with the aim being to produce a smaller molecule with high affinity for PQ (Devlin et al., 1995). However, this scFv fragment was expressed in an insoluble form and only displayed moderate PQ binding affinity. Therefore, an attempt was made to produce a soluble scFv fragment and to increase its PQ binding affinity. Unfortunately, it became clear that the supposed pH dependence of PQ binding to the scFv fragment was due to tightly bound tris-(hydroxymethyl)aminomethane (Tris) from the buffer used to purify the Ab (Bowles et al., 1997).

In a *fourth approach*, the effects of a lung surfactant-stimulating drug, ambroxol, and the administration of exogenous surfactant have been investigated. Observations that extensive alveolar collapse represents a relatively early morphological phenomenon in PQ poisonings coupled with evidence of decreased surface-active material in the BALF of PQ-treated animals (Robertson et al., 1970; Fisher et al., 1975), have prompted proposals that surfactant depletion, either through a direct action of PQ on surfactant synthesis and/or secretion or as a consequence of destruction of surfactant-producing alveolar type II cells, may be a significant event in the toxic process and in the pathophysiology of respiratory failure after PQ intoxication (Robertson et al., 1970; Silva and Saldiva, 1998). The poisoning of rats with PQ results in a surfactant-deficient state, due to surfactant inhibition by plasma proteins leaking through the damaged alveoli-capillary membrane (So et al., 1998). In addition, PQ causes a distinct reduction of lecithin fraction to 75%, leading to collapse of the alveoli (Malmqvist et al., 1973). Intratracheal instillation of exogenous surfactant almost completely restored gas exchange to normal (So et al., 1998). Similar results were also observed after intratracheal instillation of surfactant in terms of improved gas exchange and prevention of lung inflammation, which resulted in less lung damage as a consequence of PQ exposure (Chen et al., 2001, 2002a). In the study of Salmona et al. (1992), ambroxol pretreatment increased the survival rate of the animals poisoned with PQ and antagonized the reduction of total phospholipid content in the lung. Ambroxol protection also significantly reduced the animals death rate in another study (Pozzi et al., 1989). However, in the study of Nemery et al. (1992), ambroxol treatment did not prevent the PQ toxicity. The effects of these treatments have not yet been investigated in human poisonings by PQ.

*Finally*, attempts to reduce the extent of pulmonary inflammation and fibrosis, including radiotherapy and the use of anti-inflammatory and immunosuppressant agents such as cyclophosphamide (CP) and steroids, have not provided compelling evidence of clinical efficacy (Eddleston et al., 2003). Immunosuppressive treatment for PQ poisoning was first reported by Malone in 1971, which quickly stimulated further reports (Eddleston et al., 2003). As described earlier in this review, inflammation appears to constitute an early response of the

lung to PQ poisoning. It is well recognized that inflammatory cells generate ROS, including  $O_2^-$ ,  $H_2O_2$ ,  $HO^\cdot$ , and hypochlorous acid (Lang et al., 2002; Nagata, 2005; Ricciardolo et al., 2006). In addition, proteolytic enzymes (such as elastase) are also produced and secreted into this environment (Gadek et al., 1984). From a biological perspective, this array of deleterious species constitutes an efficient defense against microbiological attack. However, host cells may also be damaged by these chemical species, and there is growing evidence to suggest that the inflammatory response contributes to the pathogenic effect in certain toxic or disease states (Lang et al., 2002; Nagata, 2005; Ricciardolo et al., 2006). This may be the case especially for PQ-induced toxicity, in which oxidizing species released by stimulated inflammatory cells further increase the burden on cellular antioxidant defense systems already "stressed" by the initiating PQ redox cycling. Pulse therapy with CP and methylprednisolone (MP) shows promise in reducing PQ-related mortality. This therapy is thought to work by reducing the inflammatory process leading to pulmonary fibrosis. In a single-blinded randomized clinical trial involving 142 PQ-poisoned patients, pulse therapy reduced mortality in moderate to severe PQ poisoning from 57% to 18% (Lin et al., 1999). Pulse therapy included 15 g/kg/day of CP in 5% glucose saline, 200 ml, given for 2 days, and 1 g/day of MP in 200 ml 5% glucose saline i.v. infused during 2 h for 3 days. All patients received also dexamethasone (DEX), 10 mg i.v., every 8 h for 14 days after admission. The study was criticized for possible bias during data analysis (Buckley, 2001). Addo and co-worker (Addo et al., 1984) reported a survival rate of 75% in a group of patients treated with a combination of high-dosage CP (5 mg/kg/day, i.v.) and DEX (24 mg/day i.v.) treatments for 14 days, whereas the mortality rate in a historical control group of patients not treated with these two drugs was 80%. However, the efficacy of this treatment cannot be assessed because criteria of severity, such as plasma PQ concentrations, were not evaluated. Also, these patients were treated with routine measures, such as Fuller's earth, activated charcoal, and magnesium citrate, to eliminate PQ from the gut, forced diuresis with furosemide, triamterine, and hydrochlorothiazide, and with niacin and vitamin C as well. The same research groups subsequently reported their experience with further 52 patients, presenting 72 patients in total (Addo and Poon-King, 1986). Again, they reported a much higher survival rate, 72% compared to 32%, in patients receiving immunosuppressive therapy compared to historical controls treated with standard therapy. Perriens and colleagues subsequently reported their experience of using the Addo regimen of immunosuppression in Surinam (Perriens et al., 1992). Using a prospective study including 47 consecutive patients with PQ poisoning, there was no difference in mortality and outcome between the 14 patients who had received a standard treatment and the 33 patients who had received a high-dose CP and DEX treatment (Perriens et al., 1992). Other treatments (Lin et al., 1996, 1999) have indicated that initial pulse therapy with CP at 15 mg/kg/day for 2 days and MP at 1 g/day for 3 days simultaneously, followed by DEX

at 20 mg/day for 14 days, may be effective in treating patients with moderate to severe PQ poisoning. However, the retrospective analysis of these studies showed that they were not based on an intent-to-treat principle, as patients who died within 3 or 7 days after intoxication were excluded from the final analysis. Furthermore, the fact that these studies did not include the measurement of plasma PQ levels to assess the severity of PQ poisoning may weaken their results. Recently, Lin et al. (2006) reported a novel anti-inflammatory method, with an increase of the patients' survival rate, by repeated pulse therapy of CP (15 mg/kg/day, i.v., 2 days) and MP (1 g/day i.v., 2 days) with prolonged DEX [5 mg, i.v., every 6 h until  $PaO_2 > 11.5$  kPa (80 mm Hg)] therapy to treat severely PQ-poisoned patients with 50–90% predictive mortality. If  $PaO_2$  was  $< 8.64$  kPa (60 mm Hg), repeated pulse therapy with MP (3 days) was again administered. In addition, CP was infused for 1 day again if patients' WBC counts were  $> 3000/m^3$  and the duration was  $> 2$  weeks after initial CP pulse therapy to avoid a severe leukopenia episode. The results were similar to those obtained by previous case reports (Chen et al., 2002b; Lin et al., 2003). Although Lin et al. (2006) described some limitations of the study, such as (1) small study sample (23 PQ-poisoned patients with  $> 50\%$  and  $< 90\%$  predictive mortality assessed by PQ plasma levels), which may limit the generalization of study findings to other patients with severe PQ poisoning, (2) absence of a placebo group, and (3) all patients received additionally 2 sessions of 8 h of CHP therapy in the emergency room, the authors suggest that this therapy may be effective. Until a proper, adequately powered, randomized controlled trial confirms the benefit, this treatment should be considered "experimental." Combined repeated MP pulse therapy preceding continuous DEX is known as a strong anti-inflammatory treatment in clinical practice (McCune et al., 1988; Boumpas et al., 1992), suppressing  $O_2^-$  production by neutrophils and macrophages. Furthermore, CP exerts a wide range of immunomodulatory effects that influence virtually all components of the cellular and humoral immune response and reduce the severity of inflammation (Fox and McCune, 1994), thus contributing to the overall effect. In addition, CP-induced leukopenia 1–2 weeks later may contribute to reduce pulmonary inflammatory process of PQ-poisoned patients (Addo and Poon-King, 1986; Lin et al., 1996). Hence, the efficacy of pulse therapy may be due to prevention and/or reduction the PQ-induced severe inflammation in lungs.

Due to the prevention of fibroblasts proliferation, radiotherapy has been associated with successful reversal of PQ pulmonary damage (Webb et al., 1984) but has not been successful in preventing fatality in severe PQ poisonings (Bloodworth et al., 1986). Irradiation of the lungs was considered in patients who, after surviving the acute phase of poisoning with PQ, showed progressive deterioration of respiratory function (Shirahama et al., 1987). Studies of single-dose radiation treatment in mice have not confirmed that radiotherapy has any benefit on PQ-induced pulmonary injury (Parkins and Fowler, 1985; Salovsky and Shopova, 1993). Also in nine cases treated by Talbot et al.

(Talbot and Barnes, 1988), radiotherapy failed to show a definite benefit.

### 8.5. New Perspectives

Although many treatments have been proposed and attempted empirically based on the pathologic mechanism of toxicity, none are supported by convincing clinical efficacy. Some authors claim success based solely on the results achieved in a single patient. Few controlled trials of these interventions have been performed, and results of published case series are inconsistent. Major deficits in assessing clinical benefit from various interventions and their combinations include the lack of a uniformly used prognostic indicator that reliably predicts risk of death at an early stage in the poisoning and small numbers of patients receiving a particular intervention. In this section the new and promising treatments are discussed.

#### 8.5.1. Mechanical Ventilation with Additional Inhalation of Nitric Oxide

Over the last years, mechanical ventilation with additional inhalation of NO, a gaseous molecule that contains an unpaired electron, has been proposed for the treatment of ARDS (Troncy et al., 1997; Hart, 1999). NO has a vasodilator effect in the lung areas with a high ventilation/perfusion ratio, and this effect results in an increase in the  $\text{PaO}_2/\text{FiO}_2$  ratio (Gianetti et al., 2002). Given that PQ toxicity is increased by oxygenation, NO inhalation in human PQ poisoned patients seems to be promising. This might permit a period of survival long enough for total systemic elimination of the ingested PQ, at which time lung transplantation might be undertaken without the risk of PQ-induced fibrosis developing in the grafted lung(s). Designed to evaluate the effects of inhaled NO on the PQ-induced lung injury in rats, the study of Cho et al. (2005) showed that the inhalation of NO contributed to increase the survival rate, and also helped to reduce LPO and to inhibit pulmonary fibrosis. Awkwardly, studies performed by Berisha et al. (1994) in isolated guinea pig lungs supported the view that NO is a critical intermediary in the production of oxidant tissue damage due to PQ, since all signs of injury, including increased airway and perfusion pressures, pulmonary edema, and protein leakage into the airspaces, were dose-dependently attenuated or totally prevented by selective and competitive inhibitors of NOS such as  $N^G$ -nitro-L-arginine methyl ester or  $N^w$ -nitro-L-arginine. The underlying mechanism is thought to be due to NO rapid reaction with  $\text{O}_2^-$  to form the strong oxidant peroxynitrite ( $\text{ONOO}^-$ ) (Nemery and van Klaveren, 1995). An alternative hypothesis was subsequently proposed, based on the findings that PQ uses NOS as an electron source to generate  $\text{O}_2^-$  at the expense of NO (i.e., NOS switches from an oxygenase to a PQ reductase) (Day et al., 1999). The data reported in this last study supported the concept that NOS is, indeed, a PQ diaphorase, and suggests that toxicity associated with such redox-active compounds involves a loss of NO formation, coupled with increased  $\text{O}_2^-$  generation.

In accordance with a lower NO production and consequent inhibition of NO-induced vascular relaxation (Day et al., 1999), high systolic and diastolic pressure, measured through a catheter inserted in the carotid artery, was observed in Wistar rats exposed to PQ (35 mg/kg, i.p.) (Oliveira et al., 2005). The fact that PQ increases pulmonary artery and airway pressures emphasizes the importance of NO deficiency in the toxicological response and may explain why patients suffering from PQ poisoning improve when treated with inhaled NO (Koppel et al., 1994; Maruyama et al., 1995; Eisenman et al., 1998). No adverse consequences or tachyphylaxis was observed at the concentrations of inhaled NO used. Guidelines from the National Institute for Occupational Safety and Health state that a time-weighted average of 25 ppm for NO constitutes a permissible exposure level (Services, 1988). The use of NO in the treatment of PQ poisonings definitively deserves further studies.

#### 8.5.2. Propofol

Another promising treatment comes from the studies of Ariyama et al. (2000) and Lugo-Vallin et al. (Lugo-Vallin Ndel et al., 2002), who both observed an increase of the median survival time of mice and rats intoxicated with PQ posttreated with propofol, mainly due to its recognized scavenging activity (Murphy et al., 1992). Because of this property, propofol has been proposed for patients in intensive care units with multiorgan failure or ARDS (Smith et al., 1994).

#### 8.5.3. Induction of P-glycoprotein

More recently our research group has been paying particular attention not only to the mechanism of PQ toxicity but also to the persistent lacuna in the treatment of PQ intoxications: the release of PQ taken up by the lungs. For that purpose, we evaluated the putative usefulness of the well-known multidrug resistance (MDR) phenomena for clearing up lung PQ. MDR is characterized by the occurrence of cross-resistance of cells to a broad range of structurally and functionally unrelated xenobiotics (Gottesman and Pastan, 1993). Several mechanisms are involved in MDR. One of the most well-known mechanisms is the overexpression of a plasma membrane phosphoglycoprotein termed P-glycoprotein (P-gp). P-gp, a member of the ATP-binding cassette (ABC) transporter superfamily, was initially identified in tumor cells as an ATP-dependent transporter that can export a wide variety of unmodified substrates out of the cell (Ling et al., 1983; Chen et al., 1986; Cordon-Cardo et al., 1990; Gottesman and Pastan, 1993). Besides in tumor cells, P-gp was also found to be expressed in a polarized manner at the apical surface (or luminal, depending on the organ) in a variety of normal tissues, including the lungs (Crapo et al., 1982). The expression of P-gp in liver, brain, and intestinal tissue and also in lung tissue has been shown to be induced by DEX (Demeule et al., 1999). This increased expression is rapid, since it was observed to be maximal only 1 day posttreatment (Demeule et al., 1999). In a

first study we demonstrated that the induction of *de novo* synthesis of P-gp by DEX (100 mg/kg i.p.), 2 h after administration of a lethal dose of PQ (25 mg/kg i.p.) to Wistar rats, results in a remarkable decrease of PQ lung accumulation (to about 40% of the only PQ-exposed group in just 24 h) and an increase of its faecal excretion (Dinis-Oliveira et al., 2006a, 2006b). Verapamil [VER (10 mg/kg i.p.)], a competitive inhibitor of P-gp, given 1 h before DEX blocked its protective effects and led to an increase of PQ lung concentration (up to about twice that of the only PQ-exposed group in just 24 h) and toxicity, indicating the important role of this transporter in PQ excretion. The obtained results showed that DEX also ameliorated the biochemical and histological liver alterations induced by PQ in Wistar rats (Dinis-Oliveira et al., 2006a). On the other hand, these improvements were not observed in kidney and spleen of DEX-treated rats. The sum of these effects was clearly positive, since an increased survival rate to 50% was observed 10 days postintoxication, which indicates that high-dosage DEX treatment constitutes an impor-

tant and valuable therapeutic tool to be used against PQ-induced toxicity. Although this experimental approach is interesting and novel, our conclusions are based on a very limited data set and thus need to be confirmed by more pre-clinical studies and, more importantly, must be tested in a clinical setting.

#### 8.5.4. Sodium Salicylate

The persistent lacuna related to the nonexistence of an ideal antidote that conducts to 100% survival impelled the study described in Dinis-Oliveira et al. (2007b). We clearly showed that sodium salicylate (NaSAL, 200 mg/kg i.p.) has great potential to be used as an antidote against PQ-induced lung toxicity, mainly mediated by an effective inhibition of proinflammatory factors such as nuclear factor kappa-B (NF- $\kappa$ B), by scavenging ROS, and also through the inhibition of myeloperoxidase activity and inhibition of platelet aggregation (Dinis-Oliveira et al., 2007b) (Figure 17). Importantly, this treatment was associated with full

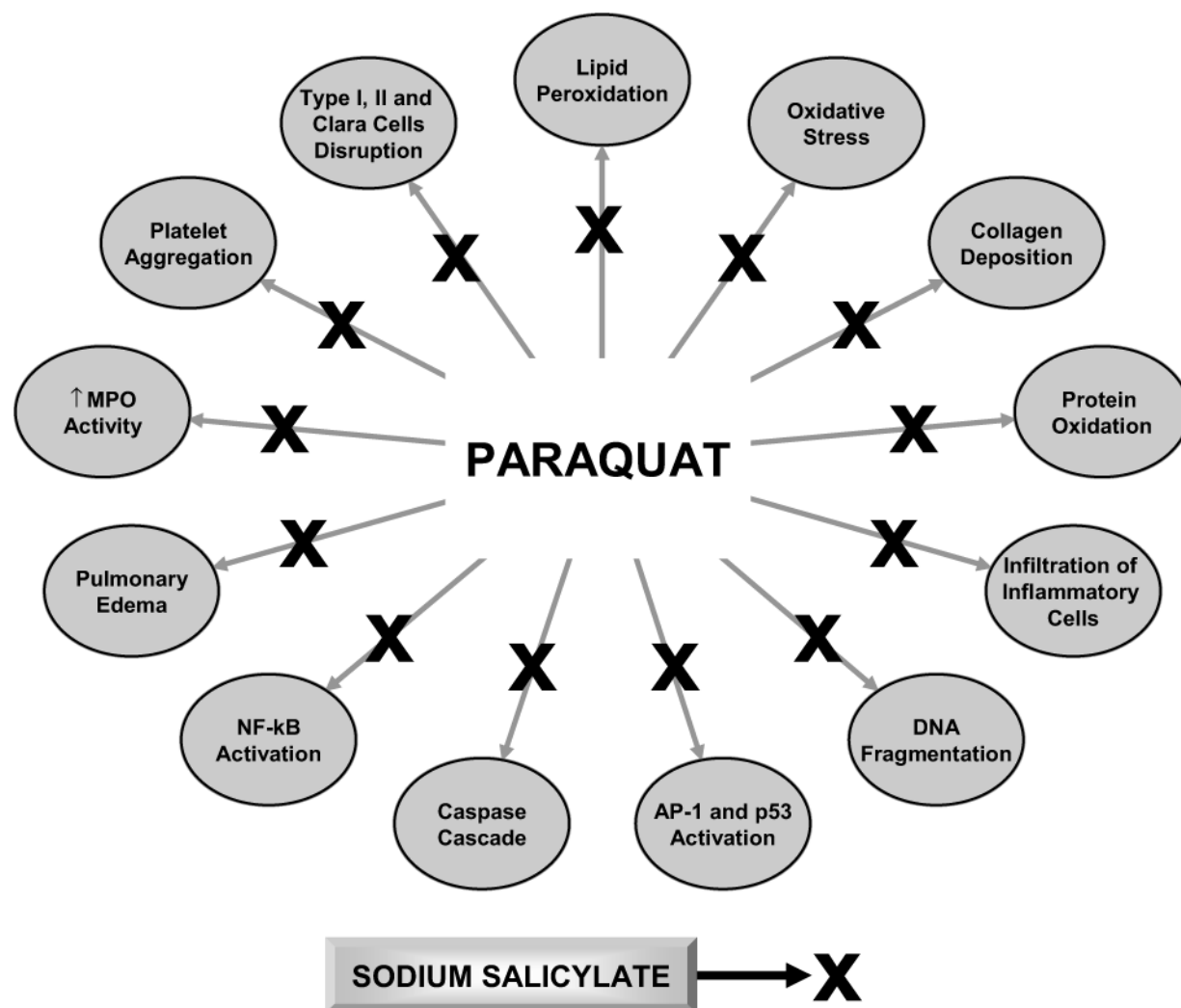


FIG. 17. Proposed protective mechanisms of sodium salicylate against pulmonary paraquat toxicity.

survival of PQ treated rats (extended for more than 30 days), in opposition to 100% mortality by day 6 in PQ-only exposed animals. NaSAL seems then to constitute a real antidote for PQ poisonings, since it is the first compound with such degree of success.

A subsequent study investigated the occurrence of apoptotic events in the lung of male Wistar rats, 24, 48, and 96 h after PQ exposure (25 mg/kg i.p.), as well as the putative healing effects provided by NaSAL (200 mg/kg i.p.) when administered 2 h after PQ (Dinis-Oliveira et al., 2007a). PQ exposure resulted in marked lung apoptosis, in a time-dependent manner, characterized by the "ladder-like" pattern of DNA observed through electrophoresis and by the presence of terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL)-positive cells (TPC) as revealed by immunohistochemistry. PQ-exposed rats suffered a time-dependent increase of caspase-3 and caspase-8 and a decrease of caspase-1 activities in the lung. Also observed was a marked mitochondrial dysfunction, evidenced by cytochrome *c* (Cyt *c*) release, and a transcriptional activation of the p53 and activator protein-1 (AP-1) transcription factors, in a time-dependent manner as a consequence of PQ exposure. Overall, this work led to a better understanding of the toxic mechanisms induced by PQ in the respiratory tract, showing that PQ induces several events involved in the apoptotic pathways, which might trigger lung toxicity. The data reported also reinforced the potential use of NaSAL in the protection against PQ-induced lung damage. NaSAL treatment resulted in the remission of the observed apoptotic signaling and consequently of lung apoptosis (Figure 17).

Of note, the administrations of DEX and NaSAL were given 2 h after intoxication of rats with PQ, a lag time that confers realism to be applied in humans, since this chronological time corresponds to longer biological time in humans and therefore may represent the actual time that passes between the herbicide ingestion and the start of medical care.

In our opinion these two last therapeutic approaches have high potential to be applied in humans. Although the doses of DEX and of NaSAL are quite high in rats and mild toxicity may ensue following the administration, clinically, PQ poisoning is an extremely frustrating condition to manage, due to the elevated morbidity and mortality observed so far, which may endorse this type of drastic treatment.

## 9. SEQUELAE IN SURVIVORS AND FULL RECOVERY

Pulmonary lesions following PQ poisoning are not invariably fatal. Most patients who survive usually do not develop obvious pulmonary complications at any stage of the intoxication or recovery phase. Nevertheless, many studies documented that victims of PQ poisoning who survived can have a residual restrictive lung disease, persistent radiological changes, and impaired gas exchange (Hudson et al., 1991). Fitzgerald et al. (1979) followed, for at least 1 year, 13 survivors of PQ poisoning to determine the prevalence of residual pulmonary disability. Of 11 adults, 5 (all nonsmokers) did not have any clinical, radio-

logical, or functional evidence of pulmonary dysfunction. Four others (all smokers) were considered normal on clinical and chest X-ray examination but had a mild deficit in pulmonary function, while the remaining two adults were known to have suffered from respiratory disability before the PQ poisoning. Bismuth et al. (1996) reported that five patients survived acute PQ ingestion, despite developing restrictive pulmonary dysfunction. Of these, two patients with documented long-term follow-up had progressive functional improvement. These authors also performed a literature review and revealed that 29 other patients survived with restrictive pulmonary dysfunction following acute PQ poisoning. Some patients who survived acute PQ poisoning may develop pulmonary fibrosis yet progressively improving over time (Bismuth et al., 1996). Similar evidences were also documented earlier (Lin et al., 1995; Papiris et al., 1995). More recently, these results were corroborated by Yamashita et al. (2000), who indicate that survivors of PQ poisoning may be left with a restrictive type of pulmonary dysfunction with progressive recovery. The authors suggested that long-term follow-up of lung function may be necessary for survivors of PQ poisoning. Renal, gastrointestinal, and hepatic manifestations return to normal following the natural course of acute necrosis (Beebejaun et al., 1971; Fisher et al., 1971).

## 10. CONCLUDING REMARKS

A few years after introduction of PQ it became clear that this was a serious hazard to humans, not with its proper use, but mainly as result of ingestion of the concentrate. In early reports (Bullivant, 1966; Campbell, 1968), accidental poisoning from drinking the dark brown concentrate, which resembled a cola drink after it had been decanted into soft-drink bottles, was common. Nowadays, however, intentional suicide deaths predominate.

Because of the well-described pulmonary adverse effects, the use of PQ had been restricted in many countries, and rigorous tolerance limits on foods have been established. Beyond its use and misuse as an herbicide and a poison for intentional suicide, PQ has become a model for pro-oxidant-induced chemical toxicity. Moreover, knowledge of the mechanism(s) of PQ toxicity has contributed significantly to the concept of cell-specific toxicity and has given rise to the notion that the cellular accumulation of a toxic agent through an endogenous transport system may underlie the observed toxic effects. Nevertheless, much remains to be elucidated in the toxicology of PQ at both pre-clinical and clinical levels. Controversy remains in the identity of the ultimate toxic agent(s) derived from PQ redox cycling and of the critical site(s) of intracellular damage. In addition, the factors that initiate and regulate the development of the fibrotic lesion are poorly understood. More than 44 years after the first reports of PQ poisoning in humans, recovery in such cases remains poor, and accepted regimens for treatment of the pulmonary effects of PQ poisoning are virtually nonexistent. Despite intensive investigation on PQ toxicity, neither the final cytotoxic mechanism nor a clinically useful antidote has been discovered. PQ toxicity

is closely related to the ingested dose. It is very rapidly accumulated in the pulmonary target cells, where it induces biochemical disturbances whose clinical manifestations are delayed. Among the possible mechanisms of toxicity, oxidative stress by generation of ROS, depletion of NADPH, formation of disulfides, protein oxidation, DNA damage, and LPO are the main features. Because of the rapidity of onset of the pulmonary lesions, extracorporeal elimination methods and most pathophysiological treatments are inefficacious in modifying the clinical course. Given the considerable toxicity of PQ, evaluation of treatments should be performed in groups of patients with a mortality rate between 30 and 80%, and not in groups with a mortality rate of 100% (Hart et al., 1984). Moreover, such evaluations should take into account the delay between ingestion and the start of treatment. Treatments that may radically improve the prognosis should be able to remove PQ from the lung rapidly, as may be achieved by the induction of *de novo* synthesis of P-gp, or should interrupt the toxic pathway (e.g., by administering NO, NaSAL, and other antioxidants and/or anti-inflammatory drugs) before irreversible pulmonary cellular damage has occurred.

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